Bangladesh J. Bot. **45**(5): 1251-1254, 2016 (December) **- Short communication**

KARYOMORPHOLOGY OF *PEPEROMIA PELLUCIDA* (L.) H. B. & K.

MAHIN AFROZ, SYEDA SHARMEEN SULTANA AND SHEIKH SHAMIMUL ALAM*

Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh

Key words: Karyomorphology, Fluorescent staining, *Peperomia pellucida*

Abstract

The present study deals with the karyomorphological features of *Peperomia pellucida* (L.) H. B. & K. after differential staining with orcein, CMA and DAPI. The interphase nuclei and prophase chromosomes were stained homogeneously with orcein. This species was found to possess 2n = 46 metacentric chromosomes. The total length of 2n chromosome complement was 148.81 µm. Individual chromosome length ranged from 2.63 to 4.31 µm. The relative length of each chromosome ranged from 0.02 to 0.03. No CMA-band was found in this species. Two similar sized bright and prominent DAPI positive bands were observed in each and every interphase, prophase and metaphase stages indicating persisting nature of these AT-rich repeats. In addition, five DAPI-positive bands were observed at different locations of metaphase chromosomes. Except 2n chromosome number the karyomorphological and fluorescent banding information probably is the first report for this species and it may help to enhance the chromosomal data base of this plant species in Bangladesh.

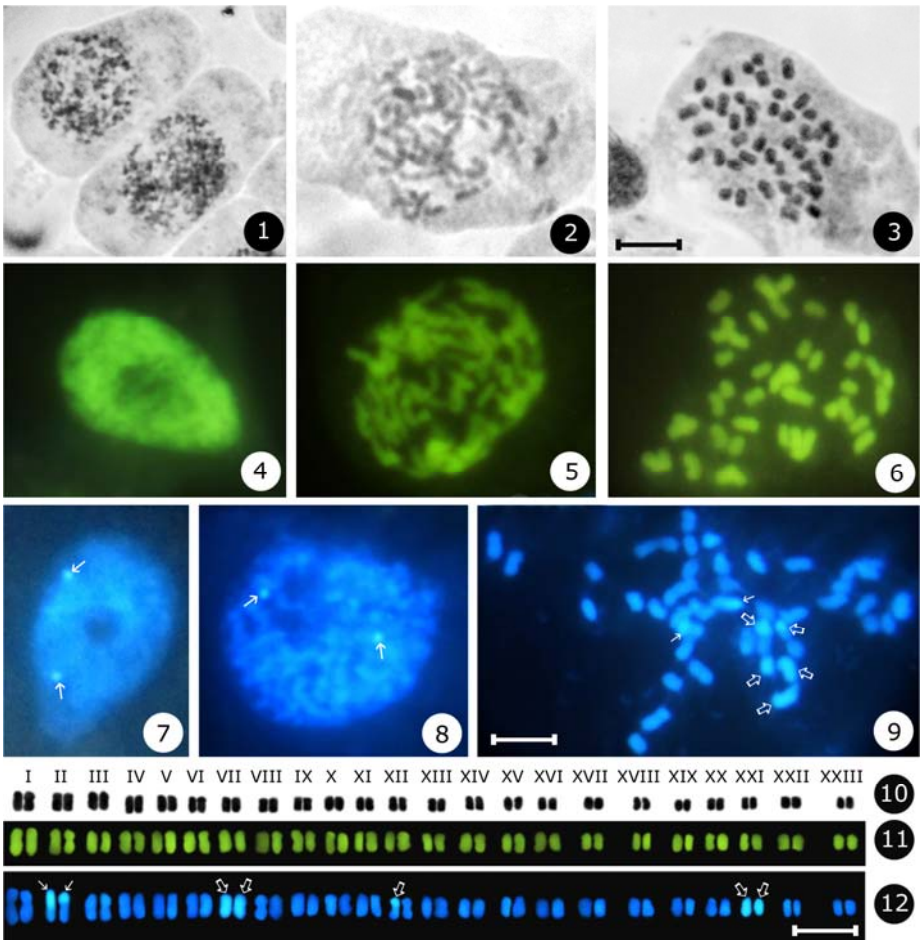
Peperomia is a pantropical genus with about 1500 - 1700 species distributed worldwide. About 90% of *Peperomia* species are from Neotropical regions, followed by Southeast Asia, Africa, Australia and New Zealand (Wanke *et al.* 2006). The majority of *Peperomia* species are epiphytic and a few terrestrial. The genus occurs mostly in humid and shady forests, and less frequently in dry forests and grasslands (Mathieu *et al.* 2011). In Bangladesh, this genus is represented by a single species *Peperomia pellucida* (L.) H. B. & K. (Ahmed *et al.* 2009).

