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QUANTITATIVE KARYOTYPE ANALYSIS OF *AGAVE AMERICANA* L. AND *A. STRIATA* ZUCC

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Abstract

Karyotype analysis of *Agave americana* L. and *A. striata* Zucc revealed that both the species had 2n = 60 with differences in individual chromosome length, total chromatin length (TCL) and total frequency % (TF%) between the complements of their chromosomes. The identified chromosome pairs were I^{m}, II^{Sm}, III^{Sm}, IV^{Sm}, V^{m}, VI^{Sm}, $VIII^{St}$, IX^{Sm}, XII^{Sm}, $XVII^{Sm}$, $XVIII^{Sm}$, XIX^{m}, XXI^{m}, $XXII^{m}$, $XXIII^{m}$, $XXIV^{St}$, XXV^{m}, $XXVI^{Sm}$, $XXVII^{Sm}$, $XXIX^{Sm}$ and XXX^{m} in *A. americana* and II^{m}, III^{St}, IV^{Sm}, VI^{sm}, VII^{St}, $VIII^{Sm}$, IX^{Sm}, XII^{m}, $XIII^{St}$, XIV^{Sm}, XV^{Sm}, XVI^{Sm}, $XVII^{Sm}$, XIV^{Sm}, XX^{m}, XXI^{Sm}, $XXIII^{Sm}$, XXV^{m}, $XXVII^{Sm}$ and $XXVIII^{m}$ in *A. striata*. The proposed standard karyotype were $1L^{1m} + 9M^{8Sm+1St} + 16S_1^{8m+7Sm+1St} + 4S_2^{2Sm+1St}$ for *A. americana* and $2L^{1m+1Sm} + 9M^{7Sm+2St} + 15S_1^{3m+11Sm+1St} + 4S_2^{2m+2Sm}$ for *A. striata*.

Introduction

Agave americana L. (Agavaceae) is commonly known as century plant. In Bangladesh, the species is treated generally as ornamental plants and a source of ethnomedicine in rural area. The heart of the plant is very rich in saccharine matters and can be eaten when backed (Facciola 1990). It is sweet and nutritious but rather fibrous (Weiner 1980).

A good number of cytological work have been done on the genus *Agave*. Schaffner (1909) worked on reduction division in the microsporocytes of *Agave*. Doughty (1936) and Vignoli (1937) reported the relative size differences and terminal spindle fiber attachments of the large chromosome of *Agave*. Granick (1944) made karyosystemic study of the genus *Agave* and reported 2n = 60 chromosomes.

Identification of individual chromosomes and their homology particularly of small size has become very complicated by the conventional method. In this context this method is very much effective particularly for small size of chromosomes. The quantitative method may help to develop a standard karyotype. With this method the variation of chromosome size from cell to cell may be determined. Thus quantitative method used by Ahmed *et al*. (1983) was adopted for karyotype analysis in two species of *Agave* in the present study.

Materials and Methods

A. americana L. and *A. striata* Zucc were obtained from Professor Sultanul Alam Cytogenetics Laboratory, Department of Botany, University of Rajshahi. The roots of 1-1.5 cm in length were collected, washed with water very carefully and pretreated with aqueous solution of Para-di-chlorobenzeen (PDB) for 4 - 5 hours at 8 - 12º C. The root tips were thoroughly washed in distilled water and fixed in 1 : 3 aceto-alcohol at room temperature for 48 hours and then preserved in 70% ethanol. Staining of root tip chromosomes was done by haematoxylin method.

Meristematic zone of the root tip was squashed in a drop of 0.5% acetocarmine. Temporary slides were examined under a compound microscope with 40 × 16 magnifications. Photomicrographs were taken from the desired preparations and chromosomes were measured from photomicrograph. During conversion of millimeter (mm) values into micrometer (µm) occular scale and stage micrometer were used for calibration at the same magnification.

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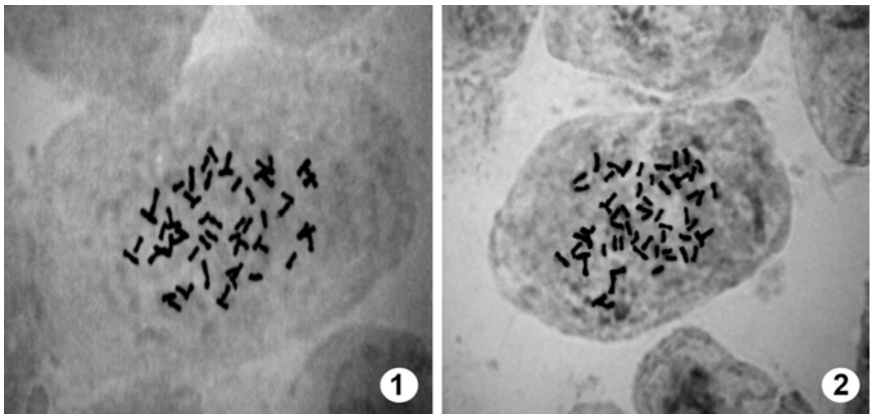
Data on chromosome morphology was determined from three cells of each species based on similar degrees of contraction of the chromosomes as described by Ahmed *et al*. (1983) with some modifications. The total frequency (TF%) was calculated using the formula of Huziwara (1962).

