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RESEARCH ARTICLE

Cytogenetic analysis of *Bienertia sinuspersici* Akhani as the first step in genome sequencing

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Abstract

Background C_4 plants are efficient in suppressing photorespiration and enhancing carbon gain as compared to C_3 plants. *Bienertia sinuspersici* Akhani is one of the few species in the family Amaranthaceae that can perform C_4 photosynthesis within individual chlorenchyma cells, without the conventional Kranz anatomy in its leaf. This plant is salt-tolerant and is well-adapted to thrive in hot and humid climates. To date, there have been no reported cytogenetic analyses yet on this species.

Objective This study aims to provide a cytogenetic analysis of *B. sinuspersici* as the first step in genome sequencing.

Methods Fluorescence in situ hybridization (FISH) karyotype analysis was conducted using the metaphase chromosomes of *B. sinuspersici* probed with 5S and 45S rDNA and *Arabidopsis*-type telomeric repeats.

Results Results of the cytogenetic analysis confirmed that *B. sinuspersici* has 2n = 2x = 18 consisting of nine pairs of metacentric chromosomes. Two loci of 45S rDNA were found on the distal regions of the short arm of chromosome 7. Nine loci of 5S rDNA were found in the pericentromeric regions of chromosomes 1, 3, 4, 6, and 8, which also colocalized with *Arabidopsis*-type telomeric repeats; while four loci in the interstitial regions of chromosome 5 and 8 can be observed. The single locus of 5S rDNA that was found in chromosome 8 appears to be hemizygous.

Conclusion The FISH karyotype analysis, based on the combination of rDNAs, telomeric tandem repeat markers and C_0t DNA chromosome landmarks, allowed efficient chromosome identification and provided useful information in characterizing the genome of *B. sinuspersici*.

Keywords *Bienertia sinuspersici* · Single-cell C_4 · FISH · rDNA · Telomeric repeats · C_0t DNA

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Abbreviations

BAC	Bacterial artificial chromosome
DNA	Deoxyribonucleic acid
dNMP	Deoxyribonucleoside monophosphate
FISH	Fluorescence in situ hybridization
GISH	Genomic in situ hybridization
NOR	Nucleolus organiser regions
rDNA	Ribosomal RNA
rpm	Revolutions per minute

Introduction

Bienertia sinuspersici is a flowering plant in the Amaranthaceae family that can be found in countries around the Persian Gulf, including Iran, Iraq, Kuwait, Saudi Arabia, and the United Arab Emirates, and is well-adapted in hot and humid climates with high salinity environment (Akhani et al. 2005). *B. sinuspersici*, a single-cell C_4 plant, performs C_4

photosynthesis within individual chlorenchyma cells without the use of the conventional Kranz anatomy (Park et al. 2010). There is a growing interest in studying its mechanism and evolution due to its efficiency in adapting to the environment but to date, only a few studies have been reported on its cytogenetic and genomic aspects.

Molecular cytogenetic techniques, particularly fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH), play an important role in mapping the chromosomes, analyzing the genome and genetic variations, studying phylogenetic relationships, detecting chromosomal rearrangements, and breeding of plants (Devi et al. 2005; She et al. 2007). The FISH technique allows the hybridization of a chromosome-specific DNA probe labeled with fluorescent dyes, producing a fluorescence signal after the probe successfully attaches to the target DNA sequence (Younis et al. 2015). It has proven to be an excellent tool in identifying chromosomes in plants using various types of DNA sequences such as genus- or species-specific repetitive sequences, tandem repeats (5S and 45S ribosomal DNA), centromeric, and telomeric repeats (Devi et al. 2005; Hizume et al. 2002; Kamstra et al. 1997; Lakshmanan et al. 2015; Page et al. 2001). Including repetitive sequences, physical mapping of single genes using FISH on chromosomes has also been beneficial in comparing the physical location of the gene and its position on the linkage map (Fukui 1990). Additionally, FISH mapping of the bacterial artificial chromosome (BAC) clones has generated a cytogenetic map that was used as supplemental information in genome sequencing (Iovene et al. 2008).

