Chromosome Karyotyping of *Senna covesii* and *S. floribunda* based on Triple-color FISH Mapping of rDNAs and Telomeric Repeats

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ABSTRACT The genus *Senna* in the family Fabaceae is distributed and cultivated around the world and has been used as sources of medicine and commercial goods. Molecular cytogenetic study based on FISH technique is useful for breeding and genomic research of the species. Triple-color FISH karyotype analyses were carried out to understand the genome structure of two *Senna* species, *S. covesii* (A. Gray) H. S. Irwin & Barneby and *S. floribunda* (Cav.) H. S. Irwin & Barneby using 5S rDNA, 45S rDNA, and telomeric repeat probes. Three and one pairs of 45S and 5S rDNA signals were detected, respectively, in *S. covesii*, whereas one pair each of 45S and 5S rDNA signals, were detected in *S. floribunda*. Telomeric signals appeared at all chromosome termini in both species. This result provides basic cytogenetic information which will be useful for future breeding and genomic research of *Senna* species.

Keywords 5S and 45S rDNAs, Telomeric repeat, *Senna covesii*, *Senna floribunda*, FISH

INTRODUCTION

The genus *Senna* in the family Fabaceae (Monkheang *et al.* 2011) has various uses such as food, flower decoration and improvement of barren land (Elaine *et al.* 2005; Resende *et al.* 2014). It is especially well known as a medicinal plant not only as ophthalmic medication but also in relieving skin diseases, alleviating poor digestion, healing of colds and even heart disease. In addition, it has expanded its usefulness as a living food such as coffee and gum (Singh *et al.* 2013). Because of these advantages, *Senna* is widely cultivated around the world including Brazil, America, Asia and Africa (Marazzi *et al.* 2006; Singh *et al.* 2013).

Cytogenetic studies provide information on chromosomal number, length, type, ploidy, and chromosomal distribution of specific sequences that are essential to elucidate genome structure and constitution in plant

species (Kim *et al.* 2005). The information can be useful in identifying species, tracing phylogenetic relationship among the related species, and breeding and development of plant species (Koo *et al.* 2003b; Kim *et al.* 2005). Despite the economic and health benefits of *Senna*, cytogenetic studies are still limited in this genus (Kim *et al.* 2005).

Fluorescence *in situ* hybridization (FISH) technique is an efficient and powerful tool in molecular cytogenetics to visualize specific DNA sequences on chromosomes and allows to construct chromosome map of the sequences. Highly repetitive DNA sequences such as rDNA, centromeric-, and telomeric-DNA have been used as FISH probes owing to their different number and distinct distribution on chromosomes according to the species (Hwang *et al.* 2009). Especially 5S rDNA, 45S rDNA, and telomeric repeat DNA are highly repetitive DNAs and usually used as primary probes for FISH experiment in

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plants (Koo *et al.* 2003a; Han *et al.* 2013).

Here, triple-color FISH karyotype was established using 5S rDNA, 45S rDNA, and *Arabidopsis*-type telomeric repeat DNA probes to elucidate the chromosomal constitutions of the genome of *S. covesii* and *S. floribunda*. This is the first report on FISH karyotype of the two species and this basic information will be useful to construct phylogenetic relationships among *Senna* species and breeding program of the medicinal species.

MATERIALS AND METHODS

Plant samples

Seeds of *S. covesii* and *S. floribunda* were obtained from Agricultural Research Service, USDA (PI 675048 and PI 601948). Seeds were germinated in the greenhouse. About 2 cm roots were harvested, pretreated with 2 mM 8-hydroxyquinoline for five hours at room temperature, fixed in aceto-ethanol (acetic acid : ethanol = 1 : 3, v/v) for more than two hours and transferred to 70% ethanol. The stock materials were kept in the refrigerator until use.

Chromosome spread preparation

Mitotic metaphase chromosome spreads were prepared using the technique described by Kato *et al.* (2004), with some modifications. Root tips (~2 cm) were digested in 1% pectolyase (Sigma, Japan) and 2% cellulase (MB Cell, Korea) solution at 37°C for 75 minutes. Meristematic tissue (~2 mm) of the roots were dissected using a needle, transferred into a 1.5 mL tube with 40 µL chilled aceto-ethanol fixative solution (acetic acid : ethanol = 1:3, v/v) and vortexed for 30 seconds at room temperature. After centrifugation, pellets were resuspended with aceto-ethanol (acetic acid : ethanol = 9 : 1, v/v) solution and drop on a slide glass in the humid chamber and then air-dried.