Although *Peperomia pellucida* has some economical, medicinal and ethnobotanical values, no report about the genetic information is available in Bangladesh (Ahmed *et al.* 2009). A few workers from different parts of the world tried to characterize this species with classical karyotype analysis but their work was confined mainly to 2n chromosome count (Kumar and Subramanium 1986, Brown 1908, Sobti and Singh 1961, Sharma and Bhattacharyya 1958, Mathew 1958, Harvey 1966, Smith 1966). Except 2n chromosome number no karyomorphological information is available for this species. Moreover, there is strong disagreement regarding 2n chromosome number. Therefore, detail karyomorphological information of *P. pellucida* is necessary to construct a chromosome data base in Bangladesh.

The plant materials were collected and maintained in the Botanic garden, Department of Botany, University of Dhaka. Healthy roots were pretreated with 0.002 M 8-hydroxyquinoline for 3 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, Alam and Kondo´s (1995) method was followed with slight modification. After hydrolyzing and dissecting, the materials were squashed with 45% acetic acid. The cover glasses were removed quickly on dry ice and allowed to air dry for at least 24 hrs before study. The air-dried slides were first pre-incubated in McIlvaine's buffer (pH 7.0) for 30 m followed by distamycin A (0.1 mg/ml) treatment for 10 m. The slides were rinsed mildly in McIlvaine's buffer supplemented with $MgSO_4$ (5 mM) for 15 m. One drop of CMA (0.1 mg/ml) was added to the materials for 15 m in a humid chamber and then

*Author for correspondence: <ssalam81@yahoo.com>.

rinsed with McIlvaine's buffer with $MgSO_4$ for 10 m. 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 Mcllvaine's buffer (pH 7.0) for 30 m and treated in actinomycin D (0.25 mg/ml) for 10 m in a humid chamber. The slides were immersed in DAPI solution (0.01 mg/ml) for 20 m and mounted with 50% glycerol. These were observed under a Nikon (Eclipse 50i) fluorescent microscope with UV filter cassette.



Figs 1-12. Different stages of mitotic cell division in *Peperomia pellucida* stained with orcein, CMA and DAPI. 1. Orcein-stained interphase nuclei, 2. Orcein-stained prophase chromosomes, 3. Orcein-stained metaphase chromosomes, 4. CMA-stained interphase nuclei, 5. CMA-stained prophase chromosomes, 6. CMA-stained metaphase chromosomes, 7. DAPI-stained interphase nuclei, 8. DAPI-stained prophase chromosomes, 9. DAPI-stained metaphase chromosomes, 10. Karyotype prepared from orcein-stained metaphase chromosomes, 11. Karyotype prepared from CMA-stained metaphase chromosomes, 12. Karyotype prepared from DAPI-stained metaphase chromosomes, → = Persistent DAPI-band, → = DAPI-band, Bar = 10 µm.

In this study, the interphase nuclei and prophase chromosomes were stained homogeneously with orcein indicating equal distribution of heterochromatins. According to Tanaka (1971), the interphase nuclei and prophase chromosomes of this species could be regarded as "Diffuse Type" and "Continuous Type", respectively.

Peperomia pellucida was found to possess 2n = 46 chromosomes (Figs. 3 and 10). Similar chromosome number for this species was reported earlier by Sharma and Bhattacharyya (1958). Besides, different chromosome numbers were also reported for this species, such as 2n = 20 - 24 (Brown 1908), 2n = 22 (Sobti and Singh 1961, Sharma and Bhattacharyya 1958) and 2n = 44 (Mathew 1958, Harvey 1966, Smith 1966). There are two probable reasons for the disagreement regarding chromosome number of *P. pellucida*, such as (i) high degree of eu- and aneuploidy tendency and (ii) existence of different cytotypes.

This species was found to possess all metacentric chromosomes with a centromeric formula of 46m (Fig. 10, Table 1). The total length of 2n chromosome complement was 148.81 µm. Individual chromosome length ranged from 2.63 to 4.31 µm. The relative length of each chromosome ranged from 0.02 to 0.03 (Table 1). In this species, the range of chromosomal length was almost negligible i.e. distance between the smallest and largest chromosomes was about 2 µm (Table 1). As a result, no gradual decrease of chromosomal length was observed in its karyotypes (Table 1). These features indicated that *P. pellucida* has a strict symmetric karyotype. Stebbins (1971) mentioned that the symmetric karyotypes indicate primitive character and from that point of view *P. pellucida* is a plant of primitive nature.

Table 1. Karyomorphological features of *Peperomia pellucida* after differential staining.

