

# First nuclear DNA amounts in diploid ( $2n=2x=14$ ) *Corchorus* spp. by flow cytometry: genome sizes in the cultivated jute species (*C. capsularis* L. and *C. olitorius* L.) are ~300% smaller than the reported estimate of 1100-1350 Mb

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**Abstract** — We report the 2C values (nuclear DNA content) and haploid genome (1C) sizes of the *Corchorus* species ( $2n=2x=14$ ), with a special reference to the two cultivated jute species, viz., *C. capsularis* L. (the white jute) and *C. olitorius* L. (the tossa jute). Flow cytometric analysis of propidium iodide-stained nuclei showed that the 2C values were between 0.502 and 0.695 pg for *C. capsularis*, and between 0.643 and 0.718 pg for *C. olitorius*. For both the cultivated species, however, mutants had higher amounts of nuclear DNA than that of the cultivars. Amongst the wild *Corchorus* species, the lowest 2C value was observed in *C. fascicularis* (0.384 pg), while the highest in *C. pseudo-olitorius* (0.712). The current 2C values of both the cultivated jute species are in disagreement with earlier estimates of 2.3 and 2.8 pg in *C. capsularis* and *C. olitorius*, respectively. Of the *Corchorus* species studied, *C. fascicularis* has the smallest haploid genome (~188 Mb) followed by *C. aestuans* (~194 Mb). Our results show that, on an average, genome sizes (1C values) of *C. capsularis* and *C. olitorius* are ~280 Mb and ~324 Mb, respectively, which are ~300% smaller than their corresponding reported estimates. The white jute cv. JRC-212 (or Branca) has the smallest genome (~246 Mb) among the economically important crop species.

**Key words:** 2C value, chromosome, *Corchorus* spp., flow cytometry, genome size, jute, nuclear DNA content.

## INTRODUCTION

The genus *Corchorus*, with over 170 species in the Index Kewensis but probably with 50-60 good species (MAHAPATRA and SAHA 2008), still continues to be extremely variable (MAHAPATRA *et al.* 1998; BENOR *et al.* 2010). It was traditionally classified within the family Tiliaceae (PURSEGLOVE 1968), which was subsequently merged with the family Malvaceae within the core Malvales based on molecular evidence of the chloro-

plast genome (ALVERSON *et al.* 1999; WHITLOCK *et al.* 2003). However, it has been recently reclassified within the family Sparrmanniaceae (HEYWOOD *et al.* 2007). The genus consists of annual or short-lived perennials (BENOR *et al.* 2010) distributed in tropical, sub-tropical and warm temperate regions of the world (EDMONDS 1990), and is represented by the two cultivated jute species, viz., *C. capsularis* L. (the white jute) and *C. olitorius* L. (the tossa jute/ Jew's mellow). Although the centre of diversity of *Corchorus* appears to be in Africa (MAHAPATRA and SAHA 2008), the origin and phylogeny of this genus still remain contended (BENOR *et al.* 2010), with little information about the genetic and evolutionary relationship between wild *Corchorus* spp. and the two cultivated species (BASU *et al.* 2004; ROY *et al.* 2006).

