Pak. J. Bot., 46(5): 1731-1734, 2014.

KARYOLOGICAL STUDIES IN TEN DIFFERENT POPULATIONS OF DESERT LILY *ALOE VERA* FROM PAKISTAN

BUSHREEN JAHAN[1*], AHSAN A. VAHIDY[2], RAFAT SAEED[1] AND AMEER AHMED MIRBAHAR[3]

[1]*Department of Botany, Federal Urdu University of Arts, Science and Technology, Gulshan-e-Iqbal Campus, Karachi, Pakistan,* [2]*Department of Genetics, University of Karachi, Karachi, Pakistan,*
[3]*Biotechnology Wing, International Centre for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*
[*]*Corresponding author's e-mail:drbushreenjahan@yahoo.com*

Abstract

To enhance theoretical basis of *Aloe* feeding and provide cytological basement, the karyotype and morphology of mitotic chromosomes, ten different populations of *Aloe vera* collected from various geographical locations of Karachi, Pakistan were studied by aceto-orcein staining technique. The results showed that chromosome number of *Aloe vera* is 2n=14. The karyotype is bimodal and consists of 14 chromosomes (8 large and 6 small) predominantly with submedian, median and subterminal centromere. Average chromosome lengths among populations ranged from 7.95-2.36 µm.

Key words: Desert lily, *Aloe vera*, Karyotype, Mitosis.

Introduction

Plant chromosomes have traditionally been a fruitful material for almost every kind of cytogenetic research. Chromosomes represent the main focus of cytogenetics. One of the main challenges of modern-day cytogenetics is to unravel the relation between the genomic content and architecture of chromosomes and their genetic function. Plant species have provided an important contribution to the knowledge of chromosome evolution, and those with large chromosomes have often been used in the study of chromosome behavior. The rapid development of molecular tools in the last few years has greatly furthered advances in elucidating the organization, behavior and evolution of chromosomes.

Aloe, a genus belonging to *Asphodelaceae* family, is distributed throughout the world and has rich medicinal ingredients. It is a perennial, evergreen, monocot crassulacean acid metabolism (CAM) plant, characterized with succulent leaf, inflorescences in bunches and liliform flowers (Ali, 2005). It grows to about three feet in height. The leaves are stiff, mottled, and lance-shaped with glabrous surfaces, sharp apices and spiny edges. Leaves send out sticky exudates when they are broken or injured. Plant takes four to five years to mature and can live up to 25 years (Anon., 2002). *Aloe vera* L. Burm. f. desert lily, nature's gift is used as a popular folk medicine with many cultivars growing naturally or in cultivation in different parts of the world. It is the most essential plant in the treatment of various ailments in the history of mankind. Since many years, it has been utilized for curing and can be consumed as a dietary supplement. It plays an important role in many aspects (Grindlay & Reynolds, 1986), such as bacteriostasis reducing blood lipid, resistance to life ageing and tumor forming. More specifically, it can provide curative effects to some extent in constipation, tuberculosis, skin diseases, heart diseases,diabetes and so on (Reynolds & Dweck, 1999; Kambizi & Afolayan, 2008). Cytological character, including chromosome number and karyotype analysis have been consider as reliable guide in study of taxonomic and evolutionary relationship by many authors (Davis & Heywood, 1963; Moore, 1968; Stace, 1980 and Elkington, 1984) showing that chromosome studies, especially when combined with hybridization and genetic analysis, have been provided essential clues in tracing the origin and the evolutionary history of plant species. The number, size and shape of chromosome can be used to characterize the karyotype of plants.

Many studies showed that the chromosome number for somatic cell of most *Aloe* is 2n = 14 and the haploid set genome consists of three short chromosomes and four long ones (Brandham & Doherty, 1998； Ji *et al.,* 2002; Alam & Khanam, 2005). Although, most *Aloe* has same chromosome number, different species display differences in karyotype. The karyotype of *Aloe* consists of near-to-terminal and near to- middle centromere chromosomes (Ji *et al*., 2002; Zheng *et al.*, 2005).

Two other genera of this tribe *viz*., *Gasteria* and *Haworthia* possess similar karyotype like *Aloe* (Sapre, 1977; Darlington & Kefallinou, 1957; Vosa & Bayer, 1986). It seems that the structural chromosomal aberrations in this tribe did not affect the morphology of the karyotype. The objectives of the present study are to standardize cytological analysis of mitotic chromosomes, construct karyotype of the species and to reveal the type and frequencies of mitotic irregularities if present.