Repetitive DNA elements, such as tandem repeats and transposable elements, comprise the largest portion of the genome in higher eukaryotes such as those of many major crop plants (He et al. 2015). The localization of these sequences using FISH can be used to identify distinct regions in the chromosomes and this information, including the distribution, genome composition, and evolutionary origin of repetitive DNA sequences, is essential to better understand the organization, evolution, behavior and functional potential of repeats in eukaryotic genomes (Mehrotra and Goyal 2014; Plohl 2010). Ribosomal DNA (rDNA) genes 5S and 45S (18S-5.8S-25S) are tandem repeats that are most frequently used probes for FISH and have been proven to be excellent chromosomal markers for karyotype analysis because they are highly conserved and have a high copy number (Hasterok et al. 2001; Kato et al. 2005; Zhou et al. 2019). In addition to rDNAs, telomeric repeats are also valuable cytogenetic markers because they are highly conserved, consisting of a short sequence similar to $TTTAGGG_{(n)}$ in tandem arrays many hundreds of units long in most eukaryotes (Heslop-Harrison 2000). The telomeres are responsible for the stability and replication of chromosomes through the enzymatic activity of telomerase (Zakian 1995) and their location in the chromosome can provide information on karyotype evolution (Hizume et al. 2000). Investigating large genomes such as those of plants has been challenging because of the abundance of the repetitive DNAs with no known function. For this reason, effective strategies to isolate and sequence the unique elements of the genomes are essential to limit the redundant sequencing of repetitive elements (Peterson et al. 2002a, b). C_0t analysis measures how much repetitive DNA is present in a DNA sample based on the principle that in a sheared genomic DNA solution, the reassociation of a specific sequence is directly proportional to the number of times it occurs in the genome (Britten and Kohne 1968; Britten et al. 1974). Based on this tool, distribution of highly repetitive (C_0t-1), high + moderately repetitive (C_0t-10), and high + moderate + low-copy DNA sequences (C_0t-100) can be identified in the chromosomes (Peterson et al. 2002a, b; Waring and Britten 1966). C_0t analysis was very useful in analyzing the complexity of the genome, especially in major crops such as maize and wheat, which have large genome sizes (Goldberg 2001; Lamoureux et al. 2005; Yuan et al. 2003).

Recently, the Rural Development Administration (RDA) in Korea launched a genome sequencing project on *B. sinuspersici* as the main material for the development of carbon metabolism engineering biotechnology to improve the yield of crop plants. The chromosome number of *B. sinuspersici* was reported to be 2n = 2x = 18, with eight pairs of metacentric chromosomes and one pair of submetacentric chromosomes (Akhani et al. 2005). However, the conventional karyotyping technique used in previous study revealed very limited cytogenetic information of the species. Cytogenetic data is necessary for the genome sequencing project because it provides basic information on the genome structure and organization of the species and also verifies sequence assemblies (Doležel et al. 2014). Whole-genome sequencing studies, genome-wide association studies on polymorphisms, and mapping of repetitive sequences using various cytogenetic approaches can clarify the structure, evolution, and function of this part of the genome, which will be of great importance in sequencing and assembling of complex plant genomes (Mehrotra and Goyal 2014).

Hence, this study aimed to construct the karyotype of *B. sinuspersici* using three useful DNA markers and major repetitive DNAs obtained from C_0t analysis in its somatic chromosomes. These data will provide an overview of the genome study of *B. sinuspersici* and will also provide the chromosomal backbone for the physical mapping of the genome sequencing results.

Materials and methods

Plant material and chromosome preparation

Young roots were harvested from the tissue culture-derived plant material of *B. sinuspersici* from the RDA, Republic

of Korea and were treated with 2 mM 8-hydroxyquinoline for 5 h at 25 °C. Carnoy's solution (3:1 acetic acid–ethanol) was used to fix the roots overnight at room temperature, and 70% ethanol at 4 °C was used to preserve the roots. The fixed root tips were washed with distilled water prior to the enzyme mixture treatment (0.3% cellulose, cytohelicase, and pectolyase) at 37 °C for 90 min. The enzyme-treated roots were transferred to a 1.5 mL tube containing Carnoy's solution and were vortexed for 20 s. The homogenized root meristems were placed on ice for 5 min and were centrifuged at 13,000 rpm to collect the pellet. The supernatant was discarded and the pellet was immediately resuspended in acetic acid–ethanol (9:1) solution. The final suspension was spread on a 70 °C pre-warmed glass slide in a humid chamber and air-dried at room temperature.

