Chromosome identification in *Cucumis anguria* revealed by cross-species single copy gene-FISH

Journal:	<i>Genome</i>
Manuscript ID	gen-2017-0235.R1
Manuscript Type:	Article
Date Submitted by the Author:	04-Feb-2018
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Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	C. anguria, cross-species FISH, single copy genes, karyotype, homeologous relationship

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27 Abstract

28 *Cucumis anguria* is a potential genetic resource for improving *Cucumis* crops due to
29 its broad-spectrum resistance. Few cytogenetic studies on *C. anguria* have been
30 reported because of its small metaphase chromosomes and the scarcity of
31 distinguished chromosomal landmarks. In this study, fourteen single copy genes from
32 cucumber and rDNAs were used as probes for FISH to identify individual
33 chromosomes of *C. anguria*. The distinctive signal distribution patterns of the probes
34 allowed us to distinguish each chromosome of *C. anguria* (A01 to A12). Further,
35 detailed chromosome characteristics were obtained through pachytene chromosome
36 FISH. The lengths of pachytene chromosomes varied from 54.80 μm to 143.41 μm . The
37 proportion of heterochromatin regions varied from 13.56% to 63.86%. Finally, the
38 chromosomal homeologous relationship between *C. anguria* and cucumber (C1-C7)
39 was analyzed. The results showed that A06 + A09, A03 + A12, A02 + A04, and A01
40 + A11, were homeologs of C1, C2, C3, and C6, respectively. Chromosomes A08, A10,
41 and A05 were homeologs of C4, C5, and C7, respectively. The chromosome
42 identification and homeologous relationship analysis between *C. anguria* and cucumber lay
43 the foundation for further research of genome structure evolution in *Cucumis* species.

44

45 **Keywords:** *C. anguria*, cross-species FISH, single copy genes, karyotype,
46 homeologous relationship

47

48 **Introduction**

49 *C. anguria* (West Indian gherkin) ($2n = 2x = 24$) is of Africa origin *Cucumis* species, and
50 cultivated now in many places especially in Brazil and the United States because its fruit is
51 rich in vitamins and minerals (Mangan et al. 2008; Matsumoto et al. 2012; Thiruvengadam
52 and Chung 2014). *C. anguria* carries broad-spectrum resistance to multiple biotic stresses,
53 including powdery mildew (Alvarez et al. 2005; Macas et al. 2007), fusarium wilt (Alvarez et
54 al. 2005; Matsumoto and Miyagi 2012) and *Meloidogyne incognita* (Fassuliotis 1970; Bhatti
55 1974; Boukema et al. 1984; Fassuliotis and Nelson 1988; Kim 2001). The cytogenetic studies
56 on *C. anguria* were mainly about repetitive sequences distribution along (Yagi et al. 2015;
57 Zhang et al. 2015; Zhang et al. 2016). Because of the small size of its chromosomes and lack
58 of distinguishing characteristics and landmarks, the karyotype and further cytological
59 characteristic of *C. anguria* have not been reported so far.

60 Within the genus *Cucumis*, only the genomes of cucumber and melon have been
61 sequenced (Huang et al. 2009; Garcia-Mas et al. 2012). The lack of genome information for
62 other species has hindered cytogenetic studies on among *Cucumis* species. Cross-species
63 fluorescence *in situ* hybridization (FISH) based on the sequence conservation between species
64 have been proved to be useful in cytological investigation for those species lacking genome
65 information in *Cucumis*, and also useful in comparative genomic researches among related
66 species (Lou et al. 2014; Yang et al. 2014). In plants, cross-species FISH technology has been
67 applied in *Sorghum*, *Brassica* and *Brachypodium* species for the researches of karyotype,

chromosomal evolution and rearrangement (Lysak et al. 2006; Tang et al. 2008; Lou et al. 2010; Wolny et al. 2013).

