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SOMATIC CHROMOSOME NUMBER AND PLOIDY LEVEL IN THREE *CURCUMA* SPP. FROM BANGLADESH

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Key words: *Curcuma,* Chromosome number, Polyploidy

Abstract

Three *Curcuma* L. species were investigated cytogenetically which represent diversed staining pattern of heterochromatins in interphase nuclei and prophase chromosomes with orcein staining. *Curcuma longa* and *C. caesia* were found to possess 2n = 3x = 63 somatic chromosomes whereas 2n = 2x = 42 chromosome number in *C. zedoaria* is reported for the first time from Bangladesh. Total chromosome length recorded in *C. longa*, *C. caesia* and *C. zedoaria* were 145.08 ± 2.85 µm, 164.93 ± 4.29 µm and 97.78 ± 2.41 µm, respectively. This was the first attempt to measure the length of the chromosomes for these species. The experiment confirmed the basic chromosome number x = 21 with triploid (*C. longa*, *C. caesia*) and diploid (*C. zedoaria*) *Curcuma* plants. Polyploidy could be employed in the evolution and diversification of the genus *Curcuma*, which is an essential factor to characterize the species of this genus.

Introduction

The genus *Curcuma* L. is an economically important perennial herb belonging to Zingiberaceae comprising 120 species[(1)] distributed throughout the Asian tropics and expands to Africa and Australia. The genus includes medicinally, culinary and ornamentally significant taxa simultaneously[(2-3)]. The species of this genus has diverse uses in Indian medicines as well as a powerful antioxidant, anti-parasitic, antispasmodic and anti-inflammatory compound, which may also inhibit carcinogenesis[(4-6)].

There are about eight *Curcuma* L. species reported from Bangladesh of which six species *viz. C. amada* Roxb., *C. angustifolia* Roxb., *C. aromatica* Salisb., *C. caesia* Roxb., *C. longa* L. and *C. zedoaria* (Christm.) Roscoe are medicinally important[(7)]. Among them the most popular and entirely cultivated species *viz. C. longa* (turmeric) is a tremendous natural food colorant with high medicinal value and *C. caesia* (commonly called black turmeric or black zedoary) shows medicinal activity as well. Moreover, *C. zedoaria* commonly known as 'Zedoary' is a native of south Asian countries including Bangladesh, used traditionally as spice, tonic and perfume[(8)].

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The genus *Curcuma* shows significant variation in somatic chromosome number which makes this taxa more complicated to study (Table 2). Therefore, thorough taxonomic revision is needed for authentic identification of different species under this genus[1,3,9]. Chromosome counting with detailed cytogenetical characterization can alleviate the ambiguity of taxonomic riddle, especially in taxa with smaller chromosomes like *Curcuma.* Moreover, cytogenetical characters are also considered as informative markers for genetics, evolution and plant conservation[10].

Different somatic chromosome numbers and diversification in the ploidy level have been investigated in various *Curcuma* species by earlier scientists[1,3,6,9,11-18]. Yet, there are no detailed cytologenetical reports about the known wild and cultivated *Curcuma* species growing in Bangladesh. Hence, the present investigation deals a chromosomal study along with ploidy levels in the available three *Curcuma* species from Bangladesh.

Materials and Methods

In this study, three *Curcuma* L. species *viz. C. longa* L., *C. caesia* Roxb. and *C. zedoaria* (Christm.) Roscoe were used as plant materials. Among these species *C. longa* L. and *C. caesia* Roxb. were collected from Chittagong University campus. *C. zedoaria* was planted in Botanical Garden, Department of Botany, University of Dhaka and maintained. For cytogenetical study, healthy roots were collected and pretreated with 0.002 M 8-hydroxyquinoline for 1 h at room temperature followed by 15 m fixation in 45% acetic acid at 4ºC and preserved in 70% alcohol for further use. These were then hydrolyzed in a mixture of 1N HCl and 45% acetic acid (2 : 1) at 65°C for 20 min. The root tips were stained and squashed in 1% aceto-orcein for 2.30 h. Afterwards the prepared slides were observed under a compound microscope (Nikon eclipse 100, Japan). The well spread and clear mitotic metaphase chromosomes were considered for the analysis. From the same slides interphase nuclei and prophase chromosomes were studied.