Genomic DNA shearing and C_0t DNA isolation

The extraction of the genomic DNA of *B. sinuspersici* was performed using the Cetyltrimethylammonium bromide (CTAB) method described by Allen et al. (2006) with minor modifications. The genomic DNA was sheared into fragments of 0.1–1 kb in length using an S220 Focused-ultrasonicator (Covaris, USA). C_0t DNA isolation was carried out following the procedure described by Zwick et al. (1997). The sheared genomic DNA was diluted to a concentration of 500 ng μL^{-1} using 5 M NaCl and distilled H_2O (Sigma, USA) to a final concentration of 0.3 M NaCl. It was then denatured at 95 °C for 10 min, cooled on ice for 10 s, and re-annealed in a heat block at 65 °C. The time required for reannealing was calculated using the following formula: $C_0t = \text{DNA concentration (mol } L^{-1}) \times T_s$, where T_s is the renaturation time in seconds. Thus, C_0t-1 DNA, $C_0t\text{-}1 = 1 = \text{mol } L^{-1} \times T_s$ and C_0t-100 DNA, $C_0t\text{-}100 = 100 = \text{mol } L^{-1} \times T_s$. The time needed for C_0t-1 was determined on the basis of the initial concentration of the DNA: (1) the sheared DNA is at a concentration of 500 ng μL^{-1}; (2) divided by the dNMP content, which is equal to an average of 339 g moL^{-1} (0.5 g moL^{-1})/339 g $moL^{-1} = 1.47 \times 10^{-3}$; and (3) for C_0t-1, $T_s = 1/(1.47 \times 10^{-3})$. Re-annealed C_0t-1 and C_0t-100 DNA were gently mixed with 10X S1 buffer (Promega, USA) and treated with the S1 enzyme (Promega, USA) for 8 min at 37 °C. Equal volumes of phenol–chloroform-isoamyl alcohol were added to stop the reaction and centrifuged at 13,000 rpm for 10 min. The supernatant containing the DNA fractions were then transferred to a new tube separately and purified using the ethanol precipitation method.

Probe preparation and fluorescence in situ hybridization

The 5S rDNA, 45S rDNA and *Arabidopsis*-type (TTT AGGG)$_4$ telomeric repeat oligoprobes were used for FISH karyotype analysis. The pre-labeled oligoprobes (PLOPs) were designed and prepared following methods described by Waminal et al. (2018). C_0t-1 and C_0t-100 DNAs were labeled with digoxigenin-11-dUTP (Roche, Germany) by nick translation according to the manufacturer's protocol.

FISH was performed using the procedures described by Lim et al. (2007) with minor modifications. The hybridization mixture consists of 50% formamide, 10% dextran sulfate, 20X SSC, 50 ng μL^{-1} of each DNA probe, and Sigma purified water. The mixture was pipetted onto prepared chromosome slides and denatured on an 80 °C slide heater for 5 min. These slides were incubated at room temperature in a humid chamber for 30 min. After hybridization, the slides were washed successively with 2X SSC at room temperature for 10 min, 0.1X SSC at 42 °C for 25 min, and 2X SSC at room temperature for 5 min, then were dehydrated through an ethanol series (70%, 90%, and 100%) at room temperature for 3 min each. The slides were counterstained with Vectashield (Vector Labs, H-1000, USA) with 1 μg mL^{-1} 4′, 6-diamidino-2-phenylindole (DAPI) and examined under an Olympus BX53 fluorescence microscope with a built-in CCD camera (CoolSNAP™cf) using an oil lens (×100 magnification). The images were enhanced using Adobe Photoshop CS6. Chromosomes were paired based on their FISH signals, morphological characteristics, and arm lengths.

Results

Karyotyping

FISH karyotype analysis using the metaphase chromosomes of *B. sinuspersici* confirmed that it has a diploid genotype, having 2n = 2x = 18 chromosomes (Fig. 1). Nine pairs of chromosomes were classified as metacentric based on the arm-ratio values of Levan et al. (1964). Table 1 summarized the characteristics of the chromosomes arranged in decreasing length. The chromosome lengths ranged from 7.56 μm for chromosome 9 being the shortest to 10.07 μm for chromosome 1 being the longest. The total length of the somatic metaphase chromosome of *B. sinuspersici* was 83.07 μm. Bright DAPI bands were intensely stained in primary constrictions of the chromosomes 1, 3, 4, 6, and 8 but were not observed in chromosome 2, 5, 7, and 9 (Fig. 1a).

