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ORCEIN AND FLUORESCENT BANDING ANALYSIS OF TWO FLORAL TYPES OF *CATHARANTHUS ROSEUS* L.

TANUSREE DATTA, MEGHLA SAHA PINKY, CHANDAN KUMAR DASH[1] AND KAZI NAHIDA BEGUM*

Department of Botany, Jagannath University, Dhaka-1100, Bangladesh

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Abstract

Two floral types of *Catharanthus roseus* L. *viz.* pink and white were studied through differential staining with orcein, CMA and DAPI for cytogenetical characterization and to assist towards updating their taxonomical status and evaluating chromosomal diversity between them. "Simple Chromocenter Type" of interphase nuclei was observed with some darkly stained small hetero-chromatic regions throughout the nuclei. Most of the prophase chromosomes of *Catharanthus roseus* (pink and white) were "Continuous Type" and a few were "Gradient Type". Although these two floral types possessed 16 metacentric chromosomes in somatic cells, they showed variation in fluorescent banding pattern considering the modification of GC- and AT-rich repetitive segments. Taking into account all the parameters of both the floral types of *C. roseus* showed strict symmetric karyotype as well as primitive nature. Therefore, the combined data of differential staining provide information to make comments on their chromosomal status with cytogenetical characterization and also create a baseline for future research.

Introduction

Catharanthus roseus L., (Synonyms: *Vinca rosea* L., *Pervinca rosea* L., *Ammocallis rosea* L. and *Lochnera rosea* L.) commonly known as periwinkle belongs to Apocynaceae is an evergreen, ever blooming, herbaceous and important medicinal plant as well as floral species in horticulture(1). The word '*Catharanthus;* derived from the Greek language meaning 'pure flower' while *'roseus*' from Latin means red, rose or rosy(2).

Generally, two floral types of *C. roseus* are available which are named on the basis of their flower color i.e. with pink flower and reddish stems 'Rosea' and with white flower and green stems 'Alba', often found growing sympatrically(3). In Bangladesh, both floral types of *C. roseus* L. are available which is commonly known as ‘Nayantara’.

*Author for correspondence: <kazinahida@yahoo.com>. [1]Department of Botany, University of Dhaka, Dhaka-1000, Bangladesh.

Catharanthus roseus is common in many tropical and subtropical regions throughout the world as a potential source of alkaloids[1]. This plant possesses many phytochemical constituents such as polyphenols, steroid, irridoid glucosides, flavonoid glucosides, carbohydrate, saponin and alkaloids. More than 400 alkaloids are present in different parts of this plant, which are used as pharmaceuticals, agrochemicals, flavor and fragrance, ingredients, food additives and pesticides. However, among all the alkaloids, only five consisting of vinblastine, vincristine, 3′, 4′-anhydrovinblastine, serpentine and ajmalicine are marketed[4]. *C. roseus*, which is also known as "an anticancerous drug yielding plant" and this effectively treats diabetes, malaria, dengue fever, dysentery, insect bites, skin infection, diarrhea, leukemia, eye irritation, dyspepsia, toothache, sore throat and lung congestion[1,5,6]. Numerous researches have been initiated in order to increase the content of alkaloids and other biologically active constituents by producing new variants with enhanced bio-productivity. So, in this case authentic identification and genetical characterization is very important to carry out any breeding program.