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*Corchorus* spp. including the two cultivated jute species have the haploid number of seven ( $n=7$ ) chromosomes, except for *C. argutus* KUNTH, *C. cunnighamii* F. MUELL, *C. birtus* L., *C. junodii* N. E. BROWN, *C. orinocensis* KUNTH, *C. pascuorum* DOMIN. and *C. siliquosus* L., which are natural polyploids with  $n=14$  chromosomes (RAO and DATTA 1953; ISLAM and QAIYUM 1961; ROY 1962; ISLAM *et al.* 1975). However, as compared to other economically important crop species, chromosome research is not much advanced in the jute species (reviewed in HAZRA and KARMAKAR 2008), and only recently karyomorphological studies (MAITY and DATTA 2009) and chromosome banding techniques (KHATUN and ALAM 2010) have been used to differentiate speciation in *Corchorus* spp. In general, interphase nuclei of both *C. capsularis* and *C. olitorius* have been reported to be chromocentric, with no differences in chromocenter numbers between the two species (SAMAD *et al.* 1992). Although chromosomes, with five median and two submedian centromeres, of both the cultivated jute species are well differentiated by euchromatin and heterochromatin (HAZRA and KARMAKAR 2008), no relationship could be established between DNA contents and heterochromatin percentages (SAMAD *et al.* 1992). The 2C DNA contents in *C. capsularis* cv. D-154, *C. olitorius* cv. O-4 and their  $F_1$  (D-154  $\times$  O-4) hybrid have been estimated to be 2.3, 2.8 and 3.1 picograms, respectively (SAMAD *et al.* 1992). And this was the basis of genome size (1C DNA amount) estimates of 1100 and 1350 Mb for *C. capsularis* and *C. olitorius*, respectively (MIR *et al.* 2009), with an average size of  $\sim 1250$  Mb jute genome (MIR *et al.* 2008; ANONYMOUS 2010). However, SAMAD *et al.* (1992) determined 2C DNA content in jute by analyzing the DNA extracted from seeds, using the combined procedures of OGUR and ROSEN (1950) and MALIK and SINGH (1980).

It is well established that determination of nuclear DNA content of an organism by chemical analysis does not represent the 2C DNA amount because the source tissue may contain cells at different cell cycle phases with varying DNA amounts (DOLEŽEL and BARTOŠ 2005). The estimates based on DNA reassociation kinetics are also not reliable because the Cot curves obtained after DNA reassociation are difficult to interpret in terms of C-values due to the presence of different types of repetitive DNA sequences. In comparison, estimation of 2C DNA amount by flow-cytometric measurement of individual nuclei that are stained with a DNA-spe-

cific fluorochrome ensures the highest degree of precision (BENNETT and LEITCH 2005; DOLEŽEL and BARTOŠ 2005).

With these in mind, we estimated the 2C DNA contents of few diploid ( $2n=2x=14$ ) *Corchorus* spp., which are endemic to the Indian subcontinent, by laser flow cytometry. The specific objective was to reassess the nuclear genome sizes (1C DNA amounts) of the two cultivated jute species (*C. capsularis* L. and *C. olitorius* L.) in relation to their wild *Corchorus* relatives.

## MATERIALS AND METHODS

**Plant material** – Eight *Corchorus* spp. were used in the present study: *C. aestuans* L. Ac. WCIJ 037; *C. capsularis* L. cvs Branca, JRC-80, JRC-212, JRC-321 and mutants CMU-002 (X-irradiated/crumpled leaf), CMU-010 (X-irradiated/soft stem), CMU-036 ( $\gamma$ -irradiated/broad leaf); *C. fascicularis* Lam. Ac. WCIJ 004; *C. olitorius* L. cvs JRO-524, S-19, Sudan Green, Tanganyika 1 and mutants OMU-043 (X-irradiated/ribbon leaf), PPO4 (X-irradiated); *C. pseudo-capsularis* SCHWEINF. Ac. WCIJ 041; *C. pseudo-olitorius* ISLAM & ZAID Ac. WCIJ 034; *C. tridens* L. Ac. WCIJ 047; and *C. trilocularis* L. Ac. WCIJ 008. Seeds were surface-disinfested by 0.01%  $HgCl_2$  for 5 min followed by several wash in sterile distilled water and germinated at 25°C in a 90 mm disposable polycarbonate Petri dish (Greiner Bio-One GmbH, Frickenhausen, Germany) over Whatman 1 filter paper moistened with sterile distilled water. All chemicals and reagents used in the study were from Sigma-Aldrich (St. Louis, USA).