FISH analysis using rDNA and telomeric repeats

A total of 13 loci of 5S rDNA were observed in six pairs of chromosomes. Four pairs of 5S rDNA sites were found in the pericentromeric regions of short arm of chromosomes 1, 3, 4, and 6, while four loci were observed in the interstitial regions of chromosome 5 and 8 (Fig. 1c, green fluorescence). An additional locus was found in the pericentromeric

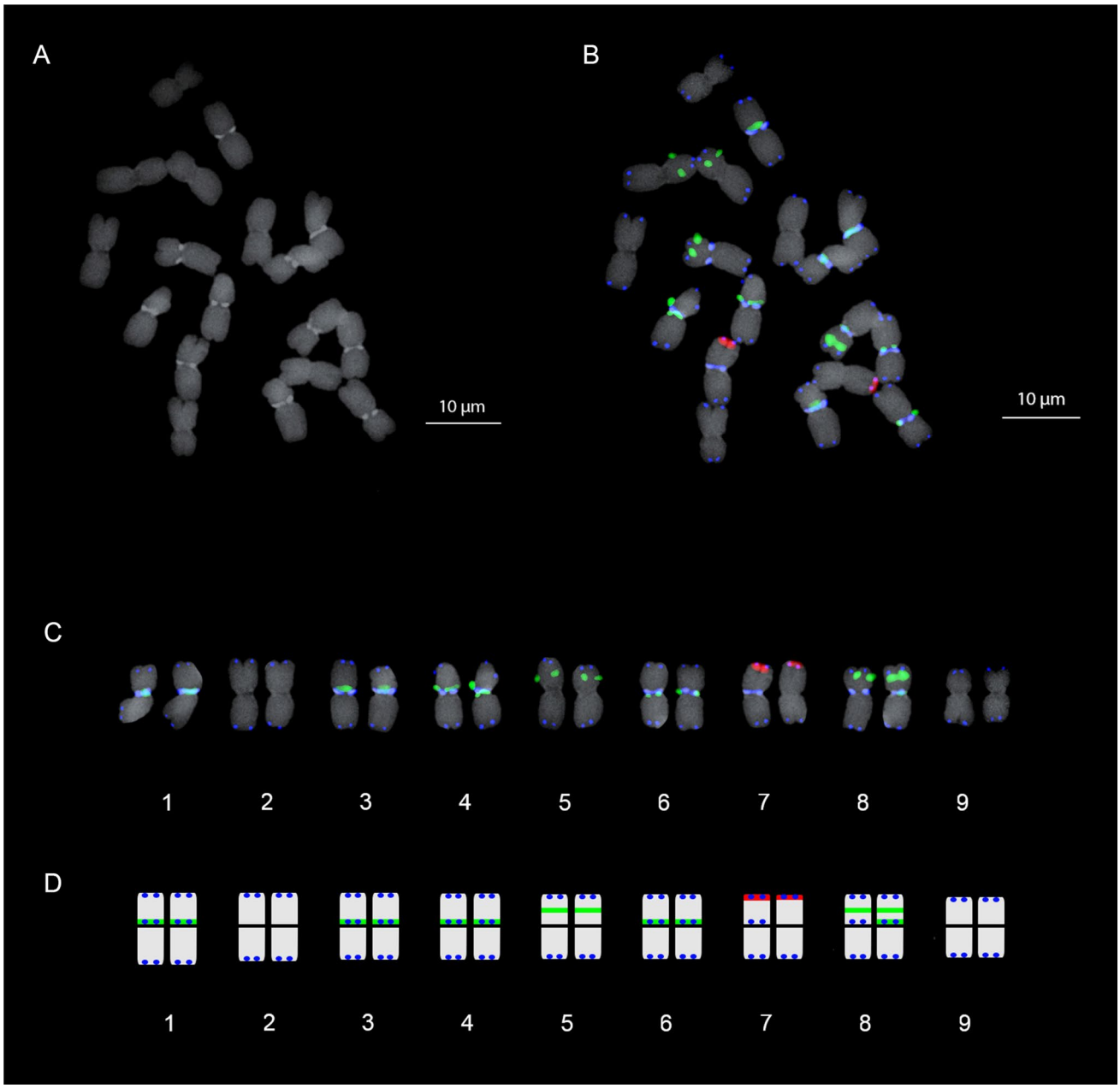

Fig. 1 *B. sinuspersici* Akhani metaphase chromosomes with DAPI staining (**a**), FISH using 5S rDNA (green fluorescence), 45S rDNA (red fluorescence), and *Arabidopsis*-type telomere (blue fluorescence-pseudo-color) as probes in *B. sinuspersici* Akhani (**b**); karyotype of the chromosomes (**c**); and idiogram of the chromosomes (**d**). Scale bar = 10 µm

region only in one homologous chromosome 8. A pair of 45S rDNA was found in the terminal region of the short arm of chromosome 7 (Fig. 1c, red fluorescence).

Signals of the *Arabidopsis*-type telomeric repeats (TTTAGGG)$_4$ were observed in the telomeric ends of both arms of all the chromosomes and in the pericentromeric regions of short arm of chromosomes 1, 3, 4, 6, and 8 (Fig. 1d, blue fluorescence-pseudo-color). Colocalization between the telomeric repeats and 5S rDNA was also observed at the pericentromeric region of these chromosomes. Additionally, 45S rDNA and telomeric repeat were colocalized at the end of the short arm of chromosome 7. Also, telomeric repeats were observed at the pericentromeric region of one of the homologous of chromosome 7.