These two floral types of *C. roseus* remain under taxonomic dilemma and also untouched for cytogenetical investigation in Bangladesh. Previous studies provided data mostly on chromosome counts of *C. roseus* which is not enough to provide detailed genomic information[7,8]. Karyosystematics is well established as important features in determination of the genetic relationship and degree of divergence among species or populations. Fluorescent chromosome banding is an excellent tool for karyotype analysis and provide information regarding the distribution of AT (Adenine - Thymine)- and GC (Guanine - Cytosine)-rich repeats in the genome[9]. Staining with DNA-base specific banding with fluorochromes such as chromomycin A_3 (CMA) and 4′, 6-diamidino-2-phenylindole (DAPI) is relatively modern method for karyotype study. CMA binds with GC-rich repetitive sequences of the genome and gives characteristics yellow colour bands. On the other hand, DAPI binds with AT-rich repeats shows characteristic blue colour bands[9]. The nature of staining properties of interphase nuclei and prophase chromosomes are generally regarded as karyomorphological parameter. Tanaka (1971) classified different types of interphase nuclei and prophase chromosomes on the basis of heterochromatin condensation[10]. The combined karyomorphology and fluorescent banding analysis enable to characterize even different forms and varieties of a species. Therefore, the present study aimed at investigating different cytological characters to revise the taxonomic status and for characterization of two foral types of *Catharanthus roseus* available in Bangladesh.

Materials and Methods

The two floral types of *Catharanthus roseus* L. i.e. pink flower and white flower type were investigated. The specimens were collected from and maintained in the Botanical garden, Department of Botany, Jagannath University, Dhaka.

The young healthy roots were collected and pretreated with 0.002 M 8-hydroxy-quinoline for 2 hrs 30 min at room temperature followed by 15 min fixation in 45% acetic acid at 4°C. These were then hydrolyzed in a mixture of 1 N HCl and 45% acetic acid (3 : 1) at 60°C for around 20 min. The root tips were stained and squashed in 1% aceto-orcein. These were observed under a Nikon Eclipse 100. For CMA- and DAPI-banding, Alam and Kondo´s[9] method was used with slight modification. After hydrolyzing and dissecting, the materials were tapped and squashed with 45% acetic acid and the cover glasses were removed quickly 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 min followed by distamycin A (0.1 mg/ml) treatment for 10 min. The slides were rinsed mildly in McIlvaine's buffer supplemented with 5 mM $MgSO_4$ for 15 min. One drop of CMA (0.1 mg/ml) was added and incubated for 1 hr in a humid chamber and then rinsed with McIlvaine's buffer containing 5 mM $MgSO_4$ for 10 min. Slides were mounted in 50% glycerol and kept at 4°C for overnight before observation 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 25 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 1 hr and mounted with 50% glycerol and observed under a Nikon (Eclipse 50i) fluorescent microscope with UV filter cassette.

Centromeric formula was determined based on the position of centromere and the number of chromosomes[11]. Different karyomorphological parameters, asymmetry and symmetry indexes were calculated, such as range of chromosomal length, total chromosome length, average chromosome length, total form (TF%) percentage[12], karyotype asymmetry index (AsK%)[13], karyotype symmetry index (Syi%)[14], degree of asymmetry of karyotypes (A)[15] and karyotypes category[16].

Results and Discussion

Although a few dividing cells were observed throughout the year, maximum number of dividing cells could be found in the root tip cells (RTCs) studied during May to August (about 60%). The number of dividing cells was very poor in extreme high or low temperature.

Some darkly stained small heterochromatic regions were found with orcein-stained interphase nuclei of both pink and white floral types of *C. roseus* and 9 - 14 sharp CMA and DAPI band were observed under fluorescent banding. According to earlier reports, these features showed "Simple Chromocenter Type" of staining property in the interphase nuclei[9]. Here, no nucleolus was observed in both types of *C. roseus* (Fig. 1).

The prophase chromosomes of the pink and white floral types were stained homogenously along the entire length i.e. "Continuous Type" and a few chromosomes

showed "Gradient Type" where chromosomes were darker in one end and gradually faint to the other end. These might be due to the more aggregation of heterochromatins on those specific locations (Fig. 1). The findings indicated that the heterochromatins were aggregated in the interphase nuclei and then homogenously or gradually distributed in the prophase chromosomes.