Single copy gene-based FISH refers to mapping the single copy gene probes onto chromosome spreads through *in situ* hybridization. Single copy gene FISH has been proved to be an valuable method for identifying individual chromosomes, and investigating chromosome rearrangements in closely related species, because of the feasibility of probe preparation (usually through PCR amplification), free of repetitive elements, and the conservation of probes among related species (Wang et al. 2006; Danilova et al. 2012, 2014). In previous research, we developed a single copy gene-based chromosome painting (ScgCP) technique which could be applied conveniently for chromosome identification in cucumber and chromosomal rearrangement analysis in three *Cucumis* species (Lou et al. 2014).

rDNAs (ribosomal RNA genes) exist universally in plants, with two families of 45S rDNA and 5S rDNA. The nucleolus organizer regions (NORs) always have 45S rDNA positioned here which contain the tandem repeat units of the 18S-5.8S-26S rRNA genes and non-transcribed spacer (Koo et al. 2010; Yagi et al. 2015; Zhang et al. 2016). The distribution patterns of 45S rDNA and 5S rDNA along chromosomes are usually used for karyotyping in plants (Han et al. 2008; Zhang et al. 2016). Based on the previous researches on *C. anguria*, two pairs of 45S rDNA and one pair of 5S rDNA loci were detected by FISH (Yagi et al. 2015; Zhang et al. 2016).

Chromosome morphology is an important component of karyotyping analysis. However, little morphological information on chromosomes could be given based on mitotic metaphase

89 chromosome preparations due to the small size of chromosomes in *C. anguria*. Generally,
90 meiotic pachytene chromosomes are 10-20 times longer than mitotic metaphase chromosomes,
91 and the resolution of FISH mapping on pachytene chromosomes is much higher than in
92 mitotic metaphase chromosomes (Cheng et al. 2002). In addition, through DAPI
93 (4',6-diamidino-2-phenylindole) staining, morphological features of chromosome associated
94 with repetitive sequence distribution could be displayed clearly, especially for pachytene
95 chromosome, which provides an important information for the identification of individual
96 chromosomes and karyotype analysis (Jong et al. 1999; Chen et al. 2000; Cheng et al. 2002;
97 Kato et al. 2004; Han et al. 2008).

98 In this study, we employed cross-species FISH technology to identify chromosomes and
99 construct *C. anguria* karyotype. Fourteen single-copy genes from cucumber, a relative species,
100 combining with 45S rDNA and 5S rDNA were mapped onto mitotic metaphase and meiotic
101 pachytene chromosome spreads to identify individual chromosomes. Distinct structural
102 differences of each chromosome were revealed by pachytene chromosome preparations after
103 DAPI staining. Further, the chromosomal homeologous relationship between *C. anguria* and
104 cucumber were inferred based on the cross-species FISH mapping of single copy genes. This
105 study will provide a foundation for further researches about comparative cytogenetics in
106 *Cucumis* species.

107

108 **Materials and Methods**

109 **Plant material and chromosome preparation**

110 *C. anguria* (accession No.: PI 249879) was used in this study. The root tips were collected
111 from the *C. anguria* plant for mitotic chromosome preparation. Mitotic chromosomes were
112 obtained using published protocols (Lou et al. 2013; Lou et al. 2014). For pachytene
113 chromosome preparation, young flower buds were harvested and fixed in 3:1 Carnoy's
114 fixative solution for at least 1 day. The anthers at the pachytene stage were digested with
115 enzyme mixtures containing 4% cellulase, 2% pectinase for 1.5 h at 37°C. The digested
116 anthers then were fixed in Carnoy's fixative solution. The slides with well-spread pachytene
117 chromosomes were obtained by 'flame dried' methods (Iovene et al. 2008).

