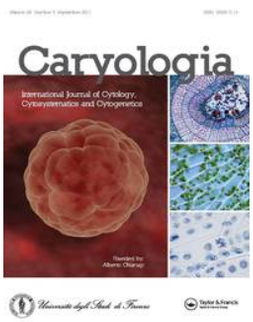

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Karyomorphological investigations on some economically important members of Zingiberaceae from Eastern India

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In this paper, karyological investigations of four economically and medicinally important Indian species of Zingiberaceae belonging to the genera *Curcuma* and *Zingiber* were performed. The somatic chromosome number of *Curcuma amada* was $2n = 42$ and that of *Curcuma longa* was $2n = 63$, while the chromosome numbers of both *Zingiber officinale* and *Zingiber zerumbet* were $2n = 22$. Chromosome morphology of the two species of *Curcuma* studied showed predominance of constrictions in the median region and absence of secondary constriction. In contrast, the two species of *Zingiber* studied showed a wide variation of constrictions from median to subterminal and six chromosomes with secondary constrictions. The results indicated presence of more symmetric karyotype in *Curcuma* spp. with respect to that of the *Zingiber* spp. studied. Detailed karyomorphological studies were undertaken and the results obtained were analyzed with respect to the published karyotype data for the four species.

Keywords: *Curcuma*; *Zingiber*; cytology; karyotype; chromosome

Introduction

The tropical family Zingiberaceae, one of the largest monocotyledonous families under order Zingiberales with over 1200 species (Kress 1990), contains many economically important plants.

The rhizomes of the plants *Curcuma amada* Roxb. (mango ginger), *Curcuma longa* L. (turmeric), *Zingiber officinale* Roscoe (ginger) and *Zingiber zerumbet* (L.) Roscoe ex Sm. (shampoo ginger), belonging to this family, are known for their medicinal properties, and have been used extensively in various traditional medicinal systems, e.g. Ayurveda (Kala et al. 2006). In addition, turmeric (*C. longa*) and ginger (*Z. officinale*) are used in different cuisines globally as spices, and India is one of the leading producers and exporters of both of them (Plant Cultures 2013a, 2013b). Rhizomes of mango ginger (*C. amada*) and shampoo ginger (*Z. zerumbet*) are used against various types of stomach ailments (Choudhary et al. 2008; Srivastava et al. 2012). Mango ginger is also used to treat leprosy (Srivastava et al. 2012) and rhizomes of shampoo ginger are used as an anti-inflammatory agent (Al–Zubairi et al. 2010).

Plants of *C. amada* are characterized by a pale yellow rhizome with the characteristic smell of green mango, lateral inflorescence with pinkish coma bracts, and peduncle covered by sheathing base of the leaves. Flowers are pale yellow with a median dark yellow band. The rhizomes of *C. longa* are bright yellow inside and strongly aromatic. The spikes of these plants are central with a whitish coma tinged with pink. The peduncle is hidden by a sheathing petiole; fertile bracts are pale green while the flowers are pale yellow with a broad, median dark yellow band. *Z. officinale* plants have grayish yellow rhizomes with a pungent flavor. The spikes are radical, growing on a long leafless peduncle with greenish bracts (lower ones turning to red at maturity). The flowers are greenish, with purplish-black lip and pale yellow corolla lobe. The rhizomes of *Z. zerumbet* are pale yellow inside. The bracts of the oblong spike are green with a paler edge, growing on a leafless peduncle. Flowers are whitish with sulfur yellow lip (Hooker 1894; Sabu 2006).

Members of Zingiberaceae are characterized by gross morphological similarity and striking chromosomal diversity. Inadequate descriptions and lack of well-preserved type specimens, coupled with the short flowering season of these plants, have led to confusion regarding their taxonomic identity (Škorničková and Sabu 2002). Variations in ploidy levels and hybridization among related genera generally lead to blurred morphological boundaries which might be a reason for the morphological instability among related taxa (Stace 2000).

Cytological studies are traditionally utilized to aid morpho-taxonomy. The concept of utilizing karyotypic asymmetry to predict evolutionary trends as proposed by Levitsky (1931) has been widely adopted as a useful method for systematic study of plants (Bennett and Leitch 2005). An asymmetric karyotype is supposed to represent an evolutionary flux, while the more symmetric ones are predicted to be more stable (Stebbins 1971).