**Chromosome analysis** – Chromosome analysis was carried out in the cultivars of both *C. capsularis* and *C. olitorius*, and in the wild *Corchorus* spp. Healthy root tips from germinated seedlings were fixed in 3:1 aceto-alcohol for 24 h at 8°C and stored in 70% ethanol for chromosome analysis. The chromosomes in root tip squash were stained with the fluorescent dye DAPI (1.0 ml of 0.35 mg ml<sup>-1</sup> DAPI stock was used to prepare a 100 ml working solution in 0.4 M Na<sub>2</sub>HPO<sub>4</sub>) following the standard procedure (SHARMA *et al.* 2010). Counts were obtained from a minimum of 30 cells and from at least five separate root tips.

**Laser flow cytometry** – Cotyledons ( $\sim 100$  mg) collected from 7-day-old germinated seedlings were finely sliced in 1.0 ml isolation solution in a 90 mm polycarbonate Petri dish to prepare nuclei

suspensions, according to ARUMUGANATHAN and EARLE (1991). The nuclei isolation solution consisted of  $\text{MgSO}_4$  buffer (10.0 mM  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 50.0 mM KCl and 5.0 mM Hepes; pH 8.0), Triton X-100 (0.25%), dithiothreitol (1.0 mg  $\text{ml}^{-1}$ ) and propidium iodide (0.1 mg  $\text{ml}^{-1}$ ). The macerates were filtered through a 30  $\mu\text{m}$  nylon net filter (NY30; Millipore India Pvt. Ltd., Bangalore, India) into a 1.5 ml Eppendorf microcentrifuge tube and centrifuged at  $5,000 \times g$  for 20 s. The supernatant was discarded, and the nuclei pellet was resuspended in 600  $\mu\text{l}$  of isolation buffer containing RNase (1.25  $\mu\text{g ml}^{-1}$ ) followed by incubation at 37°C for 15 min before running the sample on a flow cytometer.

The relative fluorescence of the propidium iodide-stained cotyledon nuclei was measured in a FACSCalibur flow cytometer (Becton Dickinson, San José, USA) using chicken (*Gallus domesticus*) red blood cell (CRBC) as an internal standard, according to SHARMA *et al.* (2010). The objects were analyzed for forward (FSC) versus side (SSC) scatter signals for at least 20,000 nuclei in each sample. Four histograms were generated: i) FL2-area versus FL2-width; ii) FL2-height versus FSC-height (Fig. 1); iii) frequency versus FL2-height; and iv) frequency versus FL2-area. The peak corresponding to the CRBC nuclei was adjusted to around channel 350 set on a linear scale of fluorescence intensity (Fig. 2-4). A direct comparison of the mean position of plant (*Corchorus* spp.) nuclear peak to the mean position of CRBC nuclear peak (2.50 pg; BENNETT *et al.* 2003) was made to calculate the nuclear DNA amount (2C values in pg). For each sample, there were six independent replicated measurements.

**Genome size estimation** – Since there is no published information on microspectrophotometric or flow cytometric estimates of genome sizes in *Corchorus* spp., relative 2C flow cytometry values were converted to 1C values (in pg), and megabases were calculated from picograms based on the conversion 0.1 pg = 98.0 Mb (CAVALIER-SMITH 1985; BENNETT *et al.* 2000).

**Statistical analysis** – Prior to univariate statistical analysis, normality and equal variance assumptions were tested. Data were analyzed by one-way analysis of variance (ANOVA) followed by multiple comparison of mean values by post-hoc Tukey's honestly significant difference test, using the statistical software MSTAT-C (Michigan State University, Michigan, USA). The term significant has been used to indicate differences for which  $P \leq 0.05$ .

## RESULTS

**Chromosome number and ploidy level in *Corchorus* spp.** – The DAPI staining of the root tip (somatic) cells showed that all *Corchorus* spp. including the cultivars of both the cultivated jute species under investigation had  $2n=14$  chromosomes with  $2x$  ploidy level. Mitotic chromosomes were extremely small. They were even much smaller in *C. aestuans* and *C. fascicularis* than those in the other *Corchorus* species, including the two cultivated jute species *C. capsularis* and *C. olitorius*. In these two cultivated species, of the seven chromosomes, five were found to be with median centromeres, while the two were with submedian centromeres. However, in wild *Corchorus* species, all chromosomes appeared to be median.

