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KARYOMORPHOLOGICAL ANALYSIS WITH DIFFERENTIAL STAINING OF NINE *CICER ARIETINUM* L. VARIETIES

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Abstract

Karyomorphological study of nine varieties of *Cicer arietinum* L. (chickpea) released from BARI was investigated. The nine varieties have 2n = 16 metacentric chromosomes revealing symmetrical karyotype. The first pair of chromosome in each variety has satellites on short arms. Nine varieties showed more or less similar karyotypes, differed in respect of other karyological features like orcein, CMA and DAPI staining property of interphase nuclei and prophase chromosomes. Therefore, the differential staining property of interphase nuclei and prophase chromosomes may be useful to distinguish nine varieties of *Cicer arietinum* L. in spite of having similar karyotypes.

Introduction

In order to characterize the variety on the basis of chromosomal information, many cytological works have been carried out on *Cicer* (Sharma and Gupta 1986, Galasso and Pignone 1992, Ocampo *et al*. 1992, Ahmad and Hymowitz 1993, Tayyar *et al*. 1994). Due to high condensation rate and small size chromosome, it is difficult to characterize the varieties. Moreover, karyotype analysis alone is unable to express the difference among different varieties of a species since the varieties of a species possess similar diploid chromosome number with similar karyotypes. Even the consideration of chromosome length, arm ratio, position and number of secondary constrictions are not always sufficient to differentiate individual chromosome (Sultana and Alam 2007, Afroz *et al.* 2013, Kondo and Hizume 1982, Hizume *et al*. 1988). In such a case, karyomorphological study provides basic genetic knowledge of an organism. The total karyomorphological behavior enables to characterize different varieties of a species. Karyomorphological study includes the nature of interphase nuclei, prophase and metaphase chromosomes. There are different parameters to study the interphase nuclei and prophase chromosomes. Tanaka (1971) was the pioneer to classify interphase nuclei and prophase chromosomes on the basis of staining property. Later a few authors tried to classify the interphase nuclei and prophase chromosomes on the basis of differential staining with orcein, CMA and DAPI (Alam and Kondo 1995, Sultana and Alam 2016). In this research, a full strength karyomorphological study has been undertaken to characterize nine varieties of *Cicer arietinum* released from BARI.

Materials and Methods

The nine varieties of *Cicer arietinum* L. *viz.* BC-1, BC-2, BC-3, BC-4, BC-5, BC-6, BC-7, BC-8 and BC-9 were collected from the Pulse Research Center (PRC) of Bangladesh Agricultural Research Institute (BARI). The accession number and pedigree of each variety is shown in Table 1. These nine varieties were maintained in the Botanic garden, Department of Botany, University of Dhaka.

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Healthy roots were collected and pretreated with 0.002 M 8-hydroxyquinoline for 1.5 hrs at 18°C followed by 15 min fixation in 45% acetic acid at 4°C. These were then hydrolyzed in a mixture of 1N HCl and 45% acetic acid (2 : 1) at 60°C for 20 sec. The root tips were stained and squashed in 1% aceto-orcein. For CMA- and DAPI banding, following Alam and Kondo's (1995) method was used with slight modification. After hydrolyzing and dissecting, the materials were squashed with 45% acetic acid. The cover glasses were removed quickly and allowed to air dry for at least 24 hrs before study. For CMA-staining, the air-dried slides were first pre-incubated in McIlvaine's buffer (pH 7.0) for 30 min followed by distamycin A (0.1 mg/ml) treatment for 10 min. The slides were rinsed mildly in McIlvaine's buffer supplemented with $MgSO_4$ (5 mM) for 15 min. One drop of CMA (0.1 mg/ml) was added to the materials for 15 min in a humid chamber and then rinsed with McIlvaine's buffer with $MgSO_4$ for 10 min. Slides were mounted in 50% glycerol and kept at 4ºC for overnight before observation. These were observed under Nikon (Eclipse 50i) fluorescent microscope with blue violet (BV) filter cassette. For DAPI-staining, after 24 hrs of air drying, the slides were first pre-incubated in McIlvaine's buffer (pH 7.0) for 27 min and treated in actinomycin D (0.25 mg/ml) for 10 min in a humid chamber. The slides were immersed in DAPI solution (0.01 mg/ml) for 20 min and mounted with 50% glycerol. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with an ultraviolet (UV) filter cassette.