2n	Total chromatin length (µm)	Range of individual chromosomal length (µm)	Range of relative length	Centromeric formula	No. of CMA-bands	% of GC-rich repeats	No. of DAPI-bands	DAPI-banded region (µm)	% of AT-rich repeats
46	148.81	2.63-4.31	0.02-0.03	46m	-	-	7	17.64	11.85

m = Metacentric chromosome.

No band was found in interphase nuclei, prophase chromosomes and metaphase chromosomes after CMA-staining revealing the lack of GC-rich repeats in the genome of this species (Schweizer 1976) (Figs. 4, 5, 6, 11). The total length of DAPI-banded region was 17.64 µm which occupied about 11.85% of the total chromatin length. Two bright and prominent DAPI positive bands were observed in each and every interphase, prophase and metaphase stages revealing the persisting nature of AT-rich repeats (Figs. 7, 8, 9, 12, → = Persistent DAPI-band). Since the size of the two bands were almost similar (~1.5 µm) in every stage of cell division, the species keeps this AT-rich domain intact in spite of high condensation at metaphase. Persistent CMA-positive band was reported earlier in different plant species such as in *Corchorus* spp. (Khatun and Alam 2010), *Coccinia grandis* (Hossain *et al.* 2016), etc. However, persistent DAPI-positive band in *P. pellucida* is probably the first report.

In addition five DAPI-positive bands were observed at different location of metaphase chromosomes (Figs. 9, 12). Both the members of chromosome pair II had bright DAPI-bands at whole short arm. Entirely DAPI-fluoresced bands were observed in both the homologue members of chromosome pair VII and XXI. DAPI-fluoresced large area indicated tandem duplication of AT-rich region. Heteromorphicity regarding banding pattern was found in chromosome pair XII where a member had terminal DAPI-band and its homologue showed no band (Fig. 12). The probable reason for heteromorphicity was due to deletion of AT-rich repeat from the respective location of the homologous chromosomes.

The detail karyomorphological information after differential staining is the first report for this species which may help to enhance the chromosomal data base of this plant species in Bangladesh.

Acknowledgement

The authors are thankful to Chromosome Research Centre, University of Dhaka, Bangladesh for providing the necessary facilities during this research work.

References

Ahmed ZU, Hasan MA, Begum ZNT, Khondker M, Kabir SMH, Ahmed M and Ahmed ATA 2009. Encyclopedia of flora and fuana of Bangladesh. Angiosperms: Dicotyledons (Magnoliaceae-Punicaceae). Asiat. Soc. Bangladesh **9**: 371-372.

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.

Brown WH 1908. The nature of the embryo sac of *Peperomia*. Bot. Gaz. **46** (12): 445-460.

Harvey BL 1966. Natural and artificial alloploids with 22 pairs of chromosomes in the genus *Carthamus* L. Dissertation abstracts, Ser. B. **27**(2): 365.

Hossain MU, Islam M, Afroz M, Sultana SS and Alam Sk S 2016. Karyotype and RAPD analysis male and female *Coccinia grandis* L. from Bangladesh. Cytologia **81** (3): 349-355.

Khatun M and Alam Sk S 2010. Conformation of species status of *Corchorus trilocularis* by differential chromosome banding and isozyme assay. Cytologia **75** (1): 83-88.

Kumar V and Subramanium B 1986. Chromosome atlas of flowering plants of the Indian subcontinent, Dicotyledons. Botanical survey of India, Calcutta **1**: 464.

Mathew PM 1958. Studies on Piperaceae. J. Indian Bot. Soc. **37**(1): 155-171.

Mathieu G, Symmank L, Callejas R, Wanke S, Neinhuis C, Goetghebeur P and Samain M 2011. New geophytic *Peperomia* (Piperaceae) species from Mexico, Belize and Costa Rica. Revista Mexicana de Biodiversidad **82**: 357-382.

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

Sharma AK and Bhattacharyya NK 1958. Chromosome studies on the two genera of the family Piperaceae. Genetica **29**(5-6): 256-289.

Smith JB 1966. Chromosome number in *Peperomia* Ruiz et Pav. (Piperaceae) and a note on chromosome number of *Piper magnificum* Trelease. Kew Bull. **20**(3): 521-528.

Sobti SN and Singh SD 1961. A chromosome survey of Indian medicinal plants (Part-1). Proc. Indian Acad. Sci., Sect. B. **54** (3): 138-144.

Stebbins GL 1971. Chromosomal evolution in higher plants. Addison-Wesley Publishing Company, California, USA. pp. 208.

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

Wanke S, Samain MS, Vanderschaeve L, Mathieu G, Goetghebeur P and Neinhuis C 2006. Phylogeny of the genus *Peperomia* (Piperaceae) inferred from the trnK/matK region (cpDNA). Pl. Biol. **8**: 93-102.

(Manuscript received on 24 October, 2016; revised on 31 October, 2016)