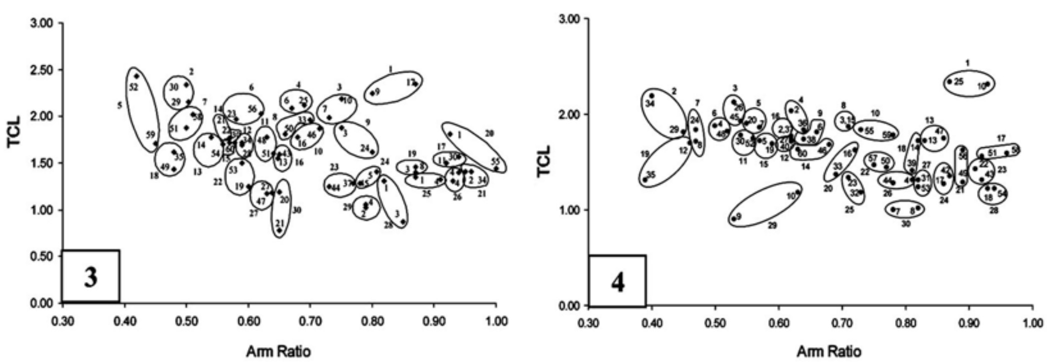
Results and Discussion

The two *Agave* species were found to possess 2n = 60 chromosomes (Figs 1 - 2). The similar chromosome number was reported earlier as well (Granick 1944, Manton 1932, Panetsos and Baker 1986, Dayal 1975, Chevallier 1996). Therefore, the present chromosome counts confirmed the earlier reports on diploid chromosome numbers of these two *Agave* species.



Figs 1 - 2. Metaphase chromosomes used for quantitative karyotype analysis: 1. *Agave americana* L. and 2. *Agave striata* Zucc.

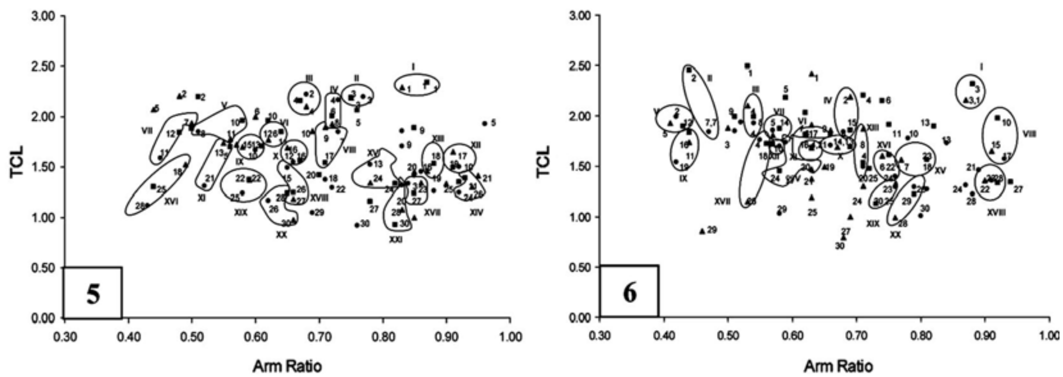
Lengths and arm ratios of respective chromosome complements of two species plotted on separate scatter diagrams are shown in Figs 3 - 4. The chromosomes of haploid complements were numbered in decreasing order of length and increasing order of arm ratio within the same length (Rhoades 1955).



Figs 3 - 4. Scatter diagrams of the respective metaphase plates: 3. *Agave americana* L. and 4. *Agave striata* Zucc (each circled point was considered to represent a homologue).