FISH with C_0t-1 DNA

C_0t-1 and C_0t-100 DNA fractions from *B. sinuspersici* were isolated, probed and were hybridized on the metaphase chromosomes (Fig. 2). Bright C_0t-1 DNA signals were observed

Table 1 Chromosome features of *B. sinuspersici* Akhani chromosomes

Chr. No.	Chromosome length (μm)			rDNA		*Ar*-type TR[b]	Arm ratio (μm) (*q*/*p*)	Type
	Short arm (*p*)	Long arm (*q*)	Total (*q* + *p*)	5S	45S			
1	4.46 ± 0.06[a]	5.70 ± 0.17	10.07 ± 0.21	+[c]	−[d]	+	1.28 ± 0.05	m[f]
2	4.83 ± 0.05	4.98 ± 0.10	9.74 ± 0.16	−	−	+	1.03 ± 0.02	m
3	4.29 ± 0.02	5.32 ± 0.12	9.58 ± 0.10	+	−	+	1.24 ± 0.03	m
4	4.51 ± 0.11	4.87 ± 0.04	9.43 ± 0.08	+	−	+	1.08 ± 0.03	m
5	4.34 ± 0.08	5.03 ± 0.06	9.32 ± 0.14	+	−	+	1.16 ± 0.03	m
6	4.35 ± 0.11	4.94 ± 0.01	9.25 ± 0.13	+	−	+	1.14 ± 0.03	m
7	4.50 ± 0.15	4.73 ± 0.06	9.19 ± 0.22	−	+	+[e]	1.05 ± 0.04	m
8	3.92 ± 0.15	5.06 ± 0.01	8.92 ± 0.17	+[e]	−	+	1.29 ± 0.05	m
9	3.36 ± 0.03	4.22 ± 0.05	7.56 ± 0.05	−	−	+	1.26 ± 0.01	m
Total	38.57	44.85	83.07					

[a]Mean ± SD

[b]*Arabidopsis*-type telomeric repeats

[c]Present

[d]Absent

[e]Heteromorphic signal

[f]Metacentric

except in the centromeric parts containing bright DAPI bands and in the telomeric regions (Fig. 2b). C_0t-100 FISH signals were dispersed throughout the entire chromosomes including telomeric regions, but still showed weak signals in the DAPI bands located in the centromeric regions (Fig. 2d).