The present study focuses on four economically important species of Zingiberaceae, all of which are cultivated throughout India, especially in the eastern and southern regions of the country. Since the 1920s

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(Sugiura 1928) there have been many reports on the chromosome number of these species from different parts of the world (Sugiura 1931; Chakraborti 1948; Sharma and Bhattacharya 1959; Sato 1960; Nambiar 1979; Das et al. 1999; Etikawati and Setyawan 2000; Eksomtramage et al. 2001; Škorničková et al. 2007; Nair et al. 2010). But there are disagreements regarding the chromosome numbers and ploidy levels reported among them. It is suggested that the major reason for this wide variation of chromosome numbers and ploidy may be due to the predominant vegetative propagation of these species (Škorničková et al. 2007).

Even among the different cultivars, there are reports of morphological and chromosomal variability (Dhamayanthi and Zachariah 1998; Nair and Sasikumar 2009; Nair et al. 2010; Daryono et al. 2012). There exists very limited information on the karyomorphology of these species, perhaps owing to the small size of chromosomes and their large number, which hamper their detailed karyotype study. Use of modern tools, such as software-assisted imaging and karyotype analysis, would be invaluable in an accurate karyomorphological study of the plants (Parai et al. 2012).

The main purpose of this study was to perform a detailed karyomorphological study of the four economically important species of the Zingiberaceae. Although the four species used in the present study are in no way representative of the family Zingiberaceae, they are widely available and offer the possibility of understanding the role of anthropogenic selection on karyomorphological evolution of these species.

Materials and methods

Several accessions of four cultivated species of Zingiberaceae – *C. amada*, *C. longa* cv. Patni, *Z. officinale* cv. Nadia and *Z. zerumbet* were included in the present study. Rhizomes of three of the plant materials, *C. amada*, *C. longa* and *Z. officinale*, were initially collected from the farmers of the local market of Kolkata, West Bengal, while rhizomes of *Z. zerumbet* were collected from the Umiam Regional Research Station of NBPGR, India. All these plants were then grown and are successfully maintained in the Experimental Garden of the Department of Botany, University of Calcutta. The plants were authenticated comparing with the herbarium specimens available at CUH (Calcutta University Herbarium) and also using relevant references.

For mitotic studies, actively growing root tips of plantlets developing from the rhizomes were harvested at different times, throughout the day, during the period of May to August. The root tips were pretreated in aqueous solution of 0.008 M 8-hydroxyquinoline (3–3.30 h at 10–12°C) and then fixed in 1: 3 propiono-ethanol (overnight at 12°C). The root tips were stained in 2% propionic orcein-HCl mixture (9:1 v/v) (1–2 h at room temperature) and squashed in 45% propionic acid in clean grease-free slides. The squashed root tips were examined under microscope (Primostar, Carl Zeiss, Germany) at a magnification of 1000×.

Well-scattered metaphase plates were photographed using AxioCam ERc5s camera (Carl Zeiss) and ZEN 2012 software (Carl Zeiss). Mitotic metaphase plates were studied from at least 10 different root tips per accession, of which five best metaphase plates were selected for karyotype analysis. Chromosome images were analyzed using Zeiss Axiovision LE 4.3 software. Based on centromeric indices and lengths of chromosomes, idiograms of individual species were drawn.

The nomenclature system proposed by Levan et al. (1964) was used for describing chromosome morphology. Karyotype asymmetry index (AI) was calculated following Paszko (2006) while the karyotype asymmetry was described following the classes proposed by Stebbins (1971). The degree of asymmetry of karyotype (A) was calculated following Watanabe et al. (1999). For numerical characterization of selected karyotypes, parameters such as length of long arm (LA), length of short arm (SA), length of chromosomes (TL = LA + SA), total length (ΣTL), relative length percentage (RL= LA + SA/ΣTL), arm ratio (AR = LA/SA), centromeric index (CI = SA/TL), value of relative chromatin (VRC = ΣTL/n), karyotype formula (KF), and, difference of relative length (DRL = Max_{RL}– Min_{RL}) were calculated (Dong et al. 2011). Total form percent (TF% = ΣSA/TL) was calculated following Huziwara (1962). The intrachromosomal and interchromosomal asymmetry indices (A_1 and A_2 respectively) were calculated according to Zarco (1986). Based on Paszko (2006), the coefficients of variation of chromosome length (CV_{CL}) and chromosome index (CV_{CI}) were calculated. The ratio of mean length of short arms to long arms (Syi) was calculated following Greilhuber and Speta (1976).