Fluorescence *in situ* hybridization

The 5S and 45S rDNAs and *Arabidopsis*-type telomeric repeat sequences (TTTAGGG)_n were used as probes for FISH karyotype analysis. The pre-labelled oligoprobes (PLOP) were designed and prepared according to Waminal

et al. (2018). Mitotic metaphase chromosome spreads were obtained following the procedure of Waminal *et al.* (2012). Thirty-two µL of a FISH hybridization master mix (50% formamide, 10% dextran sulfate, and 2× SSC) were mixed with 25 ng of each of PLOP probes. Distilled water was added to a total volume of 40 µL. Chromosomes with added PLOP probes on a glass slide were denatured at 80°C for 5 minutes. Slides were incubated at RT, 30 minutes. The slides were washed with 2× SSC at room temperature for 5 minutes, 0.1× SSC at 42°C for 20 minutes and 2× SSC for 5 minutes at room temperature. Slides were dehydrated in ethanol series of 70%, 90%, and 100%, air-dried and counterstained with a premixed 4',6-diamidino-2-phenylindole (DAPI) solution (1 µg/mL; DAPI in Vectashield, Vector Laboratories, Burlingame, CA, USA). Images were captured with a model BX53 fluorescence microscope (Olympus, Tokyo, Japan) equipped with a DFC365 FS CCD camera (Leica Microsystems, Wetzlar, Germany) and adjusted by using Cytovision ver. 7.2 (Leica Microsystems). Final images were edited using Adobe Photoshop CC (Adobe Systems, San Jose, CA, USA). Chromosome types were determined according to the description of Levan *et al.* (1964). Length of chromosomes were measured using ImageJ (NIH, USA).

RESULTS

Conventional karyotype

Both *S. covesii* and *S. floribunda* had chromosome number of $2n = 28$. The chromosome length of *S. covesii* ranged from 3.58 to 5.67 µm (Table 1) and those of *S. floribunda* ranged from 2.37 to 3.52 µm (Table 2). Both species had 10 metacentrics and four submetacentric chromosomes, but *S. floribunda* chromosomes were shorter than those of *S. covesii*. One pair of satellite chromosomes were observed in chromosome 13 of both species (Figs. 1 and 2).

Chromosomal distribution of rDNA and telomeric repeat sequences

As a result of triple-color FISH karyotype analysis using 5S rDNA, 45S rDNA, and telomeric repeat probes, three

Table 1. Karyotypic analysis of mitotic metaphase chromosome of *S. covesii*.

Chromosome number (2n = 28)	Arm length (µm)			Arm ratio (L/S)	Type
	Short (S)	Long (L)	Total (S + L)		
1	2.60	3.07	5.67	1.18	m ^{z)}
2	2.09	2.54	4.63	1.21	m
3	2.11	2.42	4.53	1.14	m
4	1.97	2.43	4.40	1.23	m
5	1.96	2.44	4.40	1.24	m
6	2.10	2.12	4.22	1.01	m
7	1.14	3.01	4.15	2.64	sm ^{y)}
8	1.84	2.28	4.12	1.23	m
9	1.70	2.30	4.00	1.35	m
10	1.79	2.17	3.96	1.21	m
11	2.02	1.90	3.92	0.94	m
12	1.35	2.49	3.84	1.84	sm
13 ^{x)}	1.10	2.53	3.63	2.30	sm
14	1.12	2.46	3.58	2.19	sm
Total	24.89	34.16	59.07		10 m + 4 sm

^{z)}metacentric, ^{y)}submetacentric, ^{x)}satellite chromosome with secondary constriction.

pairs of 45S rDNA signals were observed on chromosomes 7, 11, and 13, and one pair of 5S rDNA signals, on chromosome 1 in *S. covesii* (Figs. 1 and 2). In case of *S. floribunda*, only one pair of 45S and 5S rDNA signals were detected on chromosome 13 and 14, respectively. The satellite chromosome 13 showed major 45S rDNA signals in both species. The *Arabidopsis*-type telomeric sequence distributed to the terminal regions of all chromosomes in both species.

DISCUSSION

The chromosome number of *S. floribunda* (2n = 28) in this study corroborates the finding in previous reports (Rice *et al.* 2015; Cordeiro and Felix 2018), whereas that of *S. covesii* has not been reported yet and was revealed as 2n = 28 in this study.

Souza and Benko-Iseppon (2004) and Biondo *et al.* (2005) reported the sizes and types of chromosomes on

Table 2. Karyotypic analysis of mitotic metaphase chromosome of *S. floribunda*.