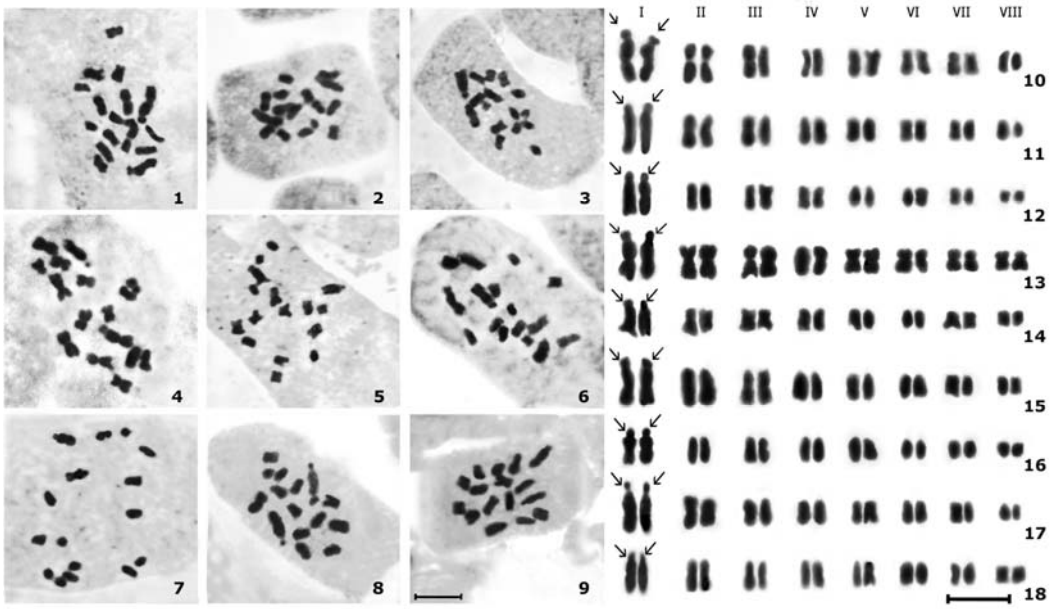
Table 1. Accession number and pedigree of nine varieties of *Cicer arietinum*.

Varieties	Accession number	Pedigree
BC-1	-	Native
BC-2	ICCL-83228	P 1231 × P 1265
BC-3	ICCL-83105	(K 850 × T 3) × (JG 62 × BEG 482)
BC-4	ICCL-85222	HMS 10 × (P 436 × H 223)
BC-5	RBH-228	ICC 14559
BC-6	ICCL-83149	(G 130 × B 108) × (NP 34 × GW 5/7)
BC-7	ICC-3274	P 3864-1
BC-8	ICCL-88003	(K 4 × Chaffa) × ICCL 81001
BC-9	ICCV-95318	ICCV 2 × ICC 7344

Results and Discussion

The nine varieties of *Cicer arietinum* L. were found to possess 2n = 16 chromosomes (Figs. 1-9). The present findings agreed with the previous reports (Sharma and Gupta 1986, Mukherjee and Sharma 1987, Galasso and Pignone 1992, Ocampo *et al*. 1992, Ahmad and Hymowitz 1993, Tayyar *et al*. 1994, Ahmad 2000). In addition, there were some reports on different 2n chromosome numbers of this species *viz.* 2n = 14 (Fedorov 1969, Dixit 1932), 2n = 24 (Meenakshi and Subramaniam 1966), 2n = 32 (Phadnis and Narkhede 1972, Oke 1955, Sen and Jana 1956) and 2n = 33 (Sen and Jana 1956). The diploid chromosome number 2n = 24 and 32 might be considered as triploid and tetraploid, respectively. On the other hand, there are two probable reasons for obtaining 2n = 33 chromosomes such as (i) either this was due to miscounting the chromosome number of 2n = 32 since chromosomes were very small in size and highly condensed or (ii) a special kind of numerical aberration. The reason for obtaining 2n = 14 chromosomes was not clear, there are also two probable explanations - (i) either the specimen was a different cytotype or (ii) might be a double monosomic. Therefore, ignoring the few exceptions, the diploid chromosome number of *Cicer arietinum* L. was almost stable and conserved.