118 **Single-copy gene probes labeling and FISH**

119 All selected gene sequences were amplified using the same PCR procedure as below. The
120 amplification procedure was as follows: 98°C for 10 sec, 60°C for 15 sec, 68°C for 4 min for
121 35 cycles, with a final extension at 68°C for 10 min. All PCR products were resolved on 1%
122 agarose gels (BIO-WEST, <http://www.genehk.com>) for 30 min at 120 V, and stained with
123 nontoxic nucleic acid dye GelRed (US Everbright Inc, <http://yuhengbio.com>). Products of the
124 expected size were cut from the gel and purified using a gel recovery kit (Promega,
125 <http://www.promega.com>).

126 The single-copy genes from the purified PCR products were used for FISH. The PCR
127 products were labeled with either biotin-16-dUTP or digoxigenin-11-dUTP using nick
128 translation, and detected using a fluorescein isothiocyanate-conjugated antibiotin antibody
129 and a rhodamine-conjugated anti-digoxigenin antibody (Roche,
130 <http://www.roche-applied-science.com>), respectively, and about 50 ng purified DNA probes

131 was used for each slide. The FISH experiments and images captured were performed as
132 previously described (Lou et al. 2013; Lou et al. 2014; Zhang et al. 2015). The chromosomes
133 lengths in the selected cells were measured using FISH view 5.5 software (Applied Spectral
134 Imaging Inc, <http://www.spectral-imaging.com>).

135 **Preparation of blocking DNAs**

136 To decrease background signals, Cot-1 DNA was used as blocking DNA during hybridization.
137 The Cot-1 DNA was isolated from *C. anguria* as described previously (Zwick et al. 1997).
138 Briefly, RNA-free genomic DNA was diluted to a concentration of 300 ng/μl using 3 M NaCl
139 and double-distilled H₂O to a final concentration of 0.3 M NaCl. Then the DNA was sheared
140 by incubating the tube containing the DNA sample in boiling water until the DNA fragment
141 size ranged from 100 bp to 1000 bp. Usually, incubation for 60–90 min is required to obtain
142 the correct size, depending on the purity of the DNA. The sheared DNA was then denatured at
143 95°C for 10 min, cooled in ice water for 10 sec, followed by incubation at 65°C in a water
144 bath for 18 min 50 sec for a re-annealing of Cot-1 DNA. Then the DNA solution was cooled
145 immediately on ice to prevent re-annealing of another DNA. S1 nuclease (1 U per 1 μg DNA)
146 was added to the cooled DNA solution to digest the single-stranded DNA at 37°C for 8 min.
147 The reaction was stopped by immediate phenol extraction using Tris-equilibrated phenol, and
148 the subsequent steps of the genomic DNA extraction method were performed until
149 resuspension of Cot-1 DNA in TE buffer (Tris-hydrochloride buffer, pH 8.0, containing 1.0
150 mM EDTA).

151

152 **Result**

153 **Selection of probes for FISH**

154 Two types of probes including single copy gene-based probes and rDNAs probes were used
155 for FISH in this study. Single copy gene probes developed from cucumber for chromosome
156 painting in our previous study (Lou et al. 2014) were used for cross-species FISH to identify
157 chromosomes of *C. anguria*. To selected single copy genes for *C. anguria* karyotyping, three
158 to seven gene probes located on the distal region of each chromosome of cucumber were
159 tested on *C. anguria* chromosome spreads. A total of 21 single copy gene probes produced
160 unique, distinguishable signals in metaphase chromosomes of *C. anguria*. Further, individual
161 chromosome-specific probes were determined through comparative FISH mapping using
162 every two probes. Finally, 14 gene probes, 45S and 5S rDNA were used for further karyotype
163 analysis of *C. anguria*. Detailed information about these single copy genes including the gene
164 codes, genes ID, primers, and fragment sizes are shown in Table 1.

