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FLUORESCENT KARYOTYPE ANALYSIS OF FOUR *ALOE* SPECIES

SHEIKH SHAMIMUL ALAM[1] AND NAHIMA KHANAM

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

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Abstract

Four *Aloe* species namely *A. zebrina*, *A. abyssinica*, *A. vera* and *A. indica* possess 2n = 14 including eight large and six small chromosomes. In *A. zebrina* two CMA-positive bands were at the terminal region in pair IVand two proximal bands at both the members of pair VI. *A. abyssinica* had two CMA-positive bands, one on a member of pair IV and the other on a member of pair VI. Two CMA-negative bands were found in *A. indica* and three such bands in *A. vera*. CMA-negative band regions of these chromosomes showed DAPI-positive bands. *A. zebrina* possess 14 DAPI-positive bands one on each chromosome. Ten and seven DAPI-positive bands were observed in *A. indica* and *A. vera*, respectively. The AT-rich areas were 12.88 μm in *A. zebrina*, 10.9 μm in *A. indica* and 6.3 μm in *A. vera*. The AT-rich areas of these three species were directly proportional to the number of bands. CMA- and DAPI-banding properties indicated the probable involvement of GC- and AT-rich base sequences in karyotypic diversification of these four *Aloe* species.

Introduction

The genus *Aloe* belongs to the tribe Aloineae, family Liliaceae. All the members of this genus possess 2n = 14 acrocentric chromosomes with a distinct bimodal karyotype, in which eight chromosomes are large and six are small (Brandham 1973, 1975, Sharma and Chatterji 1958). Two other genera of this tribe *viz. Gasteria* and *Haworthia* possess similar karyotypes like *Aloe* (Sapre 1977, Darlington and Kefallinou 1957, 1974, Vosa and Bayer 1986). It seems that the structural chromosomal aberrations in this tribe did not affect the morphology of the karyotypes.

Fluorescent staining with chromomycin A_3 (CMA) and 4-6 diamidino-2-phenyl indole (DAPI) have been used for comparative karyotype analysis of *Drosera* spp. (Alam and Kondo 1995, Alam *et al.* 1996). Fluorescent staining method effectively reveal morphological differences in the karyotypes. Moreover, by the appearence of the CMA- and DAPI-bands (positive and negative), it is possible to measure the difference in the amount and site of GC- and AT-rich base pairs on the chromosome complements and different genomes can be distinguished.

The present investigation was undertaken to study the karyotype of four *Aloe* species after staining with orcein and fluorochromes (CMA and DAPI).

Materials and Methods

Four *Aloe* speices namely *A. abyssinica* Lam., *A. zebrina* Baker, *A. indica* Royle and *A. vera* L. were selected for this study. Plants were collected from Baldha Garden, Dhaka and maintained in the Botanical Garden, Department of Botany, Dhaka University. The root tips (RTs) were pretreated with 0.002 M 8-hydroxyquinoline for 3.5 h at 25 – 30^{0}C, then hydrolysed for 10 sec at 60^{0}C in a mixture of 1N HCl and 45% acetic acid (2 : 1). The RTs were squashed in 1% aceto-orcein.

[1]Corresponding author. e-mail: ssalam@bd.drik.net / botany@udhaka.net

After hydrolysis, the RT materials were squashed with 45% acetic acid. The cover glasses were removed quickly and air dried for at least 48 h before study. The slides were incubated in McIlvaine's buffer (pH 7.0) for ten minutes followed by distamycin treatment (0.1 mg/ml). Slides were mildly rinsed in McIlvaine's buffer supplemented with 5 mM $MgSO_4$ for ten minutes. One drop of CMA (0.1 mg/ml) was placed on each slide and kept for ten minutes, and the slides were mounted in 50% glycerol and kept overnight at 4^0C. The slides were examined under a Nikon UFX-IIA fluorescent microscope with blue-violet filter cassette.

For DAPI-staining, the air dried slides were incubated in McIlvaine's buffer (pH 7.0) for ten minutes and then treated in actinomycin D (0.25 mg/ml) for ten minutes. Slides were stained with DAPI (0.1 µg/ml) for 15 minutes, the slides were mounted with 50% glycerol. These were observed under a Nikon UFX-IIA fluorescent microscope with UV filter.