The 16 chromosomes of nine varieties of chickpea found in this experiment were metacentric (Figs. 10-18, Table 2). Hejazi (2011) reported 16 metacentric chromosomes in *Cicer arietinum* L. Presence of only metacentric chromosomes is a feature of symmetric karyotype (Stebbins 1971). Symmetric karyotype is generally a sign indicating primitive in nature. Therefore, *Cicer arietinum* L. may be considered as primitive in respect of karyotype symmetry.



Figs. 1 - 9. Orcein-stained mitotic metaphase chromosomes in nine varieties of *Cicer arietinum*. 1. BC-1, 2. BC-2, 3. BC-3, 4. BC-4, 5. BC-5, 6. BC-6, 7. BC-7, 8. BC-8, 9. BC-9, Bar = 5 µm. Figs 10-18. Karyotypes prepared from orcein-stained mitotic metaphase chromosomes of nine varieties of *Cicer arietinum*. 10. BC-1, 11. BC-2, 12. BC-3, 13. BC-4, 14. BC-5, 15. BC-6, 16. BC-7, 17. BC-8, 18. BC-9, Bar = 5 µm.

A pair of prominent satellites was observed one in each member of pair I in nine varieties (Figs. 10 - 18, arrow) after orcein staining. Different numbers of satellites were also reported such as (i) two pairs and (ii) three pairs (Mukherjee and Sharma 1987). In contrast, no satellite was also reported for this species (Sharma and Gupta 1986, Ocampo *et al*. 1992). This observation clearly indicated the gradual loss of satellite portion from the genome of *Cicer arietinum*.

A conspicuous nucleolus was found in interphase, prophase and even late prophase of each variety in orcein study (Figs. 19a - 27a and 19d - 27d, arrow). This was a feature of persistent nucleolus. Persistent nucleolus was found in other species *viz. Spartocera fusca* (Cattani and Papeschi 2004) and *Zea mays* (Zirkle 1928). This nature might be due to the late transcription of rDNA to rRNA and late transportation of rRNA from the nucleus to the cytoplasm. The persistent nucleolus was a karyomorphological feature of chickpea variety.

In this study, the nine varieties showed three different types of orcein staining properties of interphase nuclei such as - (i) darkly stained small heterochromatic regions were found in each and every nucleus of all varieties except BC-2 and BC-3 (Figs. 19a, 22a - 27a). This type of staining region was named as “Simple Chromocenter Type” by Tanaka (1971), (ii) small heterochromatin

was found to be aggregated, forming large heteropycnotic bodies in BC-2 (Fig. 20a). This character was not found in the other varieties. Tanaka (1971) considered this feature as “Complex Chromocenter Type” and (iii) a few smaller heterochromatic bodies were present in the interphase nuclei of BC-3 (Fig. 21a). This type of staining was in between “Simple and Complex Chromocenter Type”.