165

166 **Karyotyping of *C. anguria* based on cross-species FISH on mitotic metaphase** 167 **chromosomes**

168 A total of 14 single copy gene probes described above, including 45S rDNA and 5S rDNA
169 were used for karyotype analysis of *C. anguria*. One or two single copy gene probes for each
170 chromosome were mapped except for chromosome 7 which was identified using 5S rDNA
171 probe. The signal patterns of these probes on the *C. anguria* mitotic metaphase chromosomes
172 are shown in Fig. 1. The relative length of each chromosome was measured in ten metaphase

173 cells. A01 to A12 were the designations for 12 different chromosome pairs of *C. anguria*
174 according to their sizes (Fig. 1M). The relative length of chromosome varied from 0.1087 to
175 0.0621 and the arm ratios from 1.07 to 2.19 (Table 2). According to the arm ratio, all
176 chromosomes of *C. anguria* were designated as metacentric chromosomes except for A01,
177 which was a submetacentric chromosome. This result revealed that two pairs of 45S rDNA
178 loci and one pair of 5S rDNA loci were mapped (Fig. 1A and G). An ideogram showing the
179 positions of 14 single copy genes, 45S rDNA and 5S rDNA on metaphase chromosomes is
180 shown in Fig. 1M. FISH patterns of each chromosome are described below.

181 Chromosome 1 (A01): The single-copy gene 6-81 was located on the short arm. The 45S
182 rDNA signal was observed at the end of the long arm.

183 Chromosome 2 (A02): The single-copy gene 3-81 was observed at the end of the long arm.

184 Chromosome 3 (A03): The single copy gene 2-54 was observed on the short arm.

185 Chromosome 4 (A04): The single copy gene 3-11 was located at the end of the short arm.

186 Chromosome 5 (A05): The single copy gene 7-5 was located on the end of the short arm
187 and 7-50 was located on the end of the long arm.

188 Chromosome 6 (A06): Single copy genes 1-51 and 1-39 were located at terminal regions of
189 the short arm and the long arm, respectively.

190 Chromosome 7 (A07): 45S rDNA was located on the distal end of short arm, 5S rDNA was
191 proximal to the primary constriction region.

192 Chromosome 8 (A08): The single copy genes 4-31 and 4-145 were located at adjacent
193 positions on the end of the long arm.

194 Chromosome 9 (A09): The single copy gene 1-44 was observed at the end of the short arm.

195 Chromosome 10 (A10): The single copy gene 5-20 was located at the middle region of the
196 long arm.

197 Chromosome 11 (A11): The single copy gene 6-1 was located at the end of the long arm.

198 Chromosome 12 (A12): The single copy gene 2-8 was close to the telomere of the long
199 arm.

200

201 **Meiotic pachytene chromosome analysis of *C. anguria* based on single copy gene FISH**

202 To obtain more information about chromosome morphology and structure of *C. anguria*,
203 meiotic pachytene chromosomes were identified based on cross-species FISH with
204 chromosome-specific single copy gene probes and rDNA probes as above. The chromosomal
205 features associated with the distribution of heterochromatin were revealed by DAPI staining
206 (Fig. 2), because DAPI staining can reflect the region of AT rich heterochromatin
207 (Kapuscinski 1995). The result showed that pachytene chromosomes have much higher
208 resolution than metaphase chromosome. For example, two single copy genes, 4-31 and 4-145,
209 with overlapping signals on chromosome 8 (A08) on metaphase chromosome spreads (Fig.
210 1H) were mapped unambiguously to adjacent sites on pachytene chromosomes (Fig. 2H).

211 The signal patterns of these probes on the *C. anguria* meiotic pachytene chromosomes
212 are shown in Fig. 2 (A-L). The relative length of each chromosome, the region of
213 heterochromatin and the location of probes were measured in ten pachytene cells. Pachytene
214 chromosomes are approximately 15 times longer than metaphase chromosome, and the

lengths of chromosomes at pachytene stage varied from 54.80 μm to 143.41 μm . The proportion of heterochromatin regions of each chromosome varied from 13.56% to 63.86% (Table 3). The pachytene chromosomes of *C. anguria* exhibited variable heterochromatin and euchromatin distribution patterns based on DAPI staining. The heterochromatin distribution patterns of different chromosomes of *C. anguria* varied greatly (Fig. 2M). On chromosomes A01, A02, and A11, the heterochromatin regions were close to the terminal region of the long arms end of chromosomes. For chromosomes A03, A05, A06, and A08, very bright DAPI signal was observed at the pericentromeric heterochromatin regions. Approximately two thirds of chromosome A04, excluding the two ends, was occupied by heterochromatin. The heterochromatin regions on chromosomes A07 and A12 were mainly located on the short arm and pericentromeric heterochromatin regions. Heterochromatin regions were observed in the middle region of both arms on chromosome A09. Chromosome A10 was mainly euchromatin, with a small amount of scattered heterochromatin.