Discussion

Karyotype analysis and distributions of rDNA and telomeric repeats

A preliminary study on the number and length of the chromosomes of *B. sinuspersici* reported that it had eight pairs of metacentric chromosomes and one pair of the submetacentric chromosome, with lengths that ranged from 3.30 to 5.71 μm (Akhani et al. 2005). In contrast, our study showed nine pairs of metacentric chromosomes with lengths ranging from 7.56 to 10.07 μm. To accurately measure chromosome size, the (1) optimization of pretreatment conditions, such as different agents, treatment time and temperature, (2) identifiable well-made metaphase slides, and the (3) sufficient number of chromosome analysis (at least ten cells) were considered as prerequisite factors (Hwang et al. 2009; Mártonfiová 2013). Despite consideration of these factors, accurate chromosome analysis has proved to be difficult due to different degrees of condensation of chromosomes during cell division. The karyotypic discrepancies between this study and the studies of Akhani et al. (2005) might be due to the different quality of metaphase chromosome spreads (Liehr et al. 2004) or accuracy of measurement (Hwang et al. 2009, 2011). Moreover, karyotype analysis of small-sized plants having high similarity in their chromosomes cannot provide accurate information if only the chromosome morphology is considered (Rho et al. 2012).

Repetitive DNAs are commonly used as primary probes to elucidate the structure of genomes at the chromosomal level. This is the first report on using rDNAs and *Arabidopsis*-type $(TTTAGGG)_4$ telomeric repeats as markers for karyotyping and identifying individual chromosomes on *B. sinuspersici*. Studies have shown that the use of 45S and 5S rDNAs combined with FISH technique can provide useful information for the breeding and genome detection in *Chrysanthemum* (Younis et al. 2015). Highly conserved 5S rDNA and 45S rDNA with the abundant number of copies have been used for the FISH analysis of *B. sinuspersici*. Interestingly, not all 5S rDNA were located in homologous chromosomal locations. A locus of 5S rDNA located in the pericentromeric region of one of the homologues of chromosome 8 appeared to be hemizygous. This may be a result of the unequal crossing-over of the 5S rDNA, causing molecular drives for genome evolution (Dover 1994; Ohta 1984; Reddy et al. 2013). Studies on the rDNA distribution of other *Bienertia* species should be conducted to consider the possibility of structural chromosome rearrangements or lost of rDNA loci during evolution, resulting in this hemizygosity (Thomas et al. 1997).

The 45S rDNA repeat unit comprises the three coding regions (18S, 5.8S, and 25S/26S/28S), internal transcribed spacers between genes (ITS1 and ITS2) and a long intergenic spacer (IGS) separating adjacent repeats (Galián et al. 2012). In this study, FISH results revealed that 45S