**Nuclear DNA content of the cultivated jute species** – There were significant ( $P \leq 0.05$ ) differences in the 2C values not only between the two cultivated jute species, but also between the genotypes and mutants of an individual species. The 2C values of *C. capsularis* (the white jute) were between 0.502 and 0.695 pg, with the lowest values recorded in cvs Branca and JRC-212 (both with 0.502 pg) and the highest in the mutant CMU-010 (0.695 pg) (Table 1; Fig. 2). In *C. capsularis* cvs JRC-80 and JRC-321, the 2C values were 0.601 and 0.685, respectively (Fig. 3-4). In general, *C. capsularis* mutants had higher amounts of nuclear DNA, particularly CMU-010 that recorded an increase of about 38.0% over cvs JRC-212 and Branca. Comparisons of the nuclear DNA contents of the *C. olitorius* (the tossa jute) cultivars showed that they had significantly ( $P \leq 0.05$ ) higher 2C values (0.643-0.674 pg) than those of *C. capsularis* (Table 1). There was no significant ( $P \leq 0.05$ ) difference in the 2C values between the two Indian cultivars of *C. olitorius*, viz., JRO-524 (0.669 pg) and S-19 (0.674 pg), however, the later had significantly higher nuclear DNA content than the exotic cv. Tanganyika 1 (0.662 pg). Of both the cultivated jute species, *C. olitorius* mutant OMU-043 (ribbon leaf mutant) had the highest 2C value (0.718 pg), recording an increase of about 12.0 and 43.0% over the smallest nuclear DNA-containing *C. olitorius* cv. Sudan Green and *C. capsularis* cv JRC-212 or Branca, respectively (Table 1).

**Nuclear DNA content of the wild *Corchorus* spp.** – There was significant ( $P \leq 0.05$ ) variation in the 2C values among the *Corchorus* wild species. The lowest 2C value was observed in *C. fascicularis* (0.384 pg), while the highest in *C.*

*pseudo-olitorius* (0.712) (Table 1). Although *C. aestuans* had low nuclear DNA content, its 2C value (0.396 pg) was significantly ( $P \leq 0.05$ ) higher than that of *C. fascicularis*. In *C. pseudo-capsularis*, *C. trilocularis* and *C. tridens*, the 2C values were 0.408, 0.425 and 0.443, respectively. Interestingly, the present study showed that there was an increase in nuclear DNA content by about 85.0% in *C. pseudo-olitorius* over the smallest nuclear DNA-containing wild species *C. fascicularis*. In general, nuclear DNA content was about 5.6-10.7% higher in *C. pseudo-olitorius* than in *C. olitorius* cultivars.

*Nuclear genome sizes of the jute and wild Corchorus spp.* – Based on the conversion 0.1 pg = 98.0 Mb, the genome sizes of the jute and wild *Corchorus* species are shown in Table 1. Except for *C. pseudo-olitorius*, all the wild *Corchorus* species had smaller nuclear genomes than those of the cultivated jute species. *C. fascicula-*

*ris* had the smallest nuclear genome (188 Mb) followed by *C. aestuans* (194 Mb). In general, between the two cultivated jute species, *C. capsularis* had smaller nuclear genomes than *C. olitorius*. The predominant Indian *C. olitorius* cv. JRO-524 had 33.0% (by 82 Mb) larger nuclear genome than its corresponding *C. capsularis* cv. JRC-212 (Table 1).

## DISCUSSION

Ours is the first report on flow-cytometric estimation of nuclear DNA contents (2C values) and genome sizes of the two cultivated jute species including their few diploid ( $2n = 2x = 14$ ) wild relatives, which are endemic to the Indian subcontinent. Before estimating the 2C values, we did characterize these *Corchorus* species for chromosome numbers and ploidy levels. Chro-

TABLE 1 — Nuclear DNA content (2C and 1C DNA amounts) and genome sizes of *Corchorus* spp. including different genotypes and mutants of *C. capsularis* (the white jute) and *C. olitorius* (the tossa jute).