Results

Curcuma amada

The somatic chromosome number of *C. amada* was found to be $2n$ = 42, the karyotype formula being $2n$ = 42= 8M + 34m (Table 1 and Figure 1 A, B). The centromeric positions of chromosomes varied from median to nearly median, the centromeric index ranging between 37.5% and 49.31%. Chromosomes with secondary constrictions were absent. The individual chromosome lengths ranged between 0.47 µm and 1.06 µm and the total length of chromosomes was 26.44 ± 0.9 µm. The lengths of short arms were between 0.22 µm and 0.49 µm, while the long arms were 0.24 µm to 0.57 µm in length. The arm ratio ranged between 1.03 and 1.67. The karyotype asymmetry index was found to be 0.013 and the karyotype asymmetry type (based on Stebbins 1971) was deduced to be 1B. The degree of karyotype asymmetry was 0.23. The relative length per cent was between 0.040% and 0.018%, their difference being

Table 1. Chromosome numbers, karyotype formulae and morphometric parameters studied.

	C. amada	*C. longa*	*Z. officinale*	*Z. zerumbet*
Somatic chromosome number (2*n*)	42	63	22	22
Karyotype formula	8M + 34m	11M + 48m + 4Sm	4M + 6m + 6Sm + 2Sm:St + 2Sm:Sm + 1m:Sm + 1St:St	2M + 10m + 3Sm + 1St + 1Sm:Sm + 2m:St + 1Sm:St + 1m:m + 1m:Sm
Short arm range (SA)	0.22–0.49	0.25–0.53	0.33–0.91	0.18–0.73
Long arm range (LA)	0.24–0.57	0.31–0.90	0.46–1.5	0.4–1.26
Total length range (TL)	0.47–1.06	0.57–1.27	0.89-2.18	0.98–1.75
Centromeric index range (CI)	37.5–49.31	28–49.51	17.97–49.09	18.37–49.51
Arm ratio range (LA : SA)	1.03–1.67	1.02–2.57	1.04–4.42	1.02–4.44
Summation of total length of chromosomes (ΣTL)	26.44 ± 0.9	53.09 ± 4.7	34.73 ± 3.4	29.22 ± 2.7
Value of relative chromatin (VRC)	1.26 ± 0.04	2.53 ± 0.05	3.16 ± 0.54	2.66 ± 0.06
Total form percentage (TF%)	44.21 ± 3.2	43.94 ± 3.08	36.14 ± 2.8	35.97 ± 2.7
Relative length percent range (RL)	0.040–0.018	0.011–0.024	0.026–0.063	0.033–0.060
Difference of relative length (DRL)	0.022	0.013	0.037	0.026
Intrachromosomal asymmetry index (A1)	0.6	0.59	0.33	0.66
Interchromosomal asymmetry index (A2)	0.17	0.18	0.22	0.16
Coefficient of variation of chromosome length (CV_{CL})	16.63	17.77	22.34	15.9
Coefficient of variation of centromeric index (CV_{CI})	7.65	9.10	22.45	27.26
Karyotype asymmetry index (AI)	0.013	0.016	0.05	0.043
Syi	79.25	78.39	63.7	65.36
Degree of asymmetry of karyotype (A)	0.23	0.18	0.38	0.38