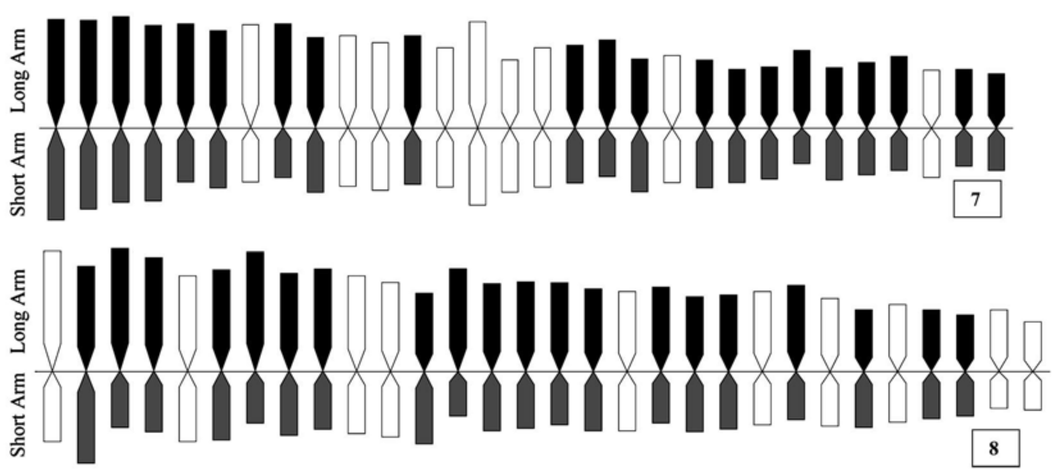
Differences were noted in respect of chromosome length, total chromatin length (TCL), centromeric position and chromosome type in both the species of *Agave*. Quantitative method helped to develop a standard karyotype. The chromosomes may not always have the same total length (Sindhu *et al*. 1982) because of variation from cell to cell and differences in fixation. The chromosome morphology varies from cell to cell and thus, major changes are associated with the cell division process.

Corresponding chromosomes in different haploid complements of each species were determined through a grouping technique applying to a combined scatter diagram of the arm ratios and standardization haploid length of chromosomes in all three cells and these are given in Figs 5 - 6.



Figs 5 - 6. Combined scatter diagram of 30 haploid chromosome values from each of the three cells: 5. *Agave americana* L. and 6. *Agave striata* Zucc. (group boundaries drawn around those chromosomes were recognizably homologous).

Each point in scatter diagram represented the specific chromosome in a particular haploid complement. The numbers of identified chromosomes were found to be only 21 in *A. americana* and only 20 in *A. striata*. Both highest (2.32 μm) and lowest (1.12 μm) mean values for identified chromosomes were found in *A. americana* (Table 1). Mean values for identified chromosomes along with statistical analysis are given in Table 1 and ideograms of two species are given in Figs 7 - 8.



Figs 7 - 8. Ideograms of the identified chromosomes: 7. *Agave americana* L. and 8. *A. striata* Zucc.

All chromosomes in three haploid components were classified in different morphological categories on the basis of total length and arm ratio within the length classes following Ahmed *et al*. (1983). The class interval 0.49 µm for length was chosen arbitrarily and the range for arm ratio as recommended by Kutarekar and Wanjari (1983) were used.

Table 1. Mean lengths (µm) and arm ratios of the identified chromosome in *Agave americana* L. and *Agave striata* Zucc. n = 3.

