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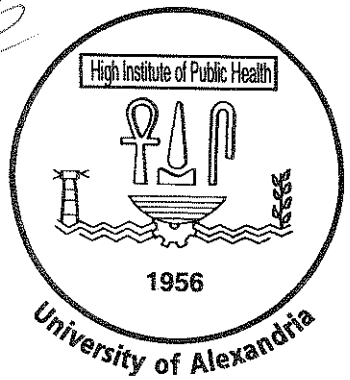

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Chromosomal Studies on the Taxonomy of some Species of the Genus *Aloe*

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Abstract: The aim of this study was to analyse the karyotype by using root tips of three species of the *Aloe* genus namely *Aloe sabaea, A. shadensis,* and *A. castellorum* and to differentiate between them from the view point of chromosomes taxonomy. In addition, the study aimed at determining the DNA concentration for the three species. Chromosome number of these three species was 2n = 14. It consisted of four pairs of large subtelocentric chromosomes and three pairs of relatively small submetacentric chromosomes. The presence of a secondary constriction was clearly visible positioned in the distal end of the long arm of the second chromosome pair in *A. shadensis,* and on the long arm of the first chromosome pair in *A. castellorum*, but not observed in *A. sabaea.* Chromosomes length, arm ratio, the relative length, and the total chromatine length were calculated. The length of the chromosome complement already indicated that the species of Aloes possess a large genome. The DNA concentrations were determined through spectrophotometer measurements, they were 3.46, 5.58, and 4.41 µg/ml in *A. sabaea, A. shadensis*, and *A. castellorum.* respectively. These significant variations in the DNA concentrations along with the observed differences in the Karyotypes would strongly support the species status of each one of them and support their current recognized taxonomic status.

INTRODUCTION

Karyotype analysis has played an important role in the identification and designation of chromosomes in many plants.

The genus *Aloe* includes over 300 species all of which originate in Africa and Arabia.[1,2] There are 25 species ,which are found in Saudia Arabia.[3]

It belongs to the Lilliaceae family. *Aloe* genus is one of the succulent plant which has many uses in medicine and commercially important plant. It is used for treating burns, abrasions, and skin irritation. When applied to scalp it prevents falling of hair and it has been indicated to be good for complexion.[4,5]

Chromosomal studies on the *Aloe* have been mostly on the determination of the chromosome counts for the purpose of cytotaxonomy classification.[6,7]

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Tylor made a comparative study of the chromosome morphology of *Aloe* emphasizing specially the number and location of satellites.[8] Resende reported the first polyploid in *Aloe citiaris* Haw and after several investigations on the Aloes showed the presence of other polyploids.[9]

The study of karyotype have clarified the minute difference in the chromosome morphology. Moreover, Almasan, *et al.*, reported that most of all species of the genus Aloe were diploid and had a chromosome number of 2n = 14.[5] Matos and Molina showed that the karyotype analysis in *A. vera* consisted of fourteen submetacentric chromosomes with length range from 5.55 to 17.76 μm.[10] Also, Brandham and Doherty suggested that most of *Aloe* species are subtelocentric.[11]

In recent years, a considerable amount of evidence has accumulated showing the variation within species and varieties in DNA amount.

MATERIAL AND METHODS:

The seeds of these species were collected by National Wildlife Research Center in Taif. The present study was performed for the first time on these species. Cytological studies were made from the root tips pretreated with the solution of colchicines 0.05% for 3 - 4 hours at 4°C, and fixed in freshly prepared acetic:alcohol [1: 3]. Root tips were then hydrolysed in 1N HCL for 8–10 minutes at 60°C, and stained in Feulgen strain for 1–2 hours in the dark according to Dyer[12] and Singh.[13] The preparations were examined under light microscope provided with camera and suitable cells were photomicrographed at magnification of x1000. Karyotype analysis was made following the system proposed by Levan et al.[14] DNA was isolated from each sample using Ultra Clean Plant DNA Isolating Kit. The concentration of DNA is read by measuring the absorbance of a sample at A260 on a spectrophotometer.