The photography was taken by using 8 mega pixels canon power shot A720 model with the magnification of 7.0xs at Auto mode. For measuring the magnification, the magnification was calculated by multiplying the magnification of objective (100xs), tube length (1.25xs) and camera lens (5xs, 3.5xs). To get an accurate measurement of chromosomes length at least three metaphase plates were measured for each species.

Results and Discussion

Staining properties of interphase nuclei and prophase chromosomes: The three *Curcuma* species showed two different staining patterns of interphase nuclei i.e. 'diffuse type' and 'simple chromocenter type' according to Tanaka 1971[19]. Among them, *C. longa* showed 'diffuse type' with homogeneously stained interphase nuclei and on the other hand, several scatterdly distributed small heterochromatic blocks were found around the

interphase nuclei of *C. caesia* and *C. zedoaria* which reflects 'simple chromocenter type' (Fig. 1, Table 1).

The prophase chromosomes of *C. longa* were found to be stained uniformly along the length and this type of prophase chromosomes are called 'continuous type'[19]. On the contrary, *C. caesia* and *C. zedoaria* exhibited 'gradient type' of prophase chromosomes where most of the chromosomes were darkly stained in one end and gradually faint to the other end (Fig. 1, Table 1). Above findings indicated the presence of homogeneous distribution of heterochromatin both in the interphase nuclei and prophase chromosomes in *C. longa*, while in *C. caesia* and *C. zedoaria* localized heterochromatins were found to be firmly aggregated in the interphase nuclei which afterwards showed gradual distribution in the prophase chromosomes (Fig. 1). Usually, localized heterochromatins are not gradually distributed, rather occupy distinct locations of prophase chromosomes and this unusuality might be due to facultative nature of heterochromatins. Therefore, the staining properties of interphase nuclei and prophase chromosomes exhibit supplementary features that help to characterize different species karyomorphologically.

Table 1. Staining properties in interphase nuclei and prophase chromosomes of three species of *Curcuma*.

Species	Type of orcein-stained interphase nuclei	Type of orcein-stained prophase chromosomes
C. longa L.	Diffuse	Continuous
C. caesia Roxb.	Simple chromocenter	Gradient
C. zedoaria (Christm.) Roscoe	Simple chromocenter	Gradient

Basic set and polyploid nature: There have been several disagreements regarding the basic chromosome number in *Curcuma* . genus. Raghavan and Venkatsubban (1943)[12] followed by Venkatasubban (1946)[20] and Ramachandran (1961)[13] first reported basic set x = 21 for this genus and they also suspected that this basic number might be originated either by combination of two basic numbers of x = 9 and x = 12 (dibasic amphidiploidy) or by secondary polyploidy. Sharma and Bhattacharya (1959)[21]proposed x = 16 as the basic number of chromosome. Leong-Sˇkornickova' *et al.* 2007[9] suggested that x = 7 should be the basic chromosome number for Indian *Curcuma* species which supports the proposal of Sato (1960)[22]. Recently, Chen and Xia (2013)[3] and Lamo and Rao (2017)[1] emphasized on the basic chromosome number x = 21. In the present investigation *C. longa* and *C. caesia* were found to possess 2n = 63 somatic chromosomes while *C. zedoaria* showed 2n = 42 chromosomes (Fig. 1, Table 3). However, the somatic chromosome numbers of three *Curcuma* species found in this study confirmed x = 21 as the basic number of chromosome and their ploidy level can be explained by this basic set (Table 3). Among the species analyzed here, *C. longa* and *C. caesia* are found to be triploids with 2n

= 3x = 63 chromosomes, meanwhile *C. zedoaria* is diploid species with chromosome number 2n = 2x = 42 (Fig. 1, Table 3).