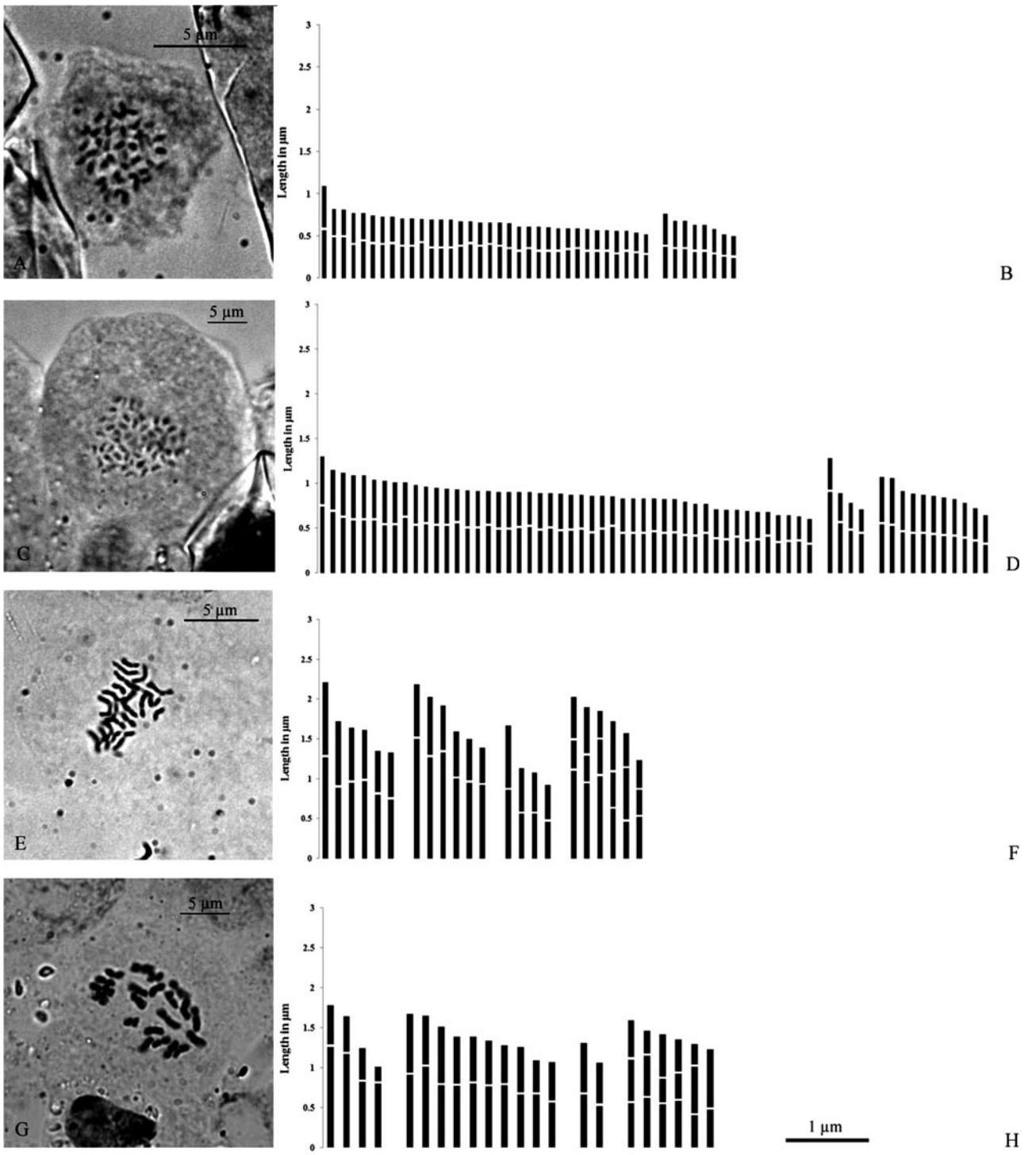

Figure 1. Somatic metaphase plates and idiograms of (A, B) *Curcuma amada* (2*n* = 42); (C, D) *Curcuma longa* (2*n* = 63); (E, F) *Zingiber officinale* (2*n* = 22); (G, H) *Zingiber zerumbet* (2*n* = 22).

0.022%. The total form percentage was 44.21 ± 3.2% and the value of relative chromatin was found to be 1.26 ± 0.04 µm. The interchromosomal and intrachromosomal asymmetry indexes of this species were found to be 0.60 and 0.17 respectively. The coefficient of variation of chromosome length was 16.63 and the coefficient of variation of centromeric index was 7.65. The Syi was found to be 79.25.

Curcuma longa

Curcuma longa had 2*n* = 63 chromosomes in the somatic cells and the karyotype formula was found to be 2*n* = 63= 11M + 48m + 4Sm (Table 1 and Figure 1 C, D). The centromeric positions included median, nearly median and submedian types. The centromeric index was 28% to 49.51%. No chromosome with secondary constriction was found. The lengths of individual

chromosomes varied from 0.57 μm to 1.27 μm while the total length was 53.09 ± 4.7 μm. The lengths of short and long arms of chromosomes were 0.25–0.53 μm and 0.31–0.90 μm, respectively. The arm ratio ranged from 1.02 to 2.57. The karyotype asymmetry index was 0.016, the karyotype asymmetry (Stebbins 1971) being of 2B type. The degree of asymmetry of karyotype was 0.18. Relative length percentage was 0.011% to 0.024% while the difference of relative lengths was 0.013%. Total form percentage was found to be 43.94 ± 3.08%. The value of relative chromatin was 2.53 ± 0.05 μm. The interchromosomal asymmetry index was 0.18 while the intrachromosomal asymmetry index was 0.60. The coefficient of variation of chromosome length was 17.77 and that of centromeric index was 9.10. The Syi of this species was found to be 78.39.