Materials and Method

Aloe vera collected from different localities of Karachi (Table 1). Root tips harvested at different times were pretreated in three was kinds of chemicals (ice water, colchicine, and 8-Hydroxyquinoline) to achieve the suitable time for best mitotic index and pretreatment reagent respectively and then fixed in freshly prepared acetic-alcohol (1:3) mixture for 24 h. The highest frequency of dividing cells was observed at 2.00p.m. Cold water treatment at 4^{o}C for 27hrs was the most suitable pretreatment. Softening of the tissue was carried out by hydrolyzing the root tips in 1N HCl in water bath at 60^{o}C for 12 minutes. The meristematic region of the root tips was squashed in a drop of 2% aceto orcein. Chromosome measurements including length of long arm, length of short arm, total chromosomal length, relative length, arm ratio and centromeric index were made from 10 enlarged well-spread metaphase cells for each population. Best observations, from an average of 50 cells, were observed using 100× oil immersion objective of a microscope and photographs were taken by Sony-DSC-W180 digital camera (resolution 4x) and then loaded in Microsoft office picture manager. Karyograms were arranged in order of decreasing length of homolog chromosome pairs (Vahidy & Jahan, 1998).

Table 1. Population of *Aloe vera* collected from different locations of Karachi, Pakistan.

Population	Location
R1	Population collected from Malir (public park)
R2	Population collected from Nazimabad (public park)
R3	Population collected from North Karachi
R4	Population collected from Landhi
R5	Population collected from Korangi
R6	Population collected from Gulistan-e-Jouhar
R7	Population collected from Mazar-e-Quaid
R8	Population collected from Safari Park
R9	Population collected from Botanical Garden of Karachi University
R10	Population collected from Department of Botany, Federal Urdu University of Arts, Sci. & Technology, Karachi

Results and Discussion

Mitotic metaphase cells of *Aloe vera* with dispersed chromosomes and the clear centromere (Fig. 1) and karyograms based on 10 different populations of *Aloe vera* have 2n=14 chromosomes, in which 8 chromosome are large and 6 are small (Figs. 2, 3 & 4).The characteristics like total length, relative length, short and long arm ratio of chromosome of each population are shown in Table 2. Results showed that the average chromosome lengths among populations ranged from 7.95-2.36 µm (Table 2). Whereas Gunjan & Roy, 2010 observed the total chromosomal length 10.42µm in the same species. Arm ratios of chromosomes ranged from 4.79-1.69 (Table 2). Brandham (1973) and Sharma & Chatterji (1958). Observed all seven acrocentric pairs of chromosome while in present work we observed a very few metacentric with arm ratio 1.0-1.7, sub metacentric (1.7-3.0) and acrocentric (3.0-7.0) chromosomes following the classification of Levan *et al.,*1964. On an average 55.71% submetacentric, 38.57% acrocentric and 5.71% metacentric chromosomes were observed in 10 different populations of *Aloe vera*. Population R1, R2, R3, R4, R7 and R8 has sub metacentric and acrocentric chromosomes whereas in population R5, R6, R9 & R10 in addition to submetacentric and acrocentric, metacentric chromosomes were also present. Chromosome pairs were made on the basis of centromeric index and are arranged in descending order with respect to total length of chromosome in each population (Figs. 2, 3 & 4).The highest and lowest relative length among all populations were 10.64 and 3.04 observed in population R4 and R2 respectively (Table 2). There were certain variations present in genome length among populations, the lowest genome length 72.87 (R2) and the highest genome length 80.32 observed in R10.

The karyotype is bimodal and consists of 14 chromosomes (eight large and six small) predominantly with submedian, median and subterminal centromere (Figs. 2, 3 & 4). Chromosome variations among populations of the same species have been observed in many plant groups (Maffei *et al.*, 1999). A dicentric chromosome has been reported in root tip cells of *Aloe vera* by Umesh & Ranganath (2003). They provided ultra structural evidence for the flawless transmission of dicentric chromosomes in the root tip cells and proposed that the presence of two functional kinetochores need not invariably lead to chromosome in stability and loss. Alam & Khanam (2005) carried out fluorescent karyotypic analysis of four *Aloe* species. Based on CMA and DAP banding properties of the chromosomes of *Aloe* species they considered CG and AT rich base sequences in chromosomes are probably involved in karyotypic diversification of these species.