Polyploidy is a usual fact in vegetatively propagated plants like *Curcuma*. Polyploidization with higher number of somatic chromosomes accompanied by smaller chromosome size is an adaptive behaviour of chromosomes[23]. In this sense, polyploid plants like *Curcuma* indicate higher grade in evolutionary trend.

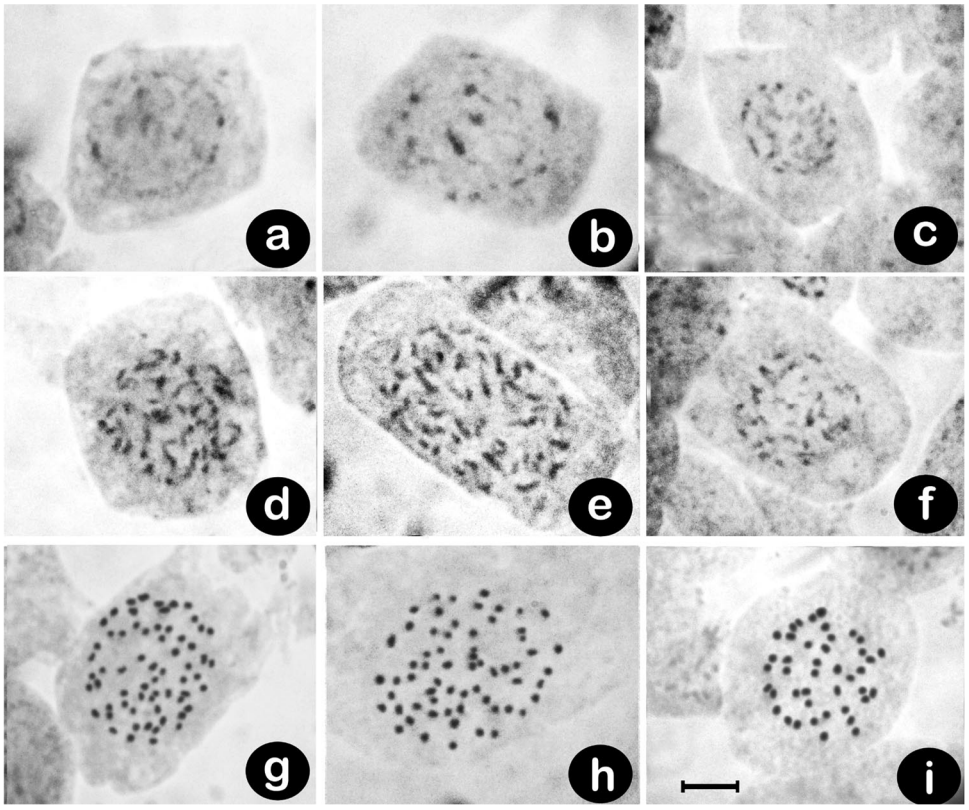

Fig. 1. Orcein-stained interphase, prophase and metaphase stages of mitotic cell division of three species of *Curcuma* (a) Interphase nuclei of *C. longa*, (b) Interphase nuclei of *C. caesia*, (c) Interphase nuclei of *C. zedoaria*, (d) Prophase chromosomes of *C. longa*, (e) Prophase chromosomes of *C. caesia*, (f) Prophase chromosomes of *C. longa*, (g) Metaphase chromosomes of *C. longa*, (h) Metaphase chromosomes of *C. caesia*, (i) Metaphase chromosomes of *C. zedoaria*. Bar = 10 µm.