Zingiber officinale

With a chromosome number of $2n = 22$ at mitotic metaphase, the karyotype formula of *Zingiber officinale* was deduced to be $2n = 22$= 4M + 6m + 6Sm + 2Sm:St + 2Sm:Sm + 1m:Sm + 1St:St (Table 1 and Figure 1 E, F). The centromeric positions were found to range from median to subterminal with the centromeric index ranging from 17.97% to 49.09%. Six chromosomes showed presence of secondary constrictions. The lengths of individual chromosomes ranged between 0.89 μm and 2.18 μm. The total chromosome length was 34.73 ± 3.4 μm with the length of short arms and the long arms 0.33–0.91 μm, and 0.46–1.5 μm, respectively. The ratio between the long and short arms ranged from 1.04 to 4.42. The karyotype asymmetry index was found to be 0.05, resulting in a karyotype asymmetry type of 2B according to Stebbins (1971). The degree of asymmetry of karyotype was 0.18. Relative length percentage ranged from 0.026% to 0.063% showing a difference of 0.037%. The total form percentage was found to be 36.14 ± 2.8% and the value of relative chromatin was 3.16 ± 0.054 μm. The interchromosomal asymmetry index was 0.22 while the intrachromosomal asymmetry index was 0.33. The coefficient of variation of chromosome length was 22.34 and the coefficient of variation of centromeric index was 22.45. The Syi was 63.70.

Zingiber zerumbet

The somatic chromosome number of this species was ascertained to be $2n = 22$. The karyotype formula was deduced to be $2n = 22$= 2M + 10m + 3Sm + 1St + 1Sm:Sm + 2m:St + 1Sm:St + 1m:m + 1m:Sm (Table 1 and Figure 1 G, H). The centromeric positions ranged from median to subterminal. Centromeric index of this species ranged from 18.37% to 49.51%. Secondary constrictions were found in six chromosomes. Lengths of individual chromosomes ranged from 0.98 μm to 1.75 μm and the total chromosome length was found to be 29.22 ± 2.7 μm. The length range of short arm was between 0.18 μm and 0.73 μm, while that of the long arm was 0.4 μm to 1.26 μm. The arm ratio varied from 1.02 to 4.44. The karyotype asymmetry index was 0.043. The karyotype asymmetry (Stebbins 1971) was found to be of 2A type while the degree of karyotype asymmetry was found to be 0.38. The relative length percentage varied between 0.033% and 0.060%, their difference being 0.026%. The total form percentage was 35.97 ± 2.7%. The value of relative chromatin was 2.66 ± 0.06 μm. The interchromosomal and intrachromosomal asymmetry indexes were found to be 0.16 and 0.66 respectively. The coefficient of variation of chromosome length was 15.9 while the coefficient of variation of centromeric index was 27.26. The Syi was found to be 65.36.

Discussion

It is apparent from earlier studies that the genus *Curcuma* L. has well-established polyploidy (Škorničková et al. 2007). The basic number of this genus was earlier reported to be $x = 21$ (Raghavan and Venkatasubban 1943; Chakraborti 1948; Sharma and Bhattacharya 1959; Ramachandran 1961, 1969; Nambiar 1979) but later on it was deduced to be $x = 7$ (Škorničková et al. 2007). If $x = 7$ is accepted as the basic chromosome number, then *C. longa* ($2n = 63$) is a nonaploid ($9x$) and *C. amada* ($2n = 42$) a hexaploid ($6x$) (Škorničková et al. 2007).

The somatic chromosome number of *C. amada* has been consistently reported to be $2n = 42$ (Raghavan and Venkatasubban 1943; Chakraborti 1948; Raghavan and Arora 1958; Sharma and Bhattacharya 1959; Ramachandran 1961, 1969; Islam 2004; Škorničková et al. 2007; Joseph 2010). Das et al. (1999), however, reported a variable number of $2n = 40$ of this plant species along with the usual $2n = 42$. The chromosome number of *Curcuma amada* was found to be $2n = 42$ in the present study.