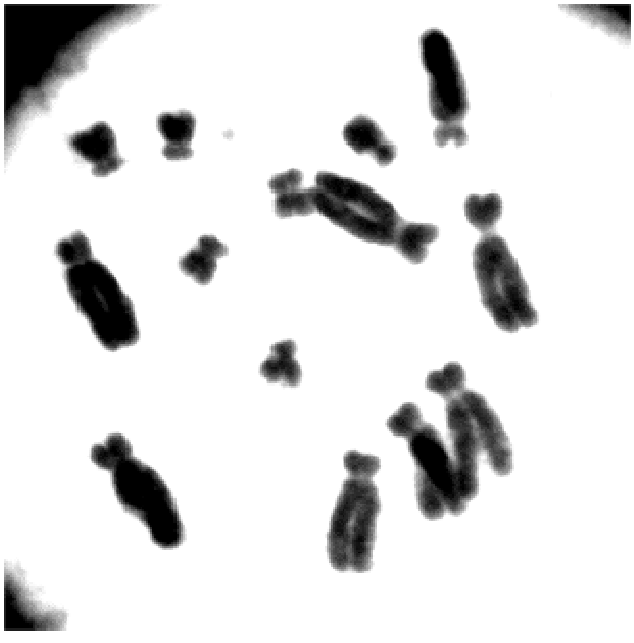

Fig. 1. Mitotic metaphase chromosomes of *Aloe vera*.

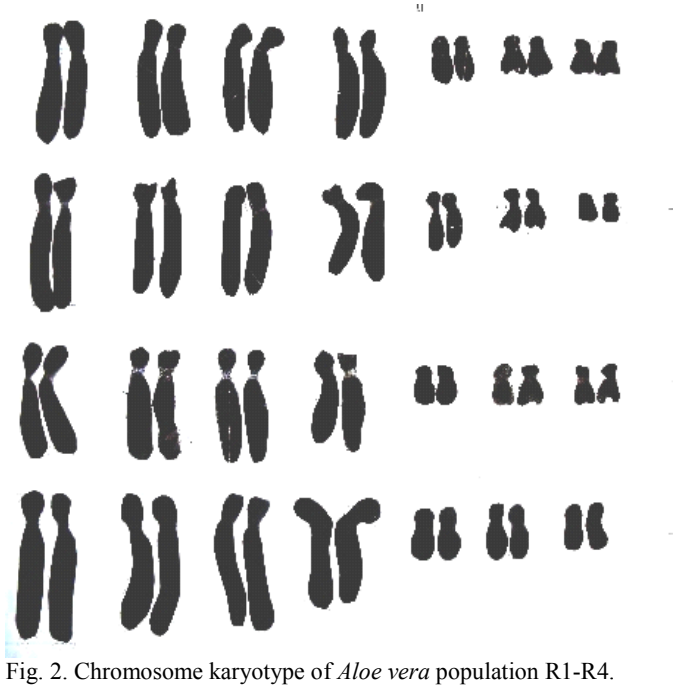

Fig. 2. Chromosome karyotype of *Aloe vera* population R1-R4.

Table 2. Karyological parameters of root tip mitotic chromosomes in 10 populations of *Aloe vera.* Reading in each column represents total length of chromosome in micro meter, relative length, and long/short arm ratio respectively. Reading represents means of ten cells.