Results and Discussion

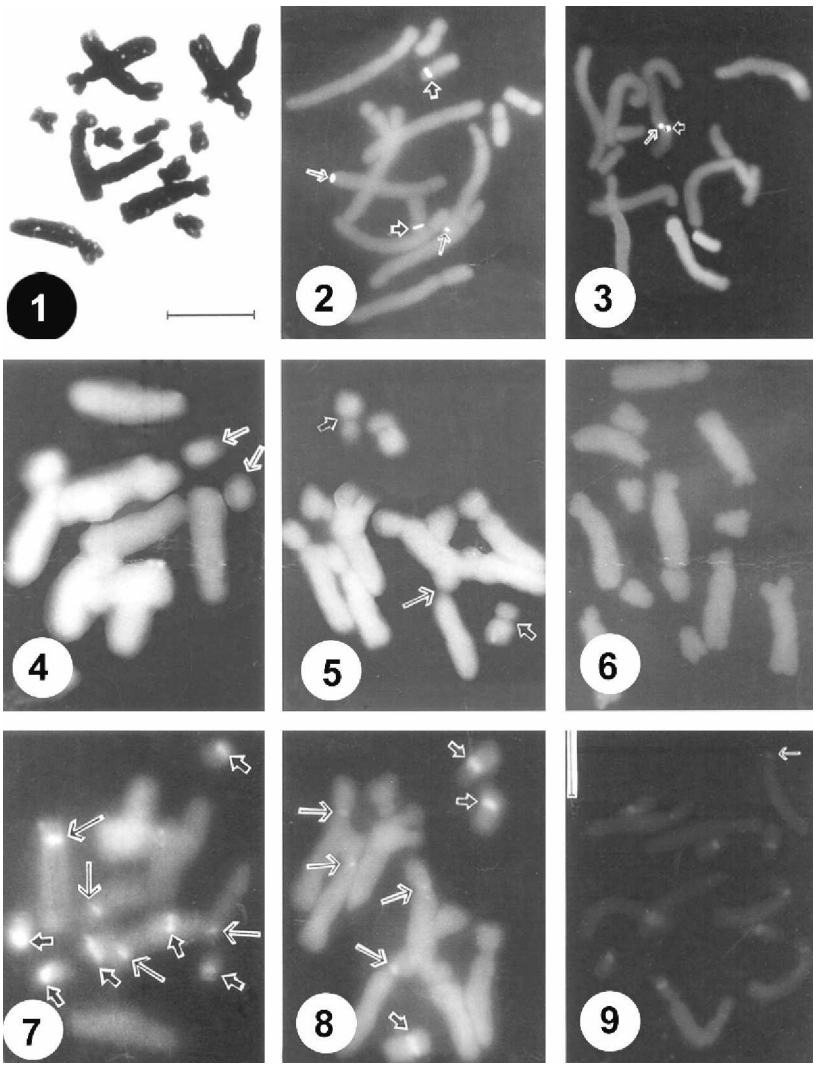
In *A. zebrina,* two large chromosomes of the pair IV showed one CMA-positive band at the terminal end of long arm (Fig.2). Both the members of the pair VI (small chromosome) possessed one CMA-positive band at the proximal region located between centromere and the short arm (Fig. 2). *A. abyssinica,* however, showed two CMA-positive bands, one at the terminal end of long arm in a member of pair IV (long chromosome) and the other at the proximal region of short arm in a member of pair VI (Fig. 3, big and small arrows, respectively). In *A. zebrina,* CMA-positive bands were present in two pairs of homologous chromosomes (pair IV and VI) (Fig. 2). On the other hand, it was found on one member of each of pair IV and VI in *A. abyssinica* (Fig. 3). Moreover, the location of bands was similar in these two species. Therefore, it is assumed that the two CMA-positve bands were deleted from *A. zebrina* and thus may evolve the karyotype of *A. abyssinica.* The total CMA-positive banded region was 3.60 µm in *A. zebrina* and 2.30 µm in *A. abyssinica* (Table 1). These differences in the number of CMA-positive bands and the amount of GC-rich areas may be related with their karyotypic diversification. No CMA-band was found in *A. indica* and *A. vera*.

Table 1. Comparative fluorescent banding in four *Aloe* species.

Species	No. of CMA-bands		Total CMA-band length (µm)	No. of DAPI bands		Total DAPI-banded length (µm)
	Positive	Negative		Positive	Negative	
Aloe abyssinica	2	–	2.30	–	–	–
A. zebrina	4	–	3.60	14	–	12.80
A. indica	–	2	–	10	–	10.90
A. vera	–	3	–	7	–	6.30

The CMA-stained slides of *A. indica* and *A. vera* were counter stained with DAPI. In the same slide the same two small chromosomes of *A. indica* showed CMA-negative band where the DAPI-positive bands had appeared (Figs. 4, 7). Since the location of CMA-negative bands was at the end, the chromosomes looked smaller as compared to DAPI-stained chromosomes. In *A. vera* two small and a large chromosomes showed CMA-negative bands exactly at the same location where DAPI-positive bands had appeared (Figs. 5, 8). Consequently, distinct gaps were found at

the centromeric regions of these chromosomes. The reversible bands indicates that these areas are fully composed of AT-rich base sequences (Alam and Kondo 1995).



Figs. 1-9: Differentially stained mitotic metaphase chromosomes of four *Aloe* species. 1. Orcein stained metaphase of *A. vera*. 2. CMA-stained metaphase of *A. zebrina*. 3. CMA-stained metaphase of *A. abyssinica*. 4. CMA-stained metaphase of *A. indica*. 5. CMA-stained metaphase of *A. vera*. 6. DAPI-stained metaphase of *A. abyssinica*. 7. DAPI-stained metaphase of *A. indica*. 8. DAPI-stained metaphase of *A. vera*. 9. DAPI-stained metaphase of *A. zebrina*. Bar = 10 µm. (In Figs. 2,3,7,8 arrows indicate positive bands, and in figs. 4 and 5 arrows indicate negative bands).

All fourteen chromosomes of *A. zebrina* possessed a DAPI-positive band revealing its AT-rich nature (Fig. 9). One such band was found in each four large and six small chromosomes of *A. indica* (Fig. 7), *A. indica* lack four bands in four large chromosomes. In *A. vera* four large and three small chromosomes showed a DAPI-positive bands (Fig. 8). Three bands from three small

chromosomes were missing in this species when compared with *A. indica*. Thus a gradual decrease of AT-rich sequences was found among the three species. The AT-rich areas were 12.80 μm in *A. zebrina*, 10.90 μm in *A. indica* and 6.30 μm in *A. vera* (Table 1). These differences in the number of bands and the length of AT-rich areas may be related to the number of DAPI bands. No DAPI-positive or negative band, however, was found in *A. abyssinica* (Fig. 6). Therefore, the distribution of GC- and AT-rich sequences might have been involved in diversification of these four *Aloe* species.

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