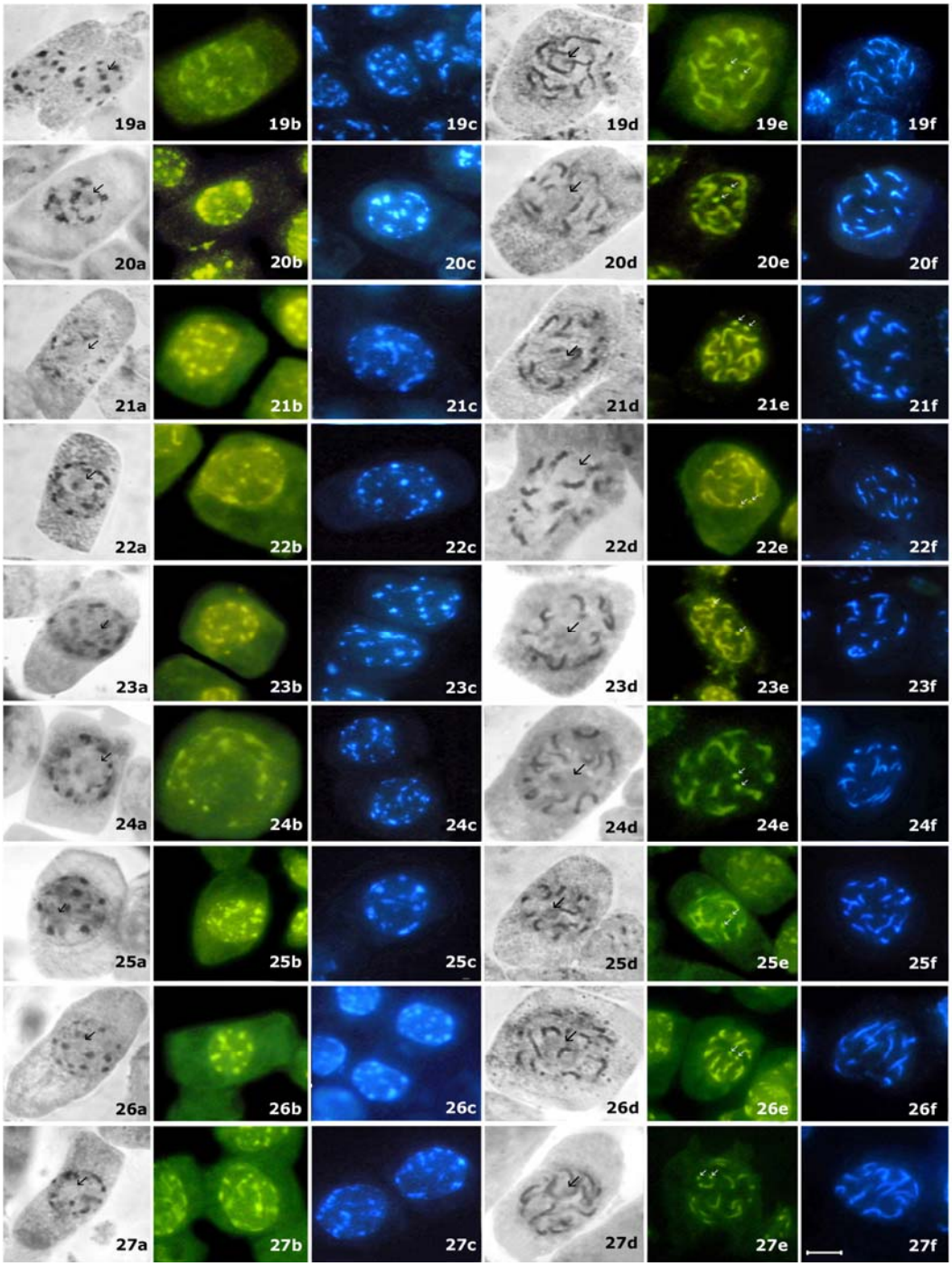
The prophase chromosomes of nine varieties could be classified into three based on orcein staining property. The prophase chromosomes of BC-1 and BC-8 were found to be stained at the interstitial region while the other regions of these chromosomes were stained faintly (Figs. 19d and 26d). This type of staining was named “Interstitial Type” by Tanaka (1971). In BC-2, BC-6 and BC-9, the prophase chromosomes were stained in one end and gradually faint to the other end

Table 2. Comparative orcein stained karyotype analysis in nine varieties of *Cicer arietinum.*

Varieties	2n	No. of satellite	Total length of 2n chromosome complement (µm)	Range of chromosomal length (µm)	Centromeric formulae
BC-1	16	2	39.19	1.47 - 3.40	16m
BC-2	16	2	36.43	1.29 - 3.31	"
BC-3	16	2	30.73	1.10 - 2.85	"
BC-4	16	2	37.21	1.61 - 3.04	"
BC-5	16	2	28.93	1.38 - 2.30	"
BC-6	16	2	38.92	1.56 - 3.22	"
BC-7	16	2	31.10	1.29 - 2.30	"
BC-8	16	2	34.59	1.43 - 3.22	"
BC-9	16	2	36.52	1.47 - 2.71	"

m = Metacentric chromosome.