Based on these result above an integrated ideogram showing the positions of the 14 selected single copy genes, 45S rDNA and 5S rDNA, euchromotain and heterochromatin is shown in Fig. 2N.

231

232 **The chromosomal homeologous relationship between *C. anguria* and cucumber**

According to the cross-species FISH, preliminary chromosomal homeologous relationship between *C. anguria* (A01-A12) and cucumber (C1-C7) was inferred (Fig. 3). Single copy gene probes 1-39 and 1-51 from the long arm of C1 were mapped on the two ends of A06 and

236 A09, separately, and 1-44 was mapped on A09. The probes 2-8 and 2-54 from the short arm
237 and long arm of C2 were mapped on the long arm of A12 and short arm of A03, respectively.
238 Two probes located on the two arms of C3 (3-11 and 3-81) were mapped on the short arm of
239 A04 and the long arm of A02, respectively. Two adjacent probes on C4, 4-31 and 4-145, were
240 mapped on adjacent positions on A08. The single copy gene 5-20 located on the long arm of
241 C5, was mapped on the long arm of A10. Two probes that were located on the short arm and
242 long arm of C6, 6-1 and 6-81, were mapped on the long arm of A11 and the short arm of A01,
243 respectively. Probes 7-5 and 7-50 located on the two arms of C7 were mapped on the two
244 ends of A05 respectively. Based on these results, the preliminary chromosomal homeologous
245 relationship between *C. anguria* and cucumber could be inferred. *C. anguria* chromosome
246 pairs A06 + A09, A03 + A12, A02 + A04, and A01 + A11 corresponded to cucumber
247 chromosome C1, C2, C3, and C6. A08, A10, and A05 corresponded to C4, C5 and C7,
248 respectively. Due to the lacking of the available single copy genes of A07, the corresponding
249 relationship of A07 with cucumber could not be inferred.

250

251 Discussion

252 Chromosome identification is importance for cytogenetic research. Nevertheless,
253 chromosomes, at metaphase stage, are usually in a highly condensed state, and thus difficult
254 to be distinguished according to their morphology, especially for those species with small size
255 of chromosomes. FISH technology has been applied to identify individual chromosomes
256 successfully and has greatly promoted to the progress of cytogenetic studies. Previous

257 cytogenetic studies of *C. anguria* were mainly focused on repetitive sequence mapping. The
258 two pairs of 45S rDNA and one pair of 5S rDNA were detected on *C. anguria* chromosomes
259 (Zhang et al. 2015; Zhang et al. 2016). However, the information from rDNA mapping is
260 insufficient for the identification of all chromosomes in *C. anguria*. FISH with chromosome
261 specific probes enables the clear identification of each chromosome and their complements.
262 Here, cross-species FISH was employed to identify chromosomes of *C. anguria*. Compared
263 with non-gene regions, single copy genes are relatively conserved among close related
264 species, and therefore probes from one species frequently could be applied effectively on its
265 relatives (Lou et al. 2010). The single copy gene probes from cucumber produced bright
266 signals in *C. anguria* chromosomes, which indicated the conservation of these probes
267 between *C. anguria* and cucumber. Because the genome sequence of *C. anguria* is not
268 available, the usability of cross-species FISH with cucumber gene probes provides a
269 convenient way to obtain *C. anguria* chromosome specific probes and then construct its
270 karyotype. This method also provided a reference for cytogenetic research in other species of
271 *Cucumis*.