Variation in somatic chromosome count: In the present investigation *C. longa* and *C. caesia* were found to possess 2n = 3x = 63 somatic chromosomes while *C. zedoaria* showed 2n = 2x = 42 chromosomes with basic chromosome number x=21 (Fig. 1, Table 3). Several chromosome counting for *C. longa* (2n = 32, 48, 62, 63, 64, 93), *C. caesia* (2n = 22 and 63)

and *C. zedoaria* (2n = 42, 63, 64, 66) were previously reported by different scientists worldwide (Table 2). However, current observations (2n = 63) of *C. longa* and *C. caesia* are in entire compliance with the reports of Ramachandran 1961[13], Prana 1977[14], Joseph *et al.* (1999)[17],Islam 2004[18], Leong-Sˇkornicˇkova´ *et al.* (2007)[9], Lamo and Rao 2014[24], Nair and Sasikumar (2009)[6]. While the observation (2n = 42) for *C. zedoaria* does not correlate with the previous report except the one of Paisooksantivatana and Thepsen (2001)[25]. However, 2n = 32, 48, 62, 64 and 93 for *C. longa*, 2n = 22 in *C. caesia* and 2n = 63, 64 and 66 in *C. zedoaria* might be due to several types of numerical chromosomal aberrations such as hypo- and hyper-aneuploidy and euploidy. Secondary modification of polyploidy might have taken place in the evolutionary pathway of this genus. Moreover, the genus *Curcuma* exhibits polyploid nature with small sized chromosomes which might be one of the probable reasons for blundering in chromosome counting or these variants might be different cytotypes of the same species.

Table 2. A summary of previously reported chromosome counts on three *Curcuma* species.

Species	(2n)	References
C. longa	32	Sato (1960)[22]
	48	Das *et al.* (1999)[16], Nayak *et al*. (2006)[26]
	62	Raghavan and Venkatsubban (1943)
	62, 63, 64	Chakravorti 1948 [27]
	63	Ramachandran (1961), Prana (1977), Islam (2004), Leong-Sˇkornicˇkova´ *et al.* (2007), Lamo and Rao (2014), Nair and Sasikumar (2009)
	64	Sugiura (1936)
	62, 93	Sharma and Bhattacharya (1959)
C. caesia	22	Das *et al.* (1999)
	63	Joseph *et al.* (1999), Islam (2004), Lamo and Rao (2014)
C. zedoaria	42	Paisooksantivatana and Thepsen (2001)
	63	Ramachandran (1961), Apavatjrut *et al.* (1996), Ardiyani 2002[28], Islam (2004), Lamo and Rao (2017)
	64	Venkatasubban (1946)
	66	Chatterjee *et al.* 1989[29]
	63, 64	Chakravorti (1948)
	63, 64, 66	Prana (1977)

In this experiment, chromosomal length was measured which revealed that all the three species possessed small sized chromosomes (Fig. 1, Table 3). Individual chromosome length range observed in *C. longa* was 1.92 - 2.72 µm, whereas the range was 2.25 - 3.34 µm and 2.11 - 2.58 µm for *C. caesia* and *C. zedoaria*, respectively (Table 3). Chen

and Xia (2013)[3] reported chromosomal length for eleven Chinese *Curcuma* species, size ranging from 0.5 to 2.1 µm which was approximately close to our present day findings. Total chromosome length recorded in *C. longa*, *C. caesia* and *C. zedoaria* were 145.08 ± 2.85 µm, 164.93 ± 4.29 µm and 97.78 ± 2.41 µm, respectively (Table 3). Nevertheless, it may be said that there is no such available record on chromosomal size for *Curcuma* species even for growing in Bangladesh. Thus, this act of approaching claims characterization of three *Curcuma* species cytogenetically for the first time in Bangladesh.

Table 3. Comparative chromosomal analysis of three *Curcuma* species.

Species Name	2n	TCL (µm)	CLR (µm)	ACL (µm)	Ploidy level (x)
C. longa	63	145.08 ± 2.85	1.92 - 2.72	2.30	3
C. caesia	63	164.93 ± 4.29	2.25 - 3.34	2.62	3
C. zedoaria	42	97.78 ± 2.41	2.11 - 2.58	2.33	2

[2n = somatic chromosome number, TCL = Total chromosome length (µm), CLR = Individual chromosome length range (µm), ACL = Average chromosome length (µm)].

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