Species/genotype	2C (pg)	1C (pg)	1C (Mb) <sup>a</sup>
<i>Corchorus capsularis</i> L. <sup>b</sup>			
cv. JRC-212	0.502 i	0.251 i	245.8 i
cv. JRC-80	0.601 g	0.301 g	294.5 g
cv. Branca	0.502 i	0.251 i	245.8 i
cv. JRC-321	0.685 c	0.343 bc	335.5 bc
mt. CMU-002	0.638 f	0.319 f	312.6 f
mt. CMU-010	0.695 b	0.347 b	340.5 b
mt. CMU-036	0.591 h	0.295 h	289.5 h
<i>Corchorus olitorius</i> L.			
cv. JRO-524	0.669 de	0.335 de	327.8 de
cv. Sudan Green	0.643 f	0.322 f	315.2 f
cv. Tanganyika 1	0.662 e	0.331 e	324.2 e
cv. S-19	0.674 cd	0.337 cd	330.5 cd
mt. OMU-043	0.718 a	0.359 a	351.8 a
mt. PPO4	0.645 f	0.322 f	315.8 f
Wild <i>Corchorus</i> spp.			
<i>C. aestuans</i> L.	0.396 m	0.198 m	194.2 m
<i>C. fascicularis</i> Lam.	0.384 n	0.192 n	188.1 n
<i>C. pseudo-capsularis</i> Schweinf.	0.408 l	0.204 l	200.1 l
<i>C. pseudo-olitorius</i> Islam & Zaid	0.712 a	0.356 a	349.0 a
<i>C. tridens</i> L.	0.443 j	0.222 j	217.1 j
<i>C. trilocularis</i> L.	0.425 k	0.212 k	208.1 k

<sup>a</sup> The factor of 0.1 pg = 98.0 Mb was used to convert picograms to Mb, according to BENNETT *et al.* (2000).

<sup>b</sup> Means with common letters within a column are not significantly different at  $P \leq 0.05$ , according to Tukey's honestly significant difference test.



mosome analysis unambiguously established them to be diploid ( $2x$ ) with  $2n=14$  chromosomes, in agreement with earlier reports (MORAKINYO and BADERINWA 1997; ALAM and RAHMAN 2000; HAZRA and KARMAKAR 2008; MAITY and DATTA 2009; KHATUN and ALAM 2010).

The results on 2C values of both the cultivated jute species, viz., *C. capsularis* and *C. olitorius*, as obtained in this study, are in sharp contradiction with their earlier estimates of 2.3 and 2.8 pg, respectively (SAMAD *et al.* 1992). These reported nuclear DNA contents (2C values) of the cultivated jute species are an overestimate by more than 300% over the current values as revealed by laser flow cytometry. These large discrepancies in the 2C values between the two estimates might have accrued from the use of two different approaches for estimating nuclear DNA content. The estima-

tion of the 2C values by chemically analyzing the DNA isolated from seeds, as employed by SAMAD *et al.* (1992), is not a reliable technique for precise determination of nuclear DNA content (DOLEŽEL and BARTOŠ 2005). In contrast, our method based on laser flow-cytometric measurement of propidium iodide-stained nuclei provides the most reliable estimate of nuclear DNA content (BENNETT *et al.* 2000; BENNETT and LEITCH 2005; DOLEŽEL and BARTOŠ 2005). Interestingly, except for *C. pseudo-olitorius*, the other wild *Corchorus* species, in the present study, have about 35.9-56.8 and 51.0-74.2% lower 2C values (nuclear DNA content) than that of *C. capsularis* and *C. olitorius*, respectively. This is in accordance with typical karyomorphology and chromosome sizes of these wild *Corchorus* spp. (MAITY and DATTA 2009), and suggests that ge-