272 Because cross-species FISH could provide the visualization proof for the sequence
273 conversation among related species, it has been used as a valuable tool for the researches of
274 comparative genomics. This technology was firstly applied in the mammalian and humans for
275 comparative genomic studies (Ried et al. 1993). In the past decades, cross-species FISH
276 technology has been applied in plant species (Lysak et al. 2003; Lysak et al. 2005; Lysak et al.
277 2006; Rens et al. 2006; Nagarajan et al. 2008; Tang et al. 2008; Liu et al. 2010; Wolny et al.

278 2013; Wang et al. 2017) . In our previous study, cross-species FISH based on single copy
279 gene has been used for comparative chromosome painting to reveal the chromosomal
280 rearrangement in three species of *Cucumis* genus (Lou et al. 2014). In this study, 14 gene
281 probes from cucumber chromosomes were mapped on *C. anguria* chromosomes successfully,
282 and based on the FISH results, chromosomal homeologous relationships between these two
283 species could be inferred. Only the homeologous relationships between *C. anguria* and
284 cucumber chromosomes was explored. The elucidation of chromosomal syntenic relationship
285 and even structural evolution between these species will rely on more probes covering all
286 chromosomes, and the high-density single copy genes molecular cytogenetic mapping of both
287 species will be constructed in the future.

288 In this study, in order to obtain more information about chromosome morphology and
289 structure of *C. anguria*, we also did the pachytene chromosomes FISH. Compared with the
290 metaphase chromosomes, the length of pachytene chromosomes are 10-20 times longer than
291 metaphase chromosomes, and therefore the resolution of pachytene chromosomes is much
292 higher than metaphase chromosomes. In tomato, two BAC of interval 1.2 Mb in
293 heterochromatin and 120 kb in euchromatic region can be distinguished on pachytene
294 chromosomes (Jong et al. 1999). BAC clones of interval 100 kb could be separated in
295 pachytene chromosomes of the euchromatic region of rice (Cheng et al. 2002). In maize,
296 two gene sequences with 100 kb interval can be resolved in pachytene chromosomes (Wang
297 et al. 2006). In cucumber, the minimum resolution of chromosomes in pachytene stage could
298 reach 250 kb using single copy genes FISH (Lou et al. 2014). Compared with the

299 chromosomes at metaphase stage, the chromosomes at pachytene have more discernible
300 cytological markers, such as euchromatin, heterochromatin and heterochromatin nodes. In
301 addition, these pachytene chromosome features are informative for further researches about
302 chromosome and genomic structure.

303

304 **Competing interests**

305 The authors declare that they have no competing interests.

306

307 **Authors' contributions**

308 Q.-F.L. and J.-F.C. conceived of the study and designed the experiments. Z.-A.L., Y.-F.B.,
309 X.-W, Y.-Z.W., S.-Q.Y., and Z.-T.Z. performed the experiments. Z.-A.L. and Q.-F.L. wrote the
310 paper. All authors read and approved the final manuscript.

311

312 **Acknowledges**

313 The authors thank Watson Atsiambo (College of Agronomy, Nanjing Agricultural
314 University, Nanjing, China) for critical reading of the manuscript. This research was
315 partially supported by the National Science Foundation of China (31772318 and 31471872),
316 The National Key Research and Development Program of China (2016YFD0100204-25), and
317 the Fund for Independent Innovation of Agricultural Science and Technology of Jiangsu
318 Province [CX(17)3016].