Previous studies indicate a huge variation in somatic chromosome numbers in *C. longa* spanning over 70 years, from Sugiura (1931) to Nair et al. (2010). Sugiura (1931, 1936) first reported the chromosome number of this species to be $2n = 64$, which was substantiated by Chakraborti (1948). Raghavan and Venkatasubban (1943), Chakraborti (1948) and Sharma and Bhattacharya (1959) reported the somatic chromosome number of this plant to be $2n = 62$. Sato (1960), however, reported the existence of a low somatic chromosome number, $2n = 32$. Das et al. (1999) and Nayak et al. (2006) have reported that some cultivars of *C. longa* show the somatic chromosome number of $2n = 48$. However, extensive studies by Ramachandran (1961, 1969) established the chromosome number of *C. longa* to be $2n = 63$ which was widely supported from the studies conducted by Chakraborti (1948), Prana (1977), Prana et al. (1978), Nambiar (1979), Nair (2000), Renjith et al. (2001), Paisooksantivatana and Thepsen (2001), Islam (2004), Škorničková et al. (2007), Nair and Sasikumar (2009) and Joseph (2010). *C. longa* was hypothesized to

be an allopolyploid by Nambiar (1979), who concluded that the species has arisen as a result of hybridization between two species with $2n = 42$ and $2n = 84$ chromosomes. Still variable chromosome numbers of *C. longa* were sporadically reported by Sharma and Bhattacharya (1959) ($2n = 93$), Renjith et al. (2001) ($2n = 84$), Nair et al. (2010) ($2n = 78, 82, 84$), and, Nair and Sasikumar (2009) ($2n = 61, 84$), although these are generally assumed to be exceptions or aberrations, rather than normal. In the present study, the cultivar of *C. longa* studied showed $2n = 63$ chromosomes in their root tip cells like a majority of earlier reports.

The genus *Zingiber* Mill., as opposed to *Curcuma*, shows constant somatic chromosome number, and very few reports of variations exist. Raghavan and Venkatasubban (1943) reported the basic number of this genus to be $x = 11$, which was confirmed by an overwhelming majority of further studies.

Earlier reports on chromosomes of the species *Z. officinale* also shows the diploid number to be $2n = 22$ (Sugiura 1928, 1931; Morinaga et al. 1929; Raghavan and Venkatasubban 1943; Chakraborti 1948; Darlington and Wylie 1955; Sharma and Bhattacharya 1959; Ramachandran 1969; Ratnambal 1979; Omanakumari and Mathew 1985; Goldblatt 1988; Rai et al. 1997; Das et al. 1998; Dhamayanthi and Zachariah 1998; Eksomtramage et al. 2002; Saensouk and Saensouk 2004; Sanpote 2004; Nayak et al. 2005; Joseph 2010). Other than this, variations in chromosome numbers were reported by some workers. The diploid number of $2n = 24$ was reported by Kihara et al. (1931), Chakraborti (1948), Sharma and Bhattacharya (1959), and Dhamayanthi and Zachariah (1998), while Etikawati and Setyawan (2000) and Daryono et al. (2012) reported the diploid number of $2n = 32$. Further variations were reported by Darlington and Janaki Ammal (1945) ($2n = 22 + 2B$), Chakraborti (1948) ($2n = 22 + 2f$, 55), Sharma and Bhattacharya (1959) ($2n = 10, 16, 21, 36, 46$), Bisson et al. (1968) ($2n = 66$), and Daryono et al. (2012) ($2n = 30$).

In *Z. zerumbet*, a well-established chromosome number of $2n = 22$ was reported by Raghavan and Venkatasubban (1943), Chakraborti (1948), Ramachandran (1969), Ratnambal (1979), Omanakumari and Mathew (1985), Eksomtramage et al. (2001), Li and Chen (2008), and Joseph (2010). In the present study, the chromosome numbers of both the species of the genus *Zingiber* (i.e. *Z. officinale* and *Z. zerumbet*) was found to be $2n = 22$, consistent with the earlier reports.

The concept of karyotype asymmetry, proposed by Levitsky (1931) and later developed by Stebbins (1971), advocated not only the study of general morphology of plant karyotypes, but also the correlation of the chromosomal changes that the plant undergoes during evolution.

