Research Article

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STUDIES ON THE CYTOTAXONOMY AMONG DIFFERENT SPECIES OF ALOE COLLECTED FROM RANCHI, JHARKHAND

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Abstract: Cytotaxonomical analysis is an important study in finding out phylogeny, evolution and interrelationships between the taxa. The present finding provides karyotype analysis on six different species of *Aloe* of Ranchi. These species differed in uniformity of their chromosome numbers. They also differed from each other in their total chromatin length, arm ratio and position of centromere.

Keywords: Cytotaxonomy, phylogeny, chromatin length, arm ratio, centromere.

INTRODUCTION

Jharkhand, a state of India, is a rich source of medicinal plants. *Aloe,* a xerophytic plant is a native of South Africa and is commonly cultivated in America either as vegetative ornamental or for its medicinal properties[1]. Several species of *Aloe* have been introduced in India. *Aloe* can flourish in a variety of climates and on the poorest soil. *Aloe* belonging to family Liliaceae [2] and tribe Aloineae are popularly known as Ghee-kunvar in Hindi, Sab-bara in Arabian and Darakhte-Sibr in Persian [3]. Aloes contain a mixture of glycosides collectively known as aloin which is the active constituent of drug and its proportion varies from species to species. *Aloe* is widely used for healing of wounds and burns, as the plant contains lupeol and salicylic acid, which increases blood flow in the wounded areas and stimulates fibroblast cells responsible for healing [4].

The following species of *Aloe* [5] were taken for the research work conducted during the year 2003-2005. *Aloe abyssinica, A barbadensis* Mill, *A. plicatalis, A. harlana, A. distance* Haw. and *A. variegata* Linn.

Objective of Research

Karyotype analysis of six species of *Aloe* and assessment of their evolutionary status. The detailed karyotype analysis of the above six species of *Aloe* collected from Ranchi district of the State Jharkhand in India was not performed earlier therefore, it was found necessary to do the cytology of the six species of *Aloe.*

MATERIALS AND METHODS

The plants of above mentioned species of *Aloe* were collected from local nurseries and from forest areas of Ranchi district of the state Jharkhand which is situated in between Latitude: 23°45'N Longitude: 85°30'E of Indian geographical area. The potted clones of six species of *Aloe* were taken which rooted readily in the compost mixture. The root apices 1-5 mm in length were excised from the pot between 1.00 pm to 2.00 pm and were treated with 0.002M-8-Hydroxyquinolene at 15°C for 4 to 5 hours. The pretreated material was then thoroughly washed with distilled water and transferred to fixative (1:3 Aceto alcohol). After 24 hours the material was transferred to 70% alcohol for preservation.

Squash preparations were made by warming the root tips in 2% acetocarmine and N-HCL solution for 45 minutes. Normal Hydrochloric acid was mixed with 2% acetocarmine in the proportion of nine-parts of dye and one part of acid [6]. Ten well-separated chromosome plates were taken for experimental purposes. The well-separated chromosomes were measured with the help of ocular and stage micrometer. The data were statistically analyzed and idiograms and histograms were prepared.

RESULTS AND DISCUSSION

The data for karyo morphological analysis have been depicted in table 1 and 2 and figure 1 to 12. The constancy in the chromosome number in *Aloe* has not been reported and different species of *Aloe* under consideration showed asymmetry in their basic chromosome numbers. The three species Aloe *abyssinica* Lam., *A. barbadensis* Mill. and *A. plicatalis* showed symmetry in chromosome number 2n=2x=14, whereas, *Aloe harlana* and *Aloe distance* Haw showed 2n=2x=21. A regular tetraploid (2n=4x=28) genetic condition was observed in *Aloe variegata* Linn. Earlier, scientists have reported polyploidy and aneuploidy in this genera [7-12].

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The greatest diversity of karyotypes are found in a single family of plants that is Liliaceae [13]. All the species of *Aloe* under consideration comprises eight long and six short chromosomes. Centromeres of all the species investigated are sub terminal to sub median showing that these species are highly asymmetrical with bimodal karyotype [13-15].