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462 **Tables**

463 **Table 1.** Summary of the single copy genes selected for cytogenetic mapping of
464 *C.anguria*.
465

Chr.	Single copy gene code*	Gene ID	Forward primer	Reverse primer	Probe size (bp)
1	6-81	Csa6M517140.1	gtgtcgtcaaatctgctggcaca	ctcaagtcaatgggttcagggtgt	8416
2	3-81	Csa3M750380.1	acagatagacagacagagaggga	agtttttaggtgagtacaggaag	8145
3	2-54	Csa2M368290.1	gacgacgctactttgttctcttt	caccttattgtttggcctctga	7618
4	3-11	Csa3M060990.1	gagccttgggattctgtttatttg	gaggttgagtgttactttgtcgg	8002
5	7-5	Csa7M067500.1	aggctcttcacacctttattatctg	ctcttttgtttctgggtttctctc	8147
5	7-50	Csa7M412850.1	gaagaacgaaagggaagtgaaca	gtgagtgaagcagaaaaaaaagg	7727
6	1-39	Csa1M421880.1	ctacgatgttctgcccattatcttg	ggggctacctctttctgtttgtct	7566
6	1-51	Csa1M570120.1	acctacttactttcacactcctctctc	tcggttacctctactttctgcttca	8546
8	4-31	Csa4M026800.1	taatgttcagtcgttctcccttc	ctcgttctcttctgggtttgttg	8019
8	4-145	Csa4M050230.1	ggctgtgttcacgccttttag	atctgttcacagccagcctct	7708
9	1-44	Csa1M480700.1	ctccataacctcactcttctcctct	actacaaccataaatgctccacact	6033
10	5-20	Csa5M396020.1	accatcataagacttcacaactcac	atctaaccctcaactattaccaacc	7738
11	6-1	Csa6M000040.1	tagaacattgtgtggaacaggagcc	gtggtatgataagattgatagagtaggg	8450
12	2-8	Csa2M022830.1	ggagagggcgcaaaaagtgaagt	ggcatcaaagaaagcaagagaaga	6310

466 *: the codes of single copy genes were from the paper published by Lou et al. (2014).

467 Chr.: chromosome
468
469

Table 2. Relative length and arm ratio of metaphase chromosomes of *C. anguria*.

Chromosome	Relative length ^a	Arm ratio ^b
1	0.1087 ± 0.0152	2.19 ± 0.21
2	0.0970 ± 0.0116	1.57 ± 0.07
3	0.0894 ± 0.0110	1.25 ± 0.16
4	0.0881 ± 0.0115	1.12 ± 0.06
5	0.0865 ± 0.0068	1.24 ± 0.11
6	0.0779 ± 0.0061	1.12 ± 0.07
7	0.0751 ± 0.0051	1.15 ± 0.12
8	0.0734 ± 0.0106	1.23 ± 0.17
9	0.0696 ± 0.0075	1.22 ± 0.21
10	0.0678 ± 0.0020	1.25 ± 0.17
11	0.0660 ± 0.0104	1.07 ± 0.02
12	0.0621 ± 0.0036	1.39 ± 0.32

^aRepresented chromosome length / total complement length^bRepresented length of the long arm / length of the short arm.

Table 3. The location of probes, the length and the proportion of heterochromatin (%) of pachytene chromosomes of *C. anguria*

Chromosome	Probes code	Location of probes (%) [*]	Total length (μm)	Heterochromatin (%)
1	6-81	0	87.57±6.12	37.76±1.07
	45S rDNA	100.00		
2	3-81	88.60	77.21±7.81	50.43±2.38
3	2-54	18.95	143.41±11.09	23.92±4.51
4	3-11	4.60	83.61±1.53	63.86±5.12
5	7-5	6.53	83.45±13.39	39.18±2.10
	7-50	75.28		
6	1-51	3.80	54.80±9.11	37.98±3.03
	1-39	81.46		
7	45S rDNA	0	73.39±10.04	42.10±4.72
	5S rDNA	26.16		
8	4-31	75.25	72.43±2.78	23.61±1.27
	4-145	87.19		
9	1-44	48.54	65.55±16.13	44.90±3.42
10	5-20	58.52	74.02±7.52	16.36±0.28
11	6-1	56.12	57.70±8.65	13.56±0.34
12	2-8	92.25	74.34±14.7	41.97±3.16

^{*}location of probes (%): The position of each probes on pachytene chromosome was measured as (S/T)×100, where S is the distance (in micrometers) from the FISH site to the end of the short arm of the chromosome and T is the total length of the chromosome (in micrometers).