Chromosome number (2n = 28)	Arm length (µm)			Arm ratio (L/S)	Type
	Short (S)	Long (L)	Total (S + L)		
1	1.31	2.21	3.52	1.68	m ^{z)}
2	1.19	2.11	3.30	1.77	sm ^{y)}
3	1.07	2.07	3.14	1.93	sm
4	1.05	2.01	3.06	1.91	sm
5	1.27	1.62	2.89	1.27	m
6	1.22	1.64	2.86	1.34	m
7	1.58	1.27	2.85	0.80	m
8	1.21	1.61	2.82	1.33	m
9	1.41	1.31	2.72	0.92	m
10	1.11	1.61	2.72	1.45	m
11	1.07	1.64	2.71	1.53	m
12	1.09	1.45	2.54	1.33	m
13 ^{x)}	0.85	1.67	2.52	1.96	sm
14	0.99	1.38	2.37	1.39	m
Total	16.42	23.6	40.02		10 m + 4 sm

^{z)}metacentric, ^{y)}submetacentric, ^{x)}satellite chromosome with secondary constriction.

eleven *Senna* species. The average length was in a range of 0.62-2.50 µm. The chromosome sizes of *S. covesii* and *S. floribunda* were larger than those of the previously reported *Senna* species. Souza and Benko-Iseppon (2004) and Biondo *et al.* (2005) analyzed the chromosome types of *Senna* species according to the ratio of long arm per short arm. Generally, prevalent chromosome types of the *Sennas* were metacentrics while submetacentrics were only few. In this research, *S. covesii* and *S. floribunda*, consisted of 10 pairs of metacentrics and 4 pairs were submetacentrics.

Senna species have been regarded as effective medicinal plants. Breeding and development of the species has been expected, and the molecular cytogenetic research based on chromosomal constitution should precede genomic research to get information of the chromosomal organization of a genome. FISH technique has been useful molecular cytogenetic tool which can localize specific DNA sequences on chromosomes making it possible to visualize chromosomal landscape of a genome and construct cytogenetic physical maps (Park *et al.* 2010). Tandem

Fig. 1. Triple-color FISH metaphases of two *Senna* species. (A) *S. covesii*, (B) *S. floribunda* using 5S rDNA, 45S rDNA and telomeric repeat probes. Green, red, and yellow signals indicate 5S rDNA, 45S rDNA, and telomeric repeats, respectively. The scale bar is 10 μ m.

Fig. 2. FISH karyotypic idiogram of *S. covesii* (A) and *S. floribunda* (B). The number stands for chromosome number. Green, red, and yellow signals indicate 5S rDNA, 45S rDNA, and telomeric repeats, respectively. The scale bar is 10 μ m.

repeat sequences or multigene families can be used as FISH probes for chromosome characterization (Waminal *et al.* 2011). 5S and 45S rDNA genes form the multi-gene clusters which commonly exist in eukaryotes as several hundred to thousand copies of highly conserved tandem repeats (Gerlach and Bedbrook 1979). *Arabidopsis*-type telomeric tandem repeats (TTTAGGG)_n distribute majorly on the eukaryotic chromosome ends (Fuchs *et al.* 1995). Owing to their sequence repetitiveness, specific distribution on the chromosomes, and varying distribution among species and populations, 5S and 45S rDNA genes and *Arabidopsis*-type telomeric repeats were used as primary FISH probes to elucidate the chromosome level genomic constitution of a species and to trace the phylogenetic and comparative genomic relationship among plant species (Koo *et al.* 2003a; Han *et al.* 2013). Nevertheless, FISH karyotype on the *Senna* chromosomes using these probes has not been reported until now. In this paper, 5S rDNA, 45S rDNA and telomeric repeat

sequences were used as probes for triple-color FISH karyotype analysis in *S. covesii* and *S. floribunda*.

The nucleolar organizing region which appeared as secondary constriction on the satellite chromosome was detected by 45S rDNA probe on the chromosome 13 in both species. Distribution of 5S rDNA was different between two species and additional two pairs of 45S rDNA repeats were detected in *S. covesii*. Due to highly copied heterochromatin regions comprising satellite DNA, varying distribution numbers of 5S and 45S rDNAs among species in the same genus may occur through unequal crossing-over (Dubcovsky and Dvorák 1995; Murata *et al.* 1997; Roy *et al.* 2005; Roa and Guerra 2012; Han *et al.* 2013). It can be deduced to undergo different chromosomal rearrangement in two species on the varying appearance of 5S and 45S rDNAs. Additional comparative repeatomics and cytogenomics studies will be helpful to explain the relationship of these two species and other species in the genus in more detail.

This result is the first FISH karyotype report applying 5S rDNA, 45S rDNA and telomeric repeat sequences as probes to *S. covesii* and *S. floribunda*. This result will be useful for *Senna* plant breeding and provide basic chromosomal backbones for *Senna* genome sequencing analysis.

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