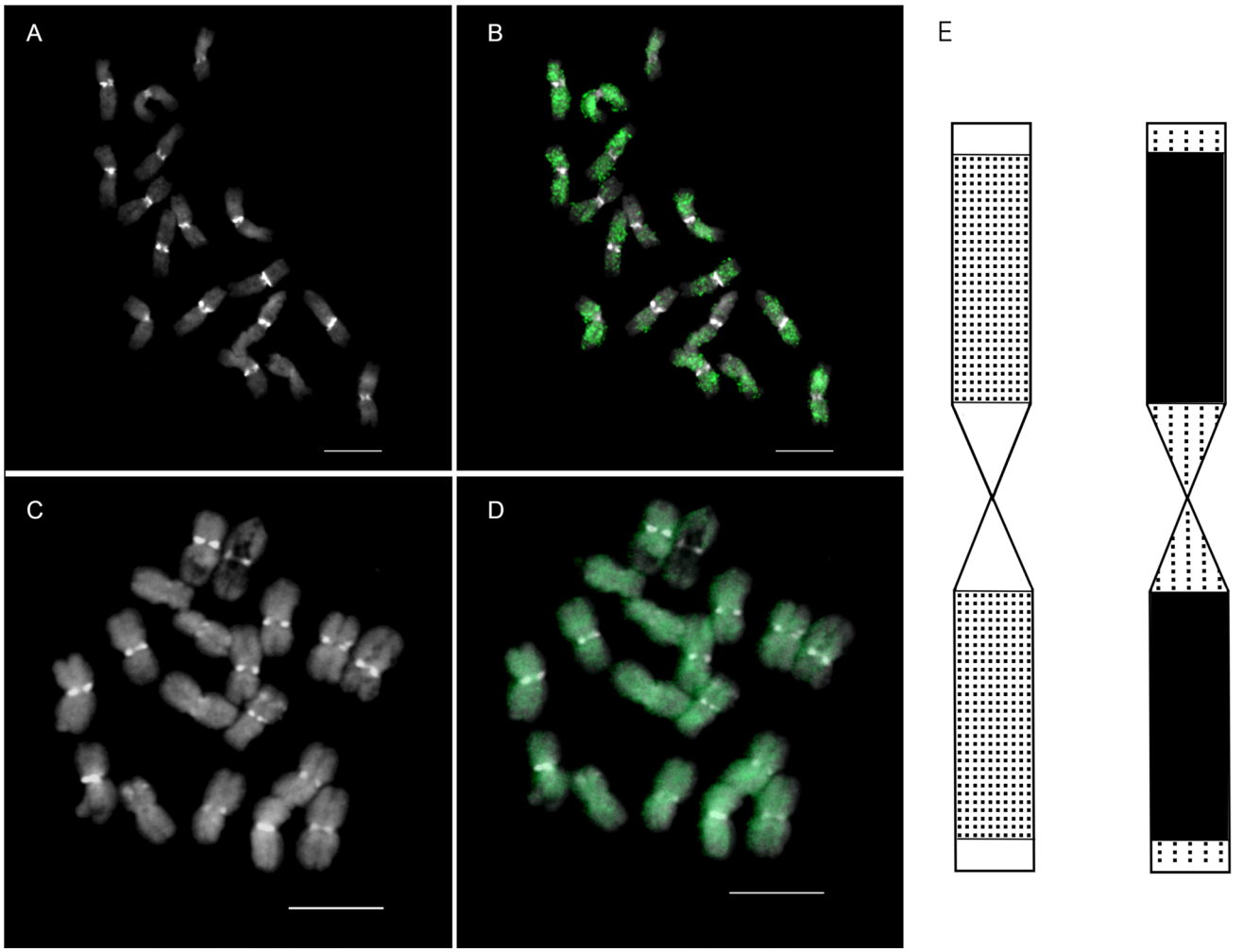

Fig. 2 FISH analysis of C_0t-1 and C_0t-100 DNA on metaphase chromosomes of *B. sinuspersici* Akhani: chromosomes with DAPI staining (gray) (**a** and **c**). The same spreads were then reprobed with C_0t-1 DNA (**b**) and C_0t-100 DNA (**d**), respectively. A representation of *B.sinuspersici* C_0t-1 and C_0t-100 DNA sites on its chromosomes (**e**). Scale bar = 10 µm

rDNA hybridized in the same region where the telomeric sequences were located. Previous reports on species such as *Giardia duodenalis* and *Sorex granarius* had shown the close relationship between the telomere repeats and the rDNA sequences (Upcroft et al. 2005; Zhdanova et al. 2007). In *Arabidopsis,* it was observed that telomeres were related with the nucleolus in interphase nuclei, suggesting that telomeres might be involved in nucleolus formation (Armstrong et al. 2001; Dvořáčková et al. 2010). Bicolor FISH on the chromosome of *Chrysanthemum segetum* revealed that 45S rDNA signals were found at the telomeric region and the telomeric repeats were interspersed into rDNA sequences. These results showed the close structural link of the rDNA with the telomere both in the cytogenetic and molecular level and it was suggested that many recombination events occurred at the terminal regions of the chromosomes (Copenhaver and Pikaard 1996; Li et al. 2012). The number, pattern and position of rDNA sites vary between species (Kolano et al. 2012). Generally, 45S rDNA signals are higher in number and variability than 5S rDNA signals such as in some species of *Cucumis* (Zhang et al. 2016), *Gossypium* (Gan et al. 2013) and *Passiflora* (De Melo and Guerra 2003) which is in contrast with our results in *B. sinuspersici*. These variations in rDNA sequences make them important in identifying species cytologically and a comparative analysis in the localization of these sequences may contribute to generalizing their patterns of distribution in certain genus and their differentiation among species (Kolano et al. 2012).

Telomeric sequences are often located at the terminal regions of the chromosomes (Fuchs et al. 1995). However, in *B. sinuspersici*, intense telomeric signals were also observed at its pericentromeric sites. There are also existing reports on plant and animal species wherein there was a non-telomeric

distribution of telomere sequence repeats (Hizume et al. 2000; Meyne et al. 1990; Schubert et al. 1995). The unusual chromosomal distribution of telomeric sequences may have resulted from multiple genome rearrangements during evolution, through different mechanisms such as chromosome fusions, arm inversions or DNA double-strand breaks (Fuchs et al. 1995). A previous report on tomato had speculated that the association of telomeric repeats with the centromere may have functional significance (Presting et al. 1996). Furthermore, in some species such as *Paramecium primaurelia* and Armenian hamster, the telomeric repeats in the interstitial regions proved to be recombination hot spots which might influence genetic variation and contribute to genomic rearrangements (Ashley and Ward 1993; Katinka and Bourgain 1992).