RESULTS

The examination of 25 cells for each species showed that the somatic chromosome number of each of the three species studied is 2n = 14. No polyploidy has been noted in any of the three species studied. A detailed karyotype analysis of the different species reveals a gross similarity in the chromosome complement. Eight chromosomes are distinctly large and six are distinctly small, based on the location of the centromere. As calculated from the chrmosome arm ratio, the chromosomes were classified into tow groups, namely:

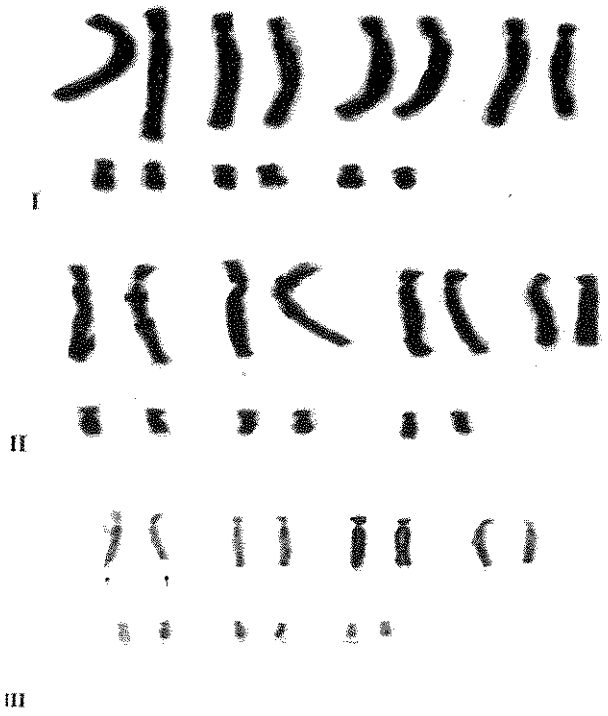

Figure [1] Karyotypes of *A. sabaea* I, *A. shadensis* II, and *A castellorum* III , arrows indicate satellites.

Table 1 : Total chromosomes mean lengths of the studied species .

Chromosome Pair no.	*A. sabaea*	*A. shadensis*	*A. castellorum*
1	17.95+4.70 =22.65 µm a=22.45 b=20.75	18.90+4.25 =23.15 µm a=22.88 b=18.35	*12.60+3.37 =15.97 µm a=21.94 b=21.10
2	17.15+4.15 =21.30 µm a=21.12 b=19.48	*17.15+4.48 =21.63 µm a=21.38 b=20.71	12.05+3.00 =15.05 µm a=20.68 b=19.93
3	16.25+3.85 =20.10 µm a=19.93 b=19.15	16.80+3.60 =20.40 µm a=20.16 b=17.64	12.10+2.50 =14.60 µm a=20.06 b=17.12
4	15.80+3.30 =19.10 µm a=18.93 b=17.27	15.70+3.15 =18.85 µm a=18.63 b=16.71	11.15+2.22 =13.37 µm a=18.37 b=16.60
5	4.35+2.15 =6.50 µm a=6.44 b=33.07	4.70+1.75 =6.45 µm a=6.37 b=27.13	3.60+1.55 =5.15 µm a=7.07 b=30.09
6	4.00+1.80 =5.80 µm a=5.75 b=31.03	4.05+1.52 =5.57 µm a=5.50 b=27.28	3.25+1.38 =4.63 µm a=6.36 b=29.80
7	3.60+1.80 =5.40 µm a=5.30 b=33.33	3.53+1.57 =5.10 µm a=5.04 b=30.78	2.85+1.15 =4.00 µm a=5.49 b=28.75

- a = Relative length - b = Centromeric Index - * = Chromosome with a satellite

group I-chromosomes possessed subterminal centromere which were observed in the large chromosomes, group II–chromosomes had submedian centromere which were observed in short chromosomes, the karyotype formula is found to be 4 st+3 sm. Satellites were found to be on the distal end of the long arm of the first chromosome in *A. castellorum* and on the long arm of the second chromosome in *A. shadensis,* while no satellites were observed in *A. sabaea.* The mean length of chromosomes ranged from [5.40 –22.65] μm in *A. sabaea*, [5.10 –23.45] μm in *A. shadensis*, and [5.04 –22.88] μm in *A. castellorum.*

DNA amount were also determined in this study. The results showed that the DNA concentration of *A. sabae, A. shadensis*, and

Table 2 : Mean differences between total DNA concentrations

		A. sabeae	A. shadensis	A . castellorum
A. sabeae	MD	–	–	–
	SE	–	–	–
A. shadensis	MD	-2.1200**	–	–
	SE	0.3372	--	–
A . castellorum	MD	-0.9500*	1.1700**	–
	SE	0.3372	0.3372	

MD = Mean Difference
SE = Standard Error
* = Significant at $P < 0.05$
** = Significant at $P < 0.01$

A. castellorum were 3.46, 5.58, and 4.41, respectively, Table 2. The mean difference was highly significant between *A. shadensis* and *A. sabaea* and between *A. shadensis* and *A. castellorum*.