While a symmetric karyotype is predominated by chromosomes of similar size and constrictions in median region, any change in either of the two parameters, namely centromeric position or size, leads to the establishment of an asymmetric karyotype, although these two parameters are independent of each other.

Though the earlier concept of a very evolved genus having a symmetric karyotype is now not accepted, still the analysis of inter- and intrachromosomal asymmetry gives valuable insights into the evolutionary potential within a selected group of plants. The karyotypes of members of *Curcuma* and *Zingiber* are generally reported to be symmetrical. Omanakumari and Mathew (1985) reported *Z. officinale* to be more symmetric than *Z. zerumbet*. But no such report is available regarding the karyotype symmetry for the two species of *Curcuma*.

Based on the chromosome types described by Levan et al. (1964), the karyotype formula analysis of the species examined in the present study revealed that most of the chromosomes of the genus *Curcuma* are of nearly median type with no secondary constriction observed in either species examined, while constrictions of the genus *Zingiber* varied from median to subterminal, both species having six chromosomes each with secondary constrictions. In the earlier studies too, chromosomes with two constrictions were reported in *Zingiber* but rarely in *Curcuma*. Joseph (2010) observed two chromosomes with secondary constriction in *C. amada* but none in *C. longa*. He also reported two pairs of chromosomes with secondary constriction in *Z. zerumbet*, while Omanakumari and Mathew (1985) had reported three pairs of the same. Reports on the number of chromosomes with secondary constriction are also available in case of *Z. officinale*. Omanakumari and Mathew (1985) and Dhamayanthi and Zachariah (1998) reported two pairs of chromosomes with secondary constrictions, and in another cultivar with variable chromosome number of $2n = 24$, Dhamayanthi and Zachariah (1998) reported only one pair of chromosomes with secondary constriction. Das et al. (1998) reported four, six and eight chromosomes with secondary constriction in eight different varieties of *Z. officinale*. Joseph (2010) also reported three pairs of chromosomes showing secondary constriction in a cultivar of *Z. officinale*.

The arm ratio (AR), the ratio between the length of the long and the short arm of the chromosomes, is an indicator of intrachromosomal asymmetry. In *Curcuma* spp. (*C. amada* and *C. longa*), AR ranged from 1.02 to 2.57, while in *Zingiber* spp. (*Z. officinale* and *Z. zerumbet*) AR was 1.02 to 4.44, indicating higher intrachromosomal symmetry of *Curcuma* spp. (Table 1). This fact was supported by the high intrachromosomal asymmetry (A_1) value of the *Zingiber* spp. High CV_{CI} and AI (Paszko 2006) values of the two *Zingiber* with respect to *Curcuma* spp. studied also indicate the existence of asymmetry in *Zingiber* with respect to *Curcuma* karyotype. High TF% and Syi value of both the species of *Curcuma* also confirm that their karyotypes are more symmetric than that of the *Zingiber* spp. studied. An analysis of the degree of asymmetry of karyotype (A) (Watanabe et al. 1999) also confirms that the karyotypes