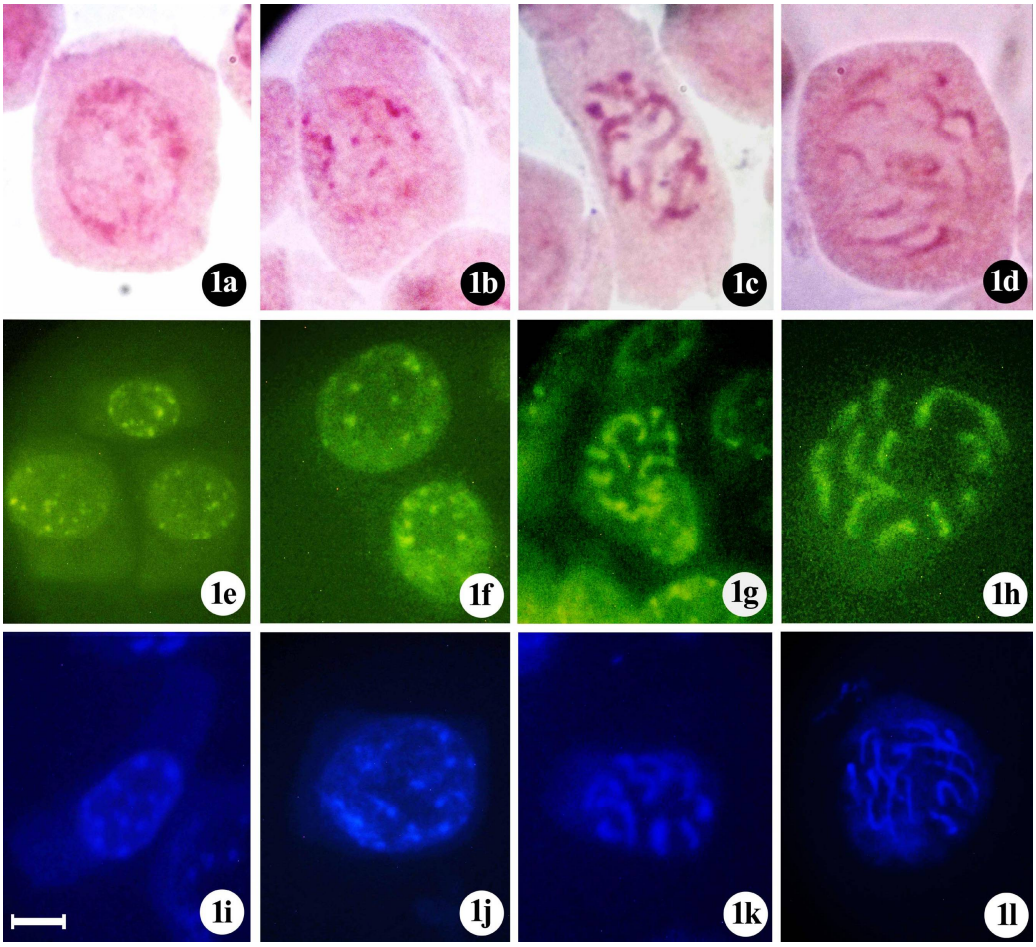

Fig. 1. Interphase nuclei and prophase chromosomes of two floral types. Orcein-stained interphase (1a) and prophase (1c), CMA-stained interphase (1e) and prophase (1g), DAPI-stained interphase (1i) and prophase (1k) of pink type. Orcein-stained interphase (1b) and prophase (1d), CMA-stained interphase (1f) and prophase (1h), DAPI-stained interphase (1j) and prophase (1l) of white type. Bar = 10 µm.

According to the recorded chromosome number, different types or varieties of *C. roseus* are characterized by the basic chromosome number i.e., x = 8[17-19]. The diploid chromosome number 2n = 2x = 16 was observed in both flower types of *C. roseus* in this investigation (Fig. 2). Similar diploid chromosome numbers were reported earlier which

correlates to the present study[7,8,20-23]. The consistency in the number of diploid chromosome complement indicated that this species is stable. Considering basic chromo-