Figs. 19-27. Mitotic interphase nuclei and prophase chromosomes of nine *Cicer arietinum* varieties. 19a. Orcein-stained interphase nuclei of BC-1, 19b. CMA-stained interphase nuclei of BC-1, 19c. DAPI-stained interphase nuclei of BC-1, 19d. Orcein-stained prophase chromosomes of BC-1, 19e. CMA-stained prophase chromosomes of BC-1, 19f. DAPI-stained prophase chromosomes of BC-1, 20a. Orcein-stained interphase nuclei of BC-2, 20b. CMA-stained interphase nuclei of BC-2, 20c. DAPI-stained interphase nuclei of BC-2, 20d. Orcein-stained prophase chromosomes of BC-2, 20e. CMA-stained prophase chromosomes of BC-2, 20f. DAPI-stained prophase chromosomes of BC-2, 21a. Orcein-stained interphase nuclei of BC-3, 21b. CMA-stained interphase nuclei of BC-3, 21c. DAPI-stained interphase nuclei of BC-3, 21d. Orcein-stained prophase chromosomes of BC-3, 21e. CMA-stained prophase chromosomes of BC-3, 21f. DAPI-stained prophase chromosomes of BC-3, 22a. Orcein-stained interphase nuclei of BC-4, 22b. CMA-stained interphase nuclei of BC-4, 22c. DAPI-stained interphase nuclei of BC-4, 22d. Orcein-stained prophase chromosomes of BC-4, 22e. CMA-stained prophase chromosomes of BC-4, 22f. DAPI-stained prophase chromosomes of BC-4, 23a. Orcein-stained interphase nuclei of BC-5, 23b. CMA-stained interphase nuclei of BC-5, 23c. DAPI-stained interphase nuclei of BC-5, 23d. Orcein-stained prophase chromosomes of BC-5, 23e. CMA-stained prophase chromosomes of BC-5, 23f. DAPI-stained prophase chromosomes of BC-5, 24a. Orcein-stained interphase nuclei of BC-6, 24b. CMA-stained interphase nuclei of BC-6, 24c. DAPI-stained interphase nuclei of BC-6, 24d. Orcein-stained prophase chromosomes of BC-6, 24e. CMA-stained prophase chromosomes of BC-6, 24f. DAPI-stained prophase chromosomes of BC-6, 25a. Orcein-stained interphase nuclei of BC-7, 25b. CMA-stained interphase nuclei of BC-7, 25c. DAPI-stained interphase nuclei of BC-7, 25d. Orcein-stained prophase chromosomes of BC-7, 25e. CMA-stained prophase chromosomes of BC-7, 25f. DAPI-stained prophase chromosomes of BC-7, 26a. Orcein-stained interphase nuclei of BC-8, 26b. CMA-stained interphase nuclei of BC-8, 26c. DAPI-stained interphase nuclei of BC-8, 26d. Orcein-stained prophase chromosomes of BC-8, 26e. CMA-stained prophase chromosomes of BC-8, 26f. DAPI-stained prophase chromosomes of BC-8, 27a. Orcein-stained interphase nuclei of BC-9, 27b. CMA-stained interphase nuclei of BC-9, 27c. DAPI-stained interphase nuclei of BC-9, 27d. Orcein-stained prophase chromosomes of BC-9, 27e. CMA-stained prophase chromosomes of BC-9, 27f. DAPI-stained prophase chromosomes of BC-9, Bar = 5 µm.



Figs. 19 - 27. Mitotic interphase nuclei and prophase chromosomes of nine *Cicer arietinum* varieties. (detailed legend in left page).