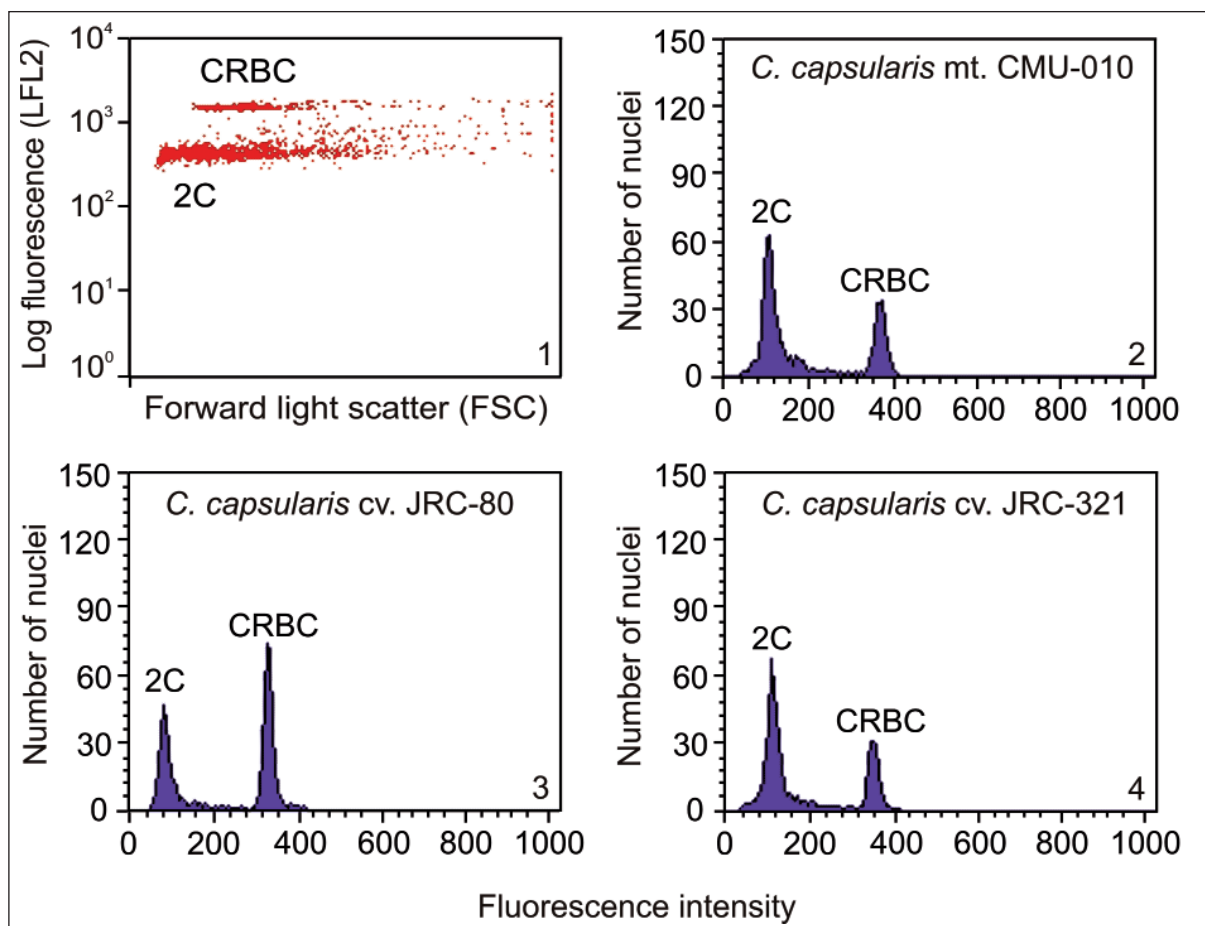


Fig. 1-4 — (1) A two-parameter histogram of log fluorescence intensity (LFL2) versus forward light scatter (FSC) generated by a FACSCalibur flow cytometer while analyzing the propidium iodide-stained jute (*C. capsularis*) nuclei (2C) using chicken red blood cell (CRBC) as an internal standard. The gating map used to eliminate the debris is not shown in the histogram. (2) A histogram of nuclei numbers as a function of fluorescence intensity from an analysis of propidium iodide-stained nuclei of *C. capsularis* mt. CMU-010 using chicken red blood cell (CRBC) as an internal standard. (3) The same as in Fig. 2, for *C. capsularis* cv. JRC-80. (4) The same as in Fig. 2, for *C. capsularis* cv. JRC-321.

nome size variation in wild species does have an evolutionary effect (PELLICER *et al.* 2009) on the speciation of the cultivated species. The variation in nuclear DNA contents within the two cultivated jute species is in agreement with results reported for several plant groups (BENNETT *et al.* 2000 and references therein). However, it is difficult to explain the reason for this intraspecific genome size variability in both the cultivated jute species, especially when all the genotypes of each of the two species are grown under the same eco-geographical conditions. It may perhaps reflect a true individual-specific genome size variability, as recently reported in *Artemisia crithmifolia* (PELLICER *et al.* 2009).

Both the cultivated jute species are characterized by chromocentric nuclear organization (SAMAD *et al.* 1992) that results from small chromosome sizes and low nuclear DNA contents (LAFONTAINE 1974; NAGL and FUSENIG 1979). Although the extent of chromocenter association has been shown to have no correlation with the genome size, but in certain species it may be dependent on various nuclear parameters (CECCARELLI *et al.* 1998). In the present study, both the cultivated jute species and particularly the wild *Corchorus* species were characterized by extremely small chromosomes, which is in agreement with earlier reports on chromosome analysis (MORAKINYO and BADERINWA 1997; ALAM and RAHMAN 2000; MAITY and DATTA 2009; KHATUN and ALAM 2010). This unequivocally supports a chromocentric interphase nuclear structure in both *C. capsularis* and *C. olitorius*, as reported by SAMAD *et al.