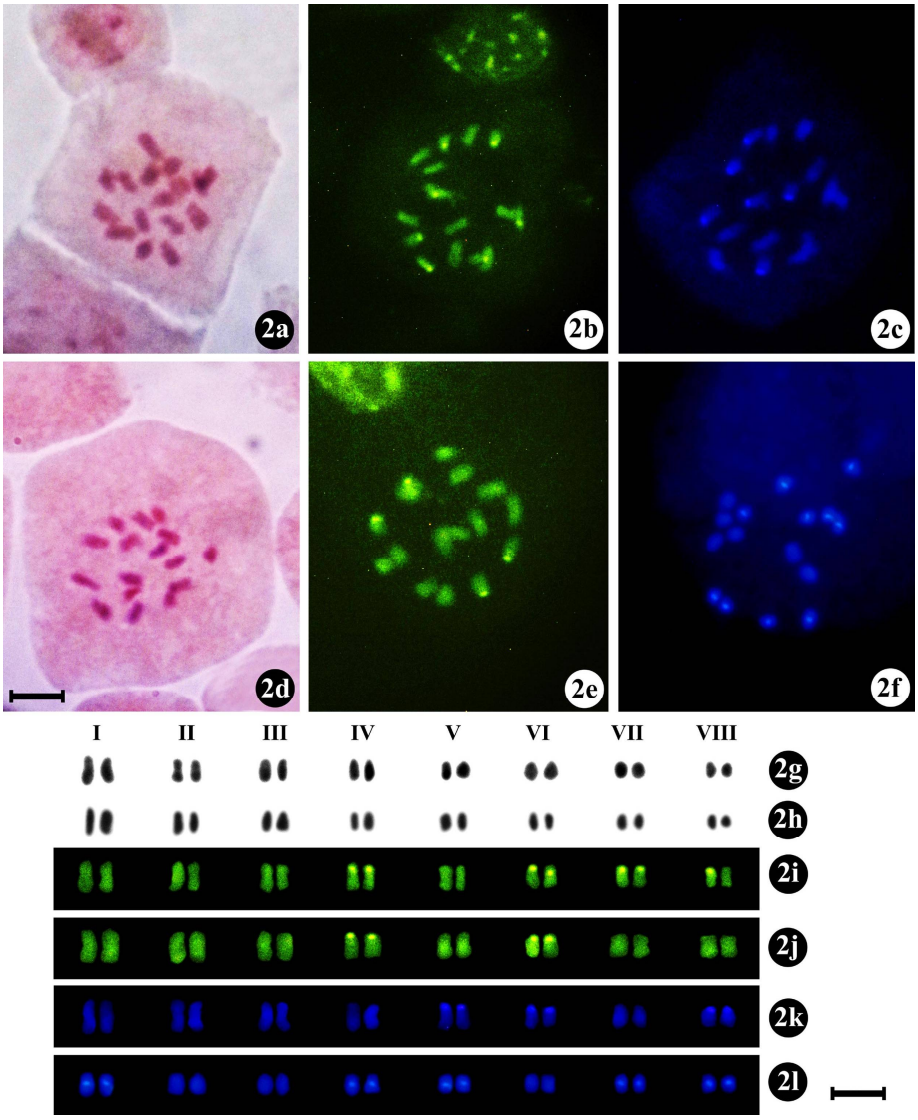

Fig. 2. Metaphase chromosomes and karyotypes of two floral types. Orcein-stained (2a, g), CMA-stained (2b, i) and DAPI-stained (2c, k) metaphase and karyotype plates of pink type. Orcein-stained (2d, h), CMA-stained (2e, j) and DAPI-stained (2f, l) metaphase and karyotype plates of white type. Bars = 10 µm.

-some number x = 8, the somatic chromosome number 2n = 24 and 32 reported for this species might be triploid and tetraploid cytotype, respectively[20,24,25]. Moreover, a few abnormal cells with 2n = 12 and 14 chromosomes were also reported for this species, which did not correlate with the present findings[24]. Some previous workers reported the

16 chromosomes of pink and white flower types with the different centromeric positions[21,22]. However, in the present study, 16 metacentric chromosomes were found in both pink and white flower type of *C. roseus* (Fig. 2). Karyotype having large number of metacentric chromosomes is usually regarded as symmetric type. Stebbins (1971) mentioned that symmetric karyotypes are of primitive features[16]. Therefore, both pink and white floral types of *C. roseus* possessed primitive karyotype in evolutionary point of view.