498 Figure Legends

499 **Fig. 1.** FISH with single copy gene, 45S rDNA and 5S rDNA probes on mitotic
500 metaphase chromosome of *C. anguria*. **(A-L)** Chromosomes were counterstained
501 with DAPI. Single copy probes are indicated by white arrows, and 45S rDNA and
502 5S rDNA in A07 are indicated by red arrows. **(M)** Individual chromosomes
503 separated from A01 to A12 are arranged according to their order. **(N)** Ideogram
504 showing the positions of single copy probes (red), 45S rDNA (green), and 5S rDNA
505 (yellow) on mitotic metaphase chromosomes of *C. anguria*. The relative lengths of the long
506 and short arms and the arm ratio of each chromosome were drawn based on the data in Table
507 2. Scale bar = 5µm.

508 **Fig. 2.** FISH with single copy gene, 45S rDNA and 5S rDNA probes on meiotic
509 pachytene chromosome of *C. anguria*. **(A-L)** FISH mapping of chromosome-specific
510 single copy gene probes and rDNAs. **(M)** Computationally straightened chromosomes
511 bivalents A01 to A12. The image has been inverted compared with that in **A-L** **(N)**
512 Ideogram showing the positions of single copy probes (red or green), 45S rDNA
513 (green), and 5S rDNA (yellow) on pachytene chromosomes of *C. anguria*. The relative
514 lengths of the chromosomes and the heterochromatin were drawn based on the data in Table 3.
515 Scale bar = 5µm.

516 **Fig. 3.** Chromosomal homeologous relationship between *C. anguria* and cucumber.
517 Red bars represent the signals of single copy genes. Green bars represent the signals

518 of 45S rDNA. Yellow bars represent the signals of 5S rDNA. The blue vertical bars
519 represent the chromosomes of cucumber, denoted C1-C7. The gray vertical bars
520 represent the chromosomes of *C. anguria*, denoted A01-A12.
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Fig. 1. FISH with single copy gene, 45S rDNA and 5S rDNA probes on mitotic metaphase chromosome of *C. anguria*. (A-L) Chromosomes were counterstained with DAPI. Single copy probes are indicated by white arrows, and 45S rDNA and 5S rDNA in A07 are indicated by red arrows. (M) Individual chromosomes separated from A01 to A12 are arranged according to their order. (N) Ideogram showing the positions of single copy probes (red), 45S rDNA (green), and 5S rDNA (yellow) on mitotic metaphase chromosomes of *C. anguria*. The relative lengths of the long and short arms and the arm ratio of each chromosome were drawn based on the data in Table 2. Scale bar = 5 μ m.

19x30mm (600 x 600 DPI)

Fig. 2. FISH with single copy gene, 45S rDNA and 5S rDNA probes on meiotic pachytene chromosome of *C. anguria*. (A-L) FISH mapping of chromosome-specific single copy gene probes and rDNAs. (M) Computationally straightened chromosomes bivalents A01 to A12. The image has been inverted compared with that in A-L (N) Ideogram showing the positions of single copy probes (red or green), 45S rDNA (green), and 5S rDNA (yellow) on pachytene chromosomes of *C. anguria*. The relative lengths of the chromosomes and the heterochromatin were drawn based on the data in Table 3. Scale bar = 5µm.

27x60mm (600 x 600 DPI)

Fig. 3. Chromosomal homeologous relationship between *C. anguria* and cucumber. Red bars represent the signals of single copy genes. Green bars represent the signals of 45S rDNA. Yellow bars represent the signals of 5S rDNA. The blue vertical bars represent the chromosomes of cucumber, denoted C1-C7. The gray vertical bars represent the chromosomes of *C. anguria*, denoted A01-A12.

1810x451mm (96 x 96 DPI)

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