(Figs. 20d, 24d, 27d). As a result, one end of these chromosomes was much darker than other end. Tanaka (1971) classified this type of staining as "Gradient Type". The prophase chromosomes of rest of the varieties (BC-3, BC-4, BC-5 and BC-7) were found to stain uniformly along the entire length called as "Continuous Type" by Tanaka (1971) (Figs. 21d, 22d, 23d, 25d).

Table 3. Types of interphase nuclei and prophase chromosomes of nine varieties of *Cicer arietinum* after staining with orcein.

Varieties	Types of interphase nuclei	Types of prophase chromosomes
BC-1	Simple chromocenter	Interstitial type
BC-2	Complex chromocenter	Gradient type
BC-3	Between simple and complex chromocenter	Continuous type
BC-4	Simple chromocenter	Continuous type
BC-5	Simple chromocenter	Continuous type
BC-6	Simple chromocenter	Gradient type
BC-7	Simple chromocenter	Continuous type
BC-8	Simple chromocenter	Interstitial type
BC-9	Simple chromocenter	Gradient type

Tanaka (1971) proposed that usually (i) the organisms possessing "Diffused Type" of staining in interphase nuclei show "Continuous Type" of staining in prophase chromosomes, (ii) the organism having "Complex Chromocenter Type" of staining in interphase nuclei possess "Interstitial Type" of staining in prophase chromosomes and (iii) the "Simple Chromocenter Type" of staining in interphase nuclei had "Gradient Type" of staining in the prophase chromosomes. The interphase nuclei and prophase chromosomes of BC-6 and BC-9 showed "Simple Chromocenter Type" and "Gradient type", respectively. These features were as per expectation. However, the other varieties did not follow the usual features proposed by Tanaka (1971). The probable reasons for the disagreement of Tanaka's (1971) proposal regarding the staining property of the interphase nuclei and prophase chromosomes might be due to the presence of facultative heterochromatins. The heterochromatin might be condensed in the interphase nuclei and then diffused along the prophase chromosomes instead of localized in a particular region (Afroz *et al*. 2013, Sultana and Alam 2016).

The above findings indicated that although the nine varieties of chickpea possessed symmetrical karyotype differed sharply in respect of staining property of interphase nuclei and prophase chromosomes. It further indicated that Tanaka's (1971) above mentioned characterization of the interphase nuclei and the prophase chromosomes may not be applicable in these chickpea varieties.

A number of brightly stained CMA-positive bands were found throughout the interphase nuclei of nine varieties. Among the bands two were prominent and distinguishable from other especially in BC-1, BC-2, BC-4, BC-5, BC-6 and BC-9 (Figs. 19b - 27b). Many DAPI-positive bands were found throughout the interphase nuclei of nine chickpea varieties (Figs. 19c - 27c). These bands were brighter than those of CMA.

The prophase chromosomes of nine chickpea varieties were brightly and uniformly stained with CMA. Two bright and distinguishable CMA-positive dots were found in every prophase stage of these varieties (Figs. 19e - 27e, arrow). Several DAPI-positive bands were distributed

within the prophase chromosomes of these varieties (Figs. 19f - 27f). The DAPI bands were different in respect of number, size and location among the varieties.

A bigger DAPI band indicates that these varieties had more AT-repeats than GC-repeats (Schweizer 1976). Moreover, numbers of DAPI bands in the interphase nuclei were more than in the prophase chromosomes. This finding revealed that the AT-rich repeats aggregated in the prophase chromosomes during the contraction of chromatin as cell cycle proceeded.

The foregoing discussion revealed that in spite of similar karyotype, the nine chickpea varieties could be differentiated with differential staining property of interphase nuclei and prophase chromosomes.

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References

Afroz M, Sultana SS and Alam SkS 2013. Karyotype and RAPD analysis of three morphological forms of *Alocasia fornicata* (Roxb.) Schott. Cytologia **78**(3): 1-7.

Ahmad F 2000. A comparative study of chromosome morphology among the nine annual species of *Cicer* L. Cytobios **101**: 37-53.