The total chromosomal and the average chromosomal length were found almost similar in pink and white floral types (43.39 and 2.71 µm; 43.54 and 2.72 µm, respectively). The range of chromosomal length was 2.05 - 3.21 µm in pink flower type and 2.26 - 3.42 µm in white flower type (Table 1). Difference between small and large chromosome was more than about 1 µm (Table 1).

Table 1. Differences in the karyomorphological parameters between the pink and white floral types.

Karyotype parameters	Pink type	White type
Orcein-stained karyotype		
Centromeric formula	16 m	16 m
Range of chromosomal length (µm)	2.05 - 3.21	2.26 - 3.42
Total chromosome length (µm)	43.39 ± 4.13	43.54 ± 0.89
Average chromosome length (µm)	2.71	2.72
Total Form (TF) value (%)	47.80	48.51
Karyotype asymmetry index (%)	52.20	51.49
Karyotype symmetry index (%)	91.57	94.20
Degree of asymmetry of karyotype (A)	0.05	0.03
Karyotype category	1A	1A
CMA-stained karyotype		
Number of CMA-band	7	4
Total CMA-banded portion	6.49	3.79
Percentage of CMA-banded portion (%)	14.96	8.70
DAPI-stained karyotype		
Number of DAPI-band	6	10
Total DAPI-banded portion	4.90	6.54
Percentage of DAPI-banded portion	11.29	15.02

m = Metacentric chromosome.

Karyotype symmetry index (Syi%) was somewhat lower in the pink flower type (91.57%) than in the white flower type (94.20%) of the plant. Whereas, karyotype asymmetry index **(**AsK%) was little higher in pink flower type (52.20%) than the white

flower type (51.49%) (Table 1). The total form percentage (TF) was more or less similar (47.80 for pink flower type and 48.51 for white flower type) for both types. The degree of karyotype asymmetry (A) is another parameter where the value was comparatively higher in pink flower type (0.05) than the white flower type (0.03). Karyotype category based on Stebbins's classification, "1A" karyotype was found for both pink and white floral types (Table 1)[16]. When the ratio of largest and smallest chromosome is less than 2 : 1, then it belongs to subtype 'A'[16]. As pink and white flower types followed this ratio and both of them were categorized as "1A" degree of difference (Table 1).

After fluorescent banding, seven terminal CMA-bands were observed in pink floral type on the short arms of both chromosomes of pair IV, VI, VII and one chromosome of pair VIII. Possible heteromorphicity occurred in chromosome pair VIII where one chromosome had terminal band and its homologue did not show any band which might be due to the deletion of banded portion from the respective chromosome. The total CMA-banded portion was 6.49 µm that occupied 14.96% of the total chromosome complements. In white floral type, four CMA-bands were found and those were observed at the terminal regions of the short arm of chromosome pair IV and VI. About 8.70% of the total somatic chromosome length was CMA-banded regions. In case of DAPI-banding, six and ten DAPI-bands were observed in pink and white floral types, respectively. In pink floral type, the DAPI-banded regions were at the terminal regions of the short arms (chromosome pair V, VI and VIII) while all the DAPI-bands were present at the centromeric regions in white floral type (chromosome pair I, IV, V, VII and VIII). The total length and percentage of the DAPI-banded regions were 4.90 µm and 11.29 in pink floral type whereas these were 6.54 µm and 15.02 in white floral type, respectively.

In spite of showing distinctiveness in fluorescent banding pattern, these two floral types had similar karyomorphology along with more or less similar total chromosome length and centromeric formula. Considering the above findings, it can be concluded that pink and white floral type possessed similar genome with some modification of banded regions (GC- and AT-rich repetitive segments). Therefore, the combined data of differential staining will be helpful for cytogenetical characterization and further investigation.

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