Table 1: Karyomorphological data of *Aloe* species

Species under consideration	Chrom No.	Arm Length		Chromosome Length (in µ)	Arm Ratio L/S	R.L (in µ)	F%	T.C.I.	Classification
		Long	Short						
1	2	3	4	5	6	7	8	9	10
	I L_1	8.05	3.50	11.55	2.30	79.98	30.30	16.99	nsm
	II L_2	8.61	3.26	11.87	2.64	82.20	27.46	17.46	nsm
	III L_3	9.91	3.26	13.17	3.03	91.20	24.75	19.37	nst
Aloe abyssinica Lam.	IV L_4	11.18	3.26	14.44	3.42	100.00	22.57	21.24	nst
	V S_1	3.20	1.45	4.65	2.20	32.20	31.18	6.84	nsm
	VI S_2	3.50	1.60	5.10	2.18	35.31	31.37	7.50	nsm
	VII S_3	4.63	2.56	7.19	1.80	49.79	35.60	10.57	nsm
	I L_1	10.06	3.76	13.82	2.67	84.31	27.20	18.58	nsm
	II L_2	11.13	3.50	14.63	3.18	82.96	23.92	19.67	nst
	III L_3	12.51	3.50	16.01	3.57	97.68	21.86	21.52	nst
Aloe barbadensis Mill.	IV L_4	12.68	3.71	16.39	3.41	100.00	22.63	22.03	nst
	V S_1	1.83	0.80	2.63	2.28	16.04	30.41	3.53	nst
	VI S_2	3.50	1.80	5.30	1.94	32.33	33.96	7.12	nsm
	VII S_3	3.76	1.83	5.59	2.05	34.10	32.73	7.51	nsm
	I L_1	9.62	3.15	12.77	3.05	74.85	24.66	17.16	nsm
	II L_2	10.50	1.75	12.25	6.00	71.80	14.28	16.60	nst
	III L_3	14.00	2.88	16.88	4.86	98.94	17.06	22.68	nst
Aloe plicatalis	IV L_4	14.00	3.06	17.06	4.57	100.00	17.93	22.93	nst
	V S_1	3.50	1.48	4.98	2.36	29.19	29.71	6.69	nst
	VI S_2	3.50	1.71	5.21	2.04	30.53	32.82	7.00	nsm
	VII S_3	3.50	1.75	5.25	2.00	30.77	33.33	7.05	nsm
	I L_1	12.83	3.50	16.33	3.66	86.44	21.43	18.50	nst
	II L_2	14.11	3.50	17.61	4.03	93.22	19.87	19.95	nst
	III L_3	14.58	3.60	18.18	4.05	96.24	19.80	20.05	nst
Aloe harlana	IV L_4	15.16	3.73	18.89	4.06	100.00	19.74	21.40	nst
	V S_1	3.73	1.75	5.48	2.13	29.01	31.93	6.20	nsm
	VI S_2	3.73	1.75	5.48	2.13	29.01	31.93	6.20	nsm
	VII S_3	4.55	1.75	6.30	2.60	33.35	27.77	7.13	nsm
	I L_1	7.00	3.50	10.50	2.00	78.94	33.33	17.69	nsm
	II L_2	8.09	3.50	11.59	2.31	87.14	30.19	19.53	nsm
	III L_3	9.10	3.50	12.60	2.60	94.73	27.77	21.23	nsm
Aloe distans Haw.	IV L_4	9.80	3.50	13.30	2.80	100.00	26.31	22.41	nsm
	V S_1	2.62	0.87	3.49	3.01	26.24	24.92	5.88	nst
	VI S_2	2.62	1.04	3.66	2.51	27.51	28.41	6.16	nsm
	VII S_3	2.97	1.22	4.19	2.43	31.50	29.11	7.06	nsm
	I L_1	10.32	2.88	13.20	3.58	75.21	21.81	18.13	nst
	II L_2	10.50	3.32	13.82	3.16	78.74	24.02	18.98	nsm
	III L_3	10.93	3.67	14.60	2.97	83.19	25.13	20.05	nsm
Aloe variegata Linn.	IV L_4	13.88	3.67	17.55	3.78	100.00	20.91	24.10	nst
	V S_1	1.92	1.09	3.01	1.76	17.15	36.21	4.13	nsm
	VI S_2	3.32	2.10	5.42	1.58	30.88	38.74	7.44	nsm
	VII S_3	3.67	1.53	5.20	2.39	29.62	29.42	7.14	nsm