* (1992). However, their estimates of 2C values in both the cultivated jute species (2.3 and 2.8 pg) and in their inter-specific hybrid (3.1 pg) do not reflect a very low nuclear DNA content, in comparison to variation in nuclear DNA amounts reported for angiosperms till date (BENNETT and LEITCH 2005; ZONNEVELD *et al.* 2005). The average 2C values for *C. capsularis* (0.602 pg) and *C. olitorius* (0.669 pg) being reported here do indeed represent a very low nuclear DNA content, and therefore, may govern a chromocentric nuclear structure. In this context, it is to be noted that SAMAD *et al.* (1992) could not find any clear relationship between DNA content and heterochromatin percentages in jute. A gross overestimation of the 2C values may be responsible for this apparent discrepancy, which merits further investigation in the future.

Our results show that, of the *Corchorus* spp., *C. fascicularis* has the smallest haploid genome

(~188 Mb). The white jute (*C. capsularis*) cvs JRC-212 and Branca, each with a genome (1C) size of ~246 Mb, are the smallest genomes among the economically important crop species (BENNETT and LEITCH 2005; ZONNEVELD *et al.* 2005). Excluding the mutants, 1C in *C. capsularis* is approximately 280 Mb, which is ~300% smaller than the reported estimate (SAMAD *et al.* 1992; MIR *et al.* 2009). Similarly, *C. olitorius* cv. Sudan Green, with a genome (1C) size of ~315 Mb, is the smallest genome among the tossa jute cultivars (studied here). The average 1C in *C. olitorius* is approximately 324 Mb, which is also ~300% smaller than the reported estimate (SAMAD *et al.* 1992; MIR *et al.* 2009). Thus our results, for the first time, provide an accurate estimate of the jute genome size, and will aid in jute genome sequencing having been initiated under the Jute Genome Project (ANONYMOUS 2010), with a highly inflated estimate (~1200 Mb) of haploid genome size.

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