C_0t DNA analysis

C_0t analysis is a cytogenetic tool that is useful in providing vital information and can be applied through other areas such as purification of DNA sequence classes, qualification of specific DNA sequences and measurement of DNA sequence polymorphism (Graham 2001). In maize, C_0t-based DNA fractionation, together with high-throughput sequencing method, showed enrichment of its genomic DNA (Palmer et al. 2003; Whitelaw et al. 2003; Yuan et al. 2003). A large genome with a high content of repetitive DNA sequences complicates the assembly of the genome (Okagaki and Phillips 2004). C_0t analysis was performed in this study to provide additional data in analyzing its genome and to locate its major repetitive sequences. *B. sinuspersici* with an anticipated large genome size of 3.8 Gb (unpublished data) has been sequenced using long-read sequencing and assembled by Kim et al. team of RDA, Korea. C_0t analysis can help analyze large-sized genome sequencing studies such as *B. sinuspersici* by visualizing the major repetitive sequences on the chromosomes.

C_0t-1 DNA is composed of highly repetitive sequences and is generally known to rDNA, centromeric and telomeric repeats (Chang et al. 2008). In the case of *Brassica* species with a small-sized genome, it has been reported that C_0t signals are mainly concentrated around the pericentromeric parts and nucleolus organiser regions (NOR) (Hwang et al. 2009; Wei et al. 2007). In large-sized genome, such as *Chrysanthemum boreale*, abundant repetitive DNAs were localized in the centromeric, pericentromeric and in telomeric regions of the chromosomes (Cuyacot et al. 2016). However, in *B. sinuspersici*, C_0t-1 DNA signals were distributed between the short and long arm of the chromosome indicated by dotted areas, while dispersed signals were observed for those in C_0t-100 with weak signals on the centromeric regions (Fig. 2e). Similar results were also found in C_0t-1 DNA analysis of *Lilium tigrinum* with large genome size where weak signals were found in the NOR (Hwang 2015). Chang et al. (2008) also reported the presence of weak C_0t-1 signals in the pericentromeric regions. Non-painting of certain parts of the telomeric regions in the *B. sinuspersici* chromosome may be due to localized non-repetitive sequences as shown in *Chrysanthemum* by Cuyacot et al. (2016) and Hwang (2015). However, further research is still needed for the differences in the distribution of the repetitive sequences among the different species (Wang et al. 2007).

In tomato, painting the chromosomes with C_0t-100 showed the distribution of all repeats along the tomato chromosomes (Chang et al. 2008). The presence of repetitive sequences in euchromatic regions are supposed to influence genome evolution, and genome sequencing has frequently revealed short and truncated copies of repetitive sequences in these regions (Biémont and Vieira 2006; Terencio et al. 2015; Timberlake 1978; Torres et al. 2011; Yuan and Wessler 2011). C_0t-100 DNA FISH analysis in *B. sinuspersici* showed results covering the whole regions in *B. sinuspersici* where the rDNAs and telomeric repeats are located in the chromosomes. These dispersed signals were due to the presence of large amounts of repetitive DNA sequences which is common to plants having large genomes such as *B. sinuspersici* (Mancia et al. 2015). Our results suggested that the C_0t DNA fractions successfully characterized the repetitive elements in *B. sinuspersici* using FISH analysis.

Nevertheless, this theory needs to be confirmed through the evaluation of retrotransposon DNA sequences. Moreover, using a C_0t value derived from a known genome size, like *B. sinuspersici*, may be adequate enough to get good quality fractions of single- and low-copy sequences if the genome size is accurate according to Lamoureux et al. (2005).

Conclusion

In conclusion, the FISH karyotype analysis in this study allowed efficient identification of the chromosome of *B. sinuspersici*. Because this species have been only recently explored, identification of highly abundant repeats using sequencing analysis may help in developing chromosome-specific markers for *B. sinuspersici* that are useful for genetic mapping and improve our understanding in its genetic constitution and genome evolution.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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