R.L : Relative Length
F% : Form Percentage
T.C.I. : Total Chromatin Index
nm : nearly median
nsm : nearly sub median
nst : nearly sub terminal

Table 2: Data related to Karyotype of *Aloe* species

Species under consideration	T.C.L. (in µ)	T.F%	G.I%	S.I%
Aloe abyssinica Lam.	67.97	27.65	32.20	38.48
Aloe barbadensis Mill.	74.37	25.41	16.04	34.07
Aloe plicatalis	74.40	21.20	29.19	26.89
Aloe harlana	88.27	22.18	29.01	28.50
Aloe distans Haw.	59.33	28.87	26.24	40.59
Aloe variegata Linn.	72.80	25.08	17.15	33.48

Karyotype formulae:

Aloe abyssinica Lam.	2n = 2x = 14	8L + 6S - 4L (nsm) + 4L (nst) + 6S (nsm)
Aloe barbadensis Mill.	2n = 2x = 14	8L + 6S - 2L (nsm) + 6L (nst) + 4S (nsm) + 2S (nst)
Aloe plicatalis	2n = 2x = 14	8L + 6S - 2L (nsm) + 6L (nst) + 4S (nsm) + 2S (nst)
Aloe harlana	2n = 3x = 21	12L + 9S - 12L (nst) + 9S (nsm)
Aloe distans Haw.	2n = 3x = 21	12L + 9S - 12L (nsm) + 3S (nst) + 6S (nsm)
Aloe variegata Linn.	2n = 4x = 28	16L + 12S - 8L (nsm) + 8L (nst) + 12S (nsm)

nsm : nearly sub median
nst : nearly sub terminal
T.C.L.: Total Chromatin Length
T.F% : Total Form Percentage
G.I. : Gradient Index
S.I. : Symmetry Index

In the present finding the presence of secondary constriction was viewed only in few metaphase plates of *Aloe variegata* Linn. and was restricted to the long chromosomes. The satellites were seen only in extremely well stained and well-spread metaphase plates. In squash preparations some chromosome plates showed them while others did not. This might perhaps be due to local variations in chromosome condensation possibly brought by diffusion gradients of pre treating agents. In condensed metaphases satellite merges with the chromosome arms and therefore, cannot be seen. The secondary constrictions in the chromosomes serve as good marker, which enables to understand the morphology of the chromosomes. The number of nucleoli in a cell is proportional to the number of SAT regions [16].

In the present investigation *Aloe harlana* is considered primitive having greatest chromatin length and *Aloe distance* Haw is advanced having the lowest chromatin length[13]. The increase or decrease in total chromatin cannot be considered criteria for primitiveness and advancement. The DNA per genome can increase or decrease during the course of evolution and it can be present among all the members of the compliment, in equal amounts or in quantitative proportional to the chromosome length or it may be confined to one or more chromosomes [17].

Apart from numerical variations of chromosomes, differences with respect to the number of nearly sub median, nearly sub terminal chromosomes were found. Total form percentage, gradient index and symmetry index values in six different species of *Aloe* under investigation were calculated. The perusals of TCI% suggest that the difference in size of the chromosome within each species of *Aloe* were not very much marked. Analysis of data of length of chromosome is of immense value in understanding the evolutionary status of the taxa. Analytical studies of the karyotype symmetry provide a valuable criterion to indicate the nature of evolutionary processes and trends in taxon in which evolution has taken place. The origin of bimodal karyotype of Aloineae is explained [13]. (Stebbins, 1971) by increasing asymmetry and heterogeneity plus the addition of a chromosome to the compliment through fixation of a centric fragment.