S. No	Population	Ploidy level	Chromosome Pairs						
			1	2	3	4	5	6	7
1	R1	2x	7.7	7.18	6.57	6.1	3.3	2.85	2.6
			10.52	10.08	9.06	8.52	4.61	4.00	3.38
			3.5	4.79	3.72	4.4	4.4	2.14	2.5
2	R2	2x	7.25	7.1	6.76	5.48	3.14	2.69	2.36
			10.29	9.94	9.28	8.76	4.04	3.46	3.04
			2.96	3.8	3.26	4.44	2.14	1.86	2.14
3	R3	2x	7.09	6.91	6.39	6.36	3.64	3.13	2.86
			9.88	9.52	8.85	8.55	4.86	4.23	3.97
			2.52	4.08	3.13	3.95	2.17	1.98	1.88
4	R4	2x	7.58	7.11	6.65	6.39	3.7	2.84	2.6
			10.64	9.78	9.14	8.78	4.33	3.79	3.56
			2.62	4.19	3.13	4.58	3.09	1.88	1.85
5	R5	2x	7.52	7.15	6.7	6.25	3.68	3.21	2.88
			10.01	9.60	8.93	8.31	4.91	4.29	3.69
			2.68	2.89	2.77	3.53	1.86	1.86	1.70
6	R6	2x	7.80	7.43	7.19	6.86	3.57	2.98	2.8
			10.09	9.60	9.31	8.87	4.61	3.85	3.61
			2.9	3.04	3.08	3.11	1.52	1.89	1.8
7	R7	2x	7.64	7.34	7.14	6.8	3.5	3.29	2.8
			9.97	9.59	9.24	8.86	4.62	4.24	3.60
			2.67	3.38	2.86	3.03	2.13	1.78	1.96
8	R8	2x	7.6	7.18	6.82	6.38	3.10	2.57	2.45
			10.54	9.93	9.39	7.42	4.35	3.77	3.35
			2.91	3.00	2.90	3.91	2.21	1.92	1.75
9	R9	2x	7.5	7.14	6.68	6.44	3.44	3.04	2.7
			10.35	9.84	9.21	8.89	4.71	4.18	3.71
			2.98	2.8	3.41	3.53	2.0	1.69	2.1
10	R10	2x	7.98	7.52	7.51	7.0	3.68	3.22	3.1
			9.94	9.54	9.42	8.61	4.56	4.02	3.84
			3.25	3.01	3.73	3.19	2.01	1.85	1.43

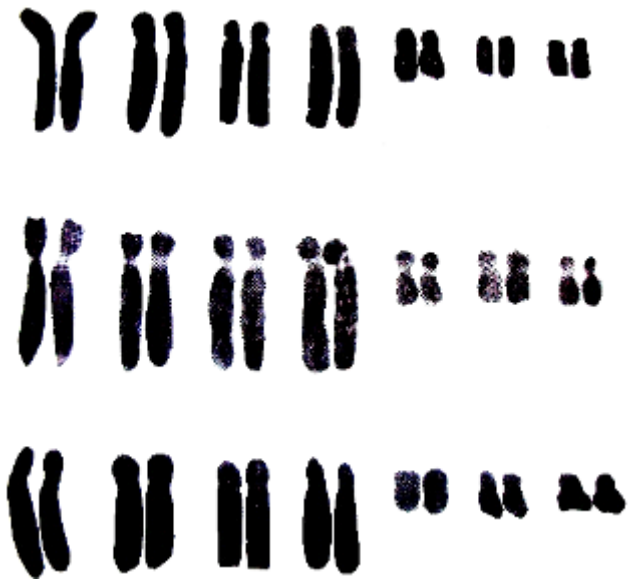

Fig. 3. Chromosome karyotype of *Aloe vera* population R5-R7.

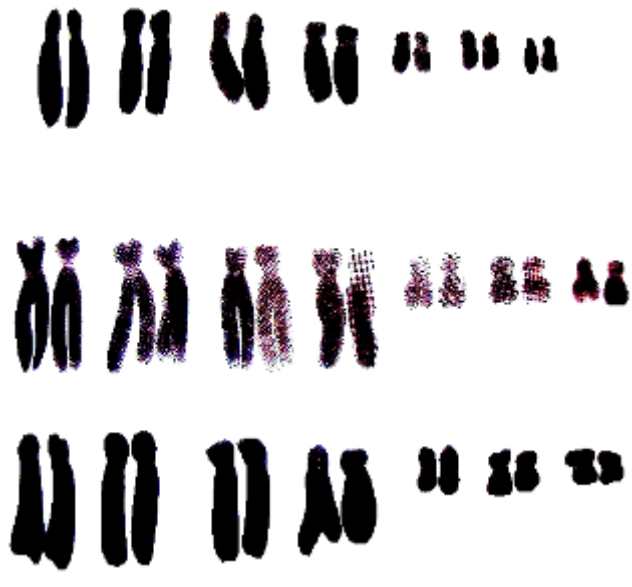

Fig. 4. Chromosome karyotype of *Aloe vera* population R8-R10.