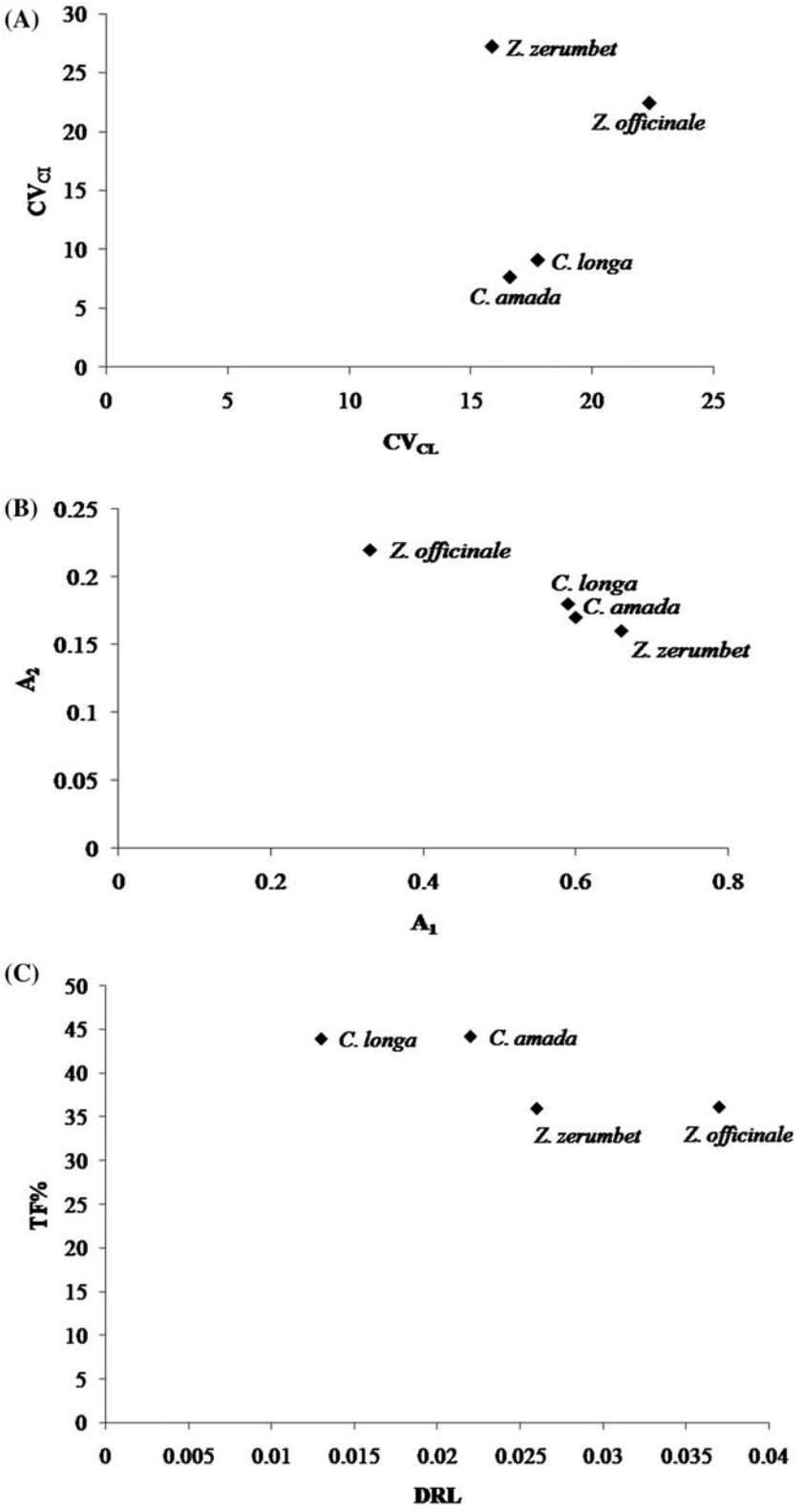

Figure 2. Scatter diagrams for the studied taxa: (A) CV_{CL} against CV_{CI}; (B) A_1 against A_2; (C) DRL against TF%.

of the two species of *Curcuma* studied are more symmetric than the two species of *Zingiber* studied. Interchromosomal asymmetry index (A_2) and difference of relative length (DRL) are low in all the four species studied, indicating similar size of the chromosome complements of each species, leading to low interchromosomal variation and thus overall karyotype symmetry. Higher intrachromosomal asymmetry and lower interchromosomal asymmetry is also indicated by the scatter diagrams based on CV_{CL} and CV_{CI}, A_1 and

A_2, and, DRL and TF% data (Figure 2). This was also supported by the typification of Stebbins' classification of karyotypes (Stebbins 1971). Since a symmetrical karyotype is considered more primitive than an asymmetric ones (Stebbins 1971), the genus *Zingiber* may be considered to be more advanced than *Curcuma*.

Conclusion

Karyomorphological studies of the plants of Zingiberaceae are especially difficult because of the small size and high number of chromosomes. The variations in chromosome numbers of these species have been reported from different parts of India as well as that of the world. In the present study, no variable chromosome number was found in the species studied. But it should also be noted that the study was confined to plants from definite geographic locations. The karyotypes of the four species studied were documented and analyzed using software-assisted imaging techniques that helped in acquiring accurate data regarding chromosome numbers and types, which is more expedient than manual karyotype analysis. Increasing the range of collection of these plants in further study might help in solving the karyological puzzle that exists in these species, especially in *C. longa*. Also, more karyomorphological studies of the plants of Zingiberaceae are needed in order to fill in the gap that exists in our present knowledge of understanding the karyological evolution and speciation in the family Zingiberaceae.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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