In the present findings all the species of *Aloe* have a low symmetrical index values, thus indicating the tendencies towards asymmetry. The gradient index in all the six species of *Aloe* are below 30, except *Aloe abyssinica* Lam. where it is 32.20. Therefore, they are considered highly asymmetrical as the GI values are less than 30. Asymmetrical karyotypes are considered advanced than symmetrical karyotypes [18]. (Levitsky, 1931). Among the six species of *Aloe*, the lowest gradient index is 16.04 in *Aloe barbadensis* Mill., which may be considered most advanced among the six species of *Aloe.* The advancement in the genus *Aloe* has been further supported by TF% and F%. The TF% gives an estimate of mean position of centromere in in different chromosomes while F% is another estimate of arm ratio. The lower values observed for these two parameters also indicate that the level of asymmetry is higher.

Fig.-1

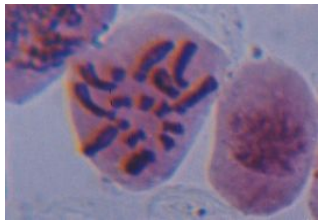

Fig.-2

Fig.-3

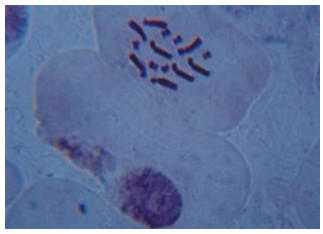

Fig.-4

Fig.-5

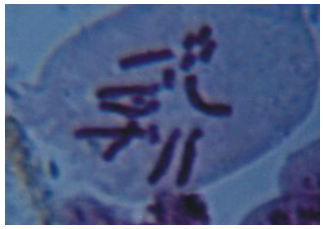

Fig.-6

Photographs and Microphotographs of metaphase plates of different species of *Aloe.* Fig.: 1&2 *Aloe abyssinica* Lam., Fig.: 3&4 *A. barbadensis* Mill., Fig.: 5&6 *A. plicatalis*

PLATE- I


Fig.-7

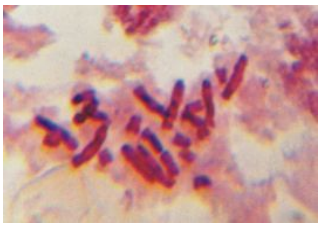
Fig.-8


Fig.-9

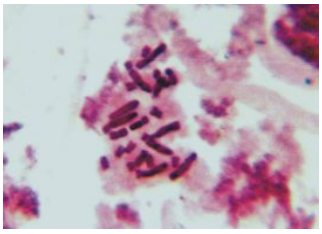
Fig.-10


Fig.-11

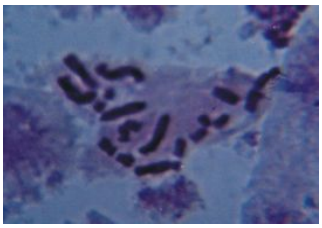
Fig.-12

Photographs and Microphotographs of metaphase plates of different species of *Aloe* Fig.:7&8 *Aloe harlana* Fig.:8&9 *Aloe distans* Haw., Fig.: 11&12 *Aloe variegata* Linn.

PLATE- II

CONCLUSION

On the basis of above findings it may be concluded that all the six species of *Aloe* under investigation were not stable due to difference in their chromosome numbers. All of them comprised a typical bimodal karyotype with asymmetrical nature, which reflects them to be advanced.

Research highlights

- The investigation revealed that the above six species of *Aloe* were not stable because they differed in their diploid chromosome numbers.
- All of them had a bimodal karyotype, a characteristic of sub tribe Aloineae.
- All the six species consisted of a asymmetrical karyotype showing their tendency towards advancement.

Limitations

All the six species of *Aloe* were locally collected from Ranchi (Jharkhand, India) and their cytological studies were performed in the laboratory of University Department of Botany, Ranchi University, Ranchi.

Recommendations

The cytological analysis gives a authentic data for the preparation of chromosome atlas.

Funding and Policy aspects

Preparation of chromosome atlas of medicinal plants of Ranchi, Jharkhand, India.

Justification of Research

Chromosome Atlas.

Authors Contribution and Competing Interests

The authors declare that they have no financial interest in making the chromosome atlas. The article is the original research work.

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