Ahmad F and Hymowitz T 1993. The fine structure of chickpea (*Cicer arietinum* L.) chromosomes as revealed by pachytene analysis. Theor. Appl. Genet. **86**: 637-641.

Alam SkS and Kondo K 1995. Differential staining with Orcein, Giemsa, CMA and DAPI for comparative chromosome study of 12 species of Australian *Drosera* (Droseraceae). Amer. J. Bot. **82**(10): 1278-1286.

Cattani MV and Papeschi AG 2004. Nucleolus organizing regions and semi-persistent nucleolus during meiosis in *Spartocera fusca* (Thunberg) (Coreidae, Heteroptera). Hereditas **140**: 105-111.

Dixit PD 1932. Studies in Indian pulses. A note on the cytology of 'Kabuli and Desi' gram types. Indian j. Agric. Sci. **2**: 385-390.

Fedorov 1969. Chromosomes number of flowering plants, ed. Fedorov. ANA Academy of science of the USSR. V.L. Komarov Botanical Institute.

Galasso I and Pignone D 1992. Characterization of chickpea chromosomes by banding techniques. Genet. Res. Crop Evol. **39**: 115-119.

Hejazi SMH 2011. Karyological study on three *Cicer* L. species (Fabaceae) in Iran. Asian J. Cell Biol. **6**(3): 97-104.

Hizume M, Abe KK and Tanaka A 1988. Fluorescent chromosome bandings in the Taxodiaceae. La Kromosomo **11-50**: 1609-1619.

Kondo T and Hizume M 1982. Banding for the chromosomes of *Cryptomeria japonica* D. Don. Japan J. Forest. Soc. **64**: 356-358.

Meenakshi G and Subramaniam MK 1966. Tetrasomy in roots of *Cicer arietinum* L. Proc. Indian Acad. Sci. Sect. **66**:192-199.

Mukherjee S and Sharma AK 1987. Structure and behaviour of chromosomes in the strains of *Cicer arietium* L. Cytologia **52**: 707-713.

Ocampo B, Venora G, Ericco A, Singh KB and Saccardo F 1992. Karyotype analysis in the genus *Cicer*. J. Genet. Breed. **46**: 229-240.

Oke JG 1955. Natural tetraploidy in *Cicer arietinum* L. Proc. Indian Acad. Sci. Sect. B. **41**:220-226.

Phadnis BA and Narkhede MN 1972. Chromosome behavior in colchicines induced autotetraploids of *Cicer arietinum* L. Cytologia **37**: 415-421.

Schweizer D 1976. Reverse fluorescent chromosome banding with Chromomycin and DAPI. Chromosoma **58**: 307-324.

Sen NK and Jana MK 1956. A spontaneous autotetraploid gram (*Cicer arietinum* L.). Curr. Sci. **25**: 231-232.

Sharma PC and Gupta PK 1986. Cytogenetics of legume genera *Cicer* L. and *Lens* L. *In:* genetics and crop improvement, Gupta PK and Bahl JR (Eds.). Rastogi and Co. Meerut, India, 321-340 pp.

Sultana SS and Alam SkS 2016. Karyomorphology of eleven varieties of *Gossypium hirsutum* L. Bangladesh J. Bot. **45**(1):151-159..

Sultana SS and Alam SkS 2007. Differential fluorescent chromosome banding of *Solanum nigrum* and *Solanum villosum* from Bangladesh. Cytologia **72**: 213-219.

Stebbins GL 1971. Chromosomal evolution in higher plants (1st Eds) Edward Arnold Ltd., London. pp. 219.

Tanaka R 1971. Types of resting nuclei in Orchidaceae. Bot. Mag. Tokyo **84**: 118-122.

Tayyar RI, Lukaszewski AJ and Waines JG 1994. Chromosome banding patterns in the annual species of *Cicer*. Genome **37**: 656-663.

Zirkle C 1928. Nucleus in root tip mitosis in *Zea mays*. Bot. Gaz. **86**: 402-418.

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