DISCUSSION

Aloe genus was characterized by chromosome number with a basic set of 7, Figure[1]. The analysis of Riely showed that the genus Aloe is characterized by mostly diploid and few are polyploid species.[6] In this study the results showed that the chromosome number for *A. sabaea, A. shadensis,* and *A. castellorum* were 2n=14. No polyploidy has been noted . Polyploidy is rare in *Aloe*. Out of 113 species studied 106 were diploid recorded by Brandham.[1] A detailed karyotype [Fig.1] is fairly asymmetrical with four pairs of large subtelocentric chromosomes and three pairs of relatively small submetacentric chromosomes. The origin of such distinctive karyotype may be traced in two alternative ways: [1] through continued deletion of certain chromosome parts resulting in marked asymmetry, or [2] through hybridization between two species with very long and very short chromosomes, respectively.[7] Based on centromere position, Adams et al.,.[2] Matos and Molina[10] went on to note that the karyotype of *A. vera* consists of 7 pairs of acrocentric chromosomes, while Almasan *et al.*,[5] found four groups of chromosomes in *A. barbadensis*. On the other hand, Brandham and Doherty[11] mentioned that the karyotype of most the Aloe species consisted of subtelocentric chromosomes. In some metaphase cells, chromosomes were so contracted that secondary constriction were not readily visible.[15] However, secondary constriction were positioned in the distal end of the long arm of the second chromosome pair in *A. shadensis*, and on the long arm of the first chromosome pair in *A. castellorum*, while no satellite was observed in *A. sabaea* [see arrows, Fig.1]. Secondary constriction has

also been noted in other *Aloe* species including *A. tenuior* and *A. scobinifoli.*[1] Quantitative estimation of DNA in plant chromosomes has led to a fuller understanding of the variation within species and varieties. DNA amount was also determined in this study. The results showed that the DNA concentration of *A. sabaea* , *A. shadensis*, and *A. castellorum* were 3.46,5.58 and 4.41, respectively. The mean differences were highly significant between *A. shadensis* and *A. sabaea* and between *A. shadensis* and *A. castellorum*. The genomic DNA content differs significantly between the species. This would serve as the basis for the variation among them. Along with the minor differences in the keryotypes of the species, this DNA variations would strongly support their current recognized taxonomic status being three different species. Further molecular studies such as DNA sequencing will be more revealing.

REFERENCES

1. Brandham P. Polyploidy and karyotype variation in the Aloineae. Kew Bull 1971; 25: 381–99.
2. Adams S, Leitch I, Bennett M, Leitch *A. Aloe* L.–a second plant family without [TTTAGGG] n telomeres. Chromosoma. 2000; 109: 201-5.
3. Collenette, D. Plant molecular systematics. New York: John Wiley and Sons; 1990.
4. Morton J. Major medicinal plant. U.S.A: Charles C .Thomas; 1977.
5. Almasan A, Barrion A, Casal, C, Dela Cruz. P. Karyotype analysis of *Aloe barbadensis* Mill, [Liliaceae]. The Philippine Agriculturist. 1991; 74 : 261-4.
6. Riley H . Polyploidy in South African species of *Aloe.* Amer. Jour. Bot 1959; 46 [2]: 126-9.
7. Sharma A, Mallick R. Interrelationship and evolution of the Tribe Aloineae as reflected in its cytology Journ. Genet. 1965; 59: 110-37.
8. Taylor W. Cytological studies on *Gasteriall, Aloe,* and *Haworthia* Amer. Jour. Bot. 1925; 12: 219-23.
9. Resende F. Gigas Formen Mit Geringerer Chromosomenzahl Als Die Stammarten Ber. Deutch. Bot. Ges. 1938; 56: 533-42.
10. Matos A, Molina J. Cytogenetic study in root tip cells of *Aloe vera* Rev. Fac. Argon. [Luz]. 1997; 14 :173-82.
11. Brandham P, Doherty M. Genome size variation in the Aloaceae, an angiosperm family displaying karyotypic orthoselection Annals of Botany. 1998; 82: 67–73 .

12. Dyer A. Investigating chromosomes. London: Edward Arnold; 1979.
13. Singh R. Plant cytogenetics London: CRC Press; 1993.
14. Levan A, Fredga K, Sandsberg A. Nomenclature for centromeric position on chromosomes. Hereditas. 1964; 52: 201 –20.
15. Liu J, Ho T, Chen S, Lu A. Karyomorphology of *Biebersteinia* Stephan [Geraniaceae] and its Systematic and taxonomic significance Bot. Bul. Acad. Sin. 2001; 42: 61-6.