Acknowledgement

The financial assistance has been supported from the Dean, Faculty of science, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan and technical assistance by Ms. Reena Aziz is gratefully acknowledged.

References

Alam, S.S. and N. Khanam. 2005. Fluorescent karyotype analysis of four *Aloe* species. *Bangladesh J. Bot*., 34(1): 17-20.

Ali, S.I. 2005. Asphodelaceae. (Eds.): Ali, S.I. and M. Qaiser. published by Department of Botany, University of Karachi & Missouri Botanical Press, *Missouri Botanical Garden*, St. Louis, Missouri, U.S.A. pp. 2.

Anonymous. 2002. Properties of aloe vera constituents. http://www.aloevera.co.uk/aloeprop.htm. Accessed 3 April, 2003.

Brandham, P.E. 1973. The chromosomes of Liliaceae III. New cases of interchange hybridity in the Aloineae. *Kew Bull*., 28: 341-348.

Brandham, P.E. and M.J. Doherty. 1998. Genomic size variation in the Aloaceae, an Angiosperm family displaying Karyotypic orthoselection. *Annals of Botany*, 82(Suppl A): 67-73.

Darlington, C.D. and M. Kefallinou. 1957. Correlated chromosome aberrations at meiosis in *Gasteria*. *Chromosoma*, 8: 364-370.

Davis, P.H. and V.H. Heywood. 1963: Principles of Angiosperm Taxonomy, Oliver 8: Boyd, Edinburgh 8. London.

Elkington, T.T. 1984. Cytogenetic variation in the British flora: Origins and significance. *New Phytol*., 98: 101-118.

Grindlay, D. and T. Reynolds. 1986. The *Aloe vera* phenomenon, a review of the properties and modern uses of the leaf parenchyma gel. *J. Ethnopharmacol*., 16: 117-151.

Gunjan, K. and B.K. Roy. 2010. Karyotype studies in dominant species of *Aloe* from eastern India. *Caryologia*, 63(1): 41-49.

Ji, C.Y., Y.X. Ma and D.T. Cui. 2002. Chromosomes and karyotype analyses of the four type's plants in *Aloe* L. *Quarterly of Forest By-Product and Speciality in China,* 3: 15-16.

Kambizi, L. and A.J. Afolayan. 2008. Extracts from *Aloe ferox* and *Withania somnifera* inhibit *Candida albicans* and *Neisseria gonorrhoea*. *Afr. J. Biotechnol*., 7(1): 12-15.

Levan, A., K. Fredga and A. Sandberg. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, 52: 201-220.

Maffei, E.M.D., M.A. Marin-Morales, P.M. Ruas, C.F. Ruas and N.I. Matzenbacher. 1999.Chromosomal polymorphism in 12 populations of Mikania micrantha (Compositae) *Genetics and Molecular Biology*, 22(3): 433-444.

Moore, D.M. 1968. The karyotype in Taxonomy. In: *Modern methods of plant taxonomy*. (Ed.): Heywood, V.H. Academic Press. London and New York, pp. 61-75.

Reynolds, T. and A.C. Dweck. 1999. *Aloe vera* leaf gel: a review update. *J. Ethnopharmacol*., 68: 3-37.

Sharma, A.K. and A.K. Chatterji. 1958. Chromosome studies as a means of detecting the method of speciation in some members of Liliaceae. *Genetica Iberica*, 10: 149-178.

Sapre, A.B. 1977. An interchange mosaic in *Aloe barbadensis* Mill. *Caryologia*, 30: 423-427.

Stace, C.A. 1980. Plant taxonomy and biosystematics. Arnold, E. Ltd., London.

Umesh, T.G. and R.M. Ranganath. 2003. Ultrastructural evidence for the flawless transmission of dicentric chromosome in *Aloe vera* L. *Cytologia*, 68(3): 211-217.

Vahidy, A.A. and B. Jahan. 1998. Karyological studies in some species of *Hordeum* L. *Pak. J. Bot*., 30: 101-115.

Vosa, C.G. and M.B. Bayer. 1986. Chromosome studies in the Southern African flora. *Caryologia*, 39: 325-334.

Zheng, M., X. Sh. Yu, Y. Li, H. Wu and S.Z. Zhang. 2005. Karyotype analysis of 14 species and 2 varieties in *Aloe* L. *J. Wuhan Bot. Res*., 23(6): 535-540.

(Received for publication 16 April 2013)