No. of identified chromosome	Chro-mosme type	*Agave americana* L.				*Agave striata* Zucc				
		Chromo-some length	CV (%)	Arm ratio	CV (%)	Chromo-some type	Chromo-some. length	CV (%)	Arm ratio	CV (%)
I	m	2.32 ± 0.0136	1.01	0.85 ± 0.012	2.22	m	2.26 ± 0.043	3.34	0.87 ± 0.0027	0.54
II	m	2.19 ± 0.0027	0.21	0.75 ± 0.0054	1.26	Sm	2.06 ± 0.1608	13.52	0.47 ± 0.014	5.22
III	m	2.16 ± 0.028	2.27	0.67 ± 0.0027	0.70	m	2.00 ± 0.041	3.51	0.53 ± 0.0027	0.89
IV	m	2.03 ± 0.058	4.92	0.72 ± 0.0027	0.65	m	1.96 ± 0.092	8.18	0.68 ± 0.0027	0.69
V	m	1.83 ± 0.0241	2.29	0.52 ± 0.0151	5.05	Sm	1.94 ± 0.021	1.93	0.42 ± 0.0047	1.94
VI	m	1.82 ± 0.0233	2.21	0.63 ± 0.0047	1.29	m	1.86 ± 0.023	2.19	0.66 ± 0.0141	3.71
VII	Sm	1.79 ± 0.0859	8.22	0.47 ± 0.0118	4.37	m	1.84 ± 0.015	1.43	0.57 ± 0.0027	0.83
VIII	m	1.76 ± 0.0919	9.05	0.73 ± 0.0144	3.42	m	1.73 ± 0.102	10.25	0.92 ± 0.0005	0.89
IX	m	1.73 ± 0.0262	2.63	0.61 ± 0.0309	8.78	m	1.70 ± 0.069	7.13	0.43 ± 0.0054	2.19
X	m	1.60 ± 0.0368	3.98	0.66 ± 0.0005	1.24	Sm	1.69 ± 0.0054	0.56	0.67 ± 0.072	1.86
XI	m	1.58 ± 0.1119	12.27	0.54 ± 0.0098	3.14	m	1.68 ± 0.00	0.0	0.63 ± 0.00	0.0
XII	m	1.54 ± 0.0422	4.75	0.91 ± 0.0027	0.52	m	1.65 ± 0.465	4.88	0.59 ± 0.015	4.45
XIII	m	1.48 ± 0.0205	2.40	0.87 ± 0.0047	0.94	m	1.63 ± 0.098	10.45	0.71 ± 0.00	0.00
XIV	m	1.31 0.0334	4.42	0.93 ± 0.0047	0.88	m	1.56 ± 0.00	0.0	0.79 ± 0.01088	2.38
XV	m	1.31 ± 0.0205	2.72	0.82 ± 0 .0237	5.01	m	1.56 ± 0.074	8.22	0.60 ± 0.0178	5.15
XVI	Sm	1.31 ± 0.0944	12.48	0.45 ± 0.0151	5.83	m	1.55 ± 0.422	4.72	0.75 ± 0.00047	1.08
XVII	m	1.30 ± 0.0259	3.46	0.85 ± 0.0047	0.96	m	1.54 ± 0.161	18.09	0.54 ± 0.0072	2.31
XVIII	m	1.30 ± 0.0766	10.21	0.63 ± .0151	4.17	m	1.35 ± 0.0082	1.04	0.91 ± 0.0047	0.89
XIX	m	1.25 ± 0.0151	2.09	0.65 ± .3033	14.15	m	1.24 ± 0.0482	6.42	0.75 ± 0.012	2.74
XX	m	1.13 ± 0.0634	9.42	0.64 ± 0.0098	2.65	m	1.16 ± 0.074	11.04	0.78 ± 0.0082	1.81
XXI	m	1.12 ± 0.0823	12.73	0.78 ± 0.0462	10.27	-	-	-	-	-
		$\bar{X}$ = 1.61		$\bar{X}$ = 0.70			$\bar{X}$ = 1.70		$\bar{X}$ = 0.66	

Standard length was used in plotting which resulted in vertical displacement of the points in the combined scatter diagram. The unidentified chromosomes were distributed to the various morphological classes. All the 1-30 haploid chromosome complements were numbered in decreasing order of total length and increasing order of arm ratio within each length classes following the convention of Rhoades (1955). The allocations of unidentified chromosomes of each species are given in Table 2.

Table 2. Allocation of unidentified chromosomes in two species of *Agave*.

Arm ratio classes (Y)	Mean no. of chromosomes per haploid set	No. of identified chromosomes with names	Proposed no. of unidentified chromosomes	Total no. of chromo-somes	Paired no. of identified chromosomes	Assigned no. of chromosomes
***Agave americana* L.**						
$0.76 \le Y \le 1.0$	1	1m		1	1m	1m
$0.51 \le Y \le 0.75$	0	0	0			
$Y \le 0.50$	0	0	0	0		
$0.76 \le Y \le 1.0$	1.33	0	0	0	0	(2,3,4,5,6,8,9,10)S
$0.51 \le Y \le 0.75$	7.33	6Sm	2Sm	8	II,III,IV,V,VISm	m
$Y \le 0.50$	1	1St	0	1	VIIISt	-7St
$0.76 \le Y \le 1.0$	6.33	5m	3m	8	XIXm,XXI,XXII,	(11,12,13,14,
					XXIII,XXVm	17,18,26)Sm
$0.51 \le Y \le 0.75$	0.33	4Sm	3Sm	7	XII,XVII,XVIII,	(15,16,19,20,
					XXVISm	21,22,23,25)m
$Y \le 0.50$	1.66	1St	0	1	XXIVSt	(24)St
$0.76 \le Y \le 1.0$	3	1m	1m	2	XXXm	(27,29)Sm
$0.51 \le Y \le 0.75$	2.67	2Sm	0	2	XXVII,XXIXSm	(28,30)m
$Y \le 0.50$	0.33	0	0	0	0	
	29.98	21	9	30		
***Agave striata* Zucc.**						
$0.76 \le Y1.0$	0.66	1m	0	1	2m	
$0.51 \le Y \le 0.75$	1.00	1Sm	1Sm	2	1m	1m,2m
$Y \le 0.50$	0.33	0	0	0	0	
$0.76 \le Y \le 1.0$	1.66	1m	0	1	12m	3,4,5,6,
$0.51 \le Y \le 0.75$	8	5Sm	5m,10m,11m	8	4,6,5,8,9,10,11m	7,8,9,
$Y \le 0.50$	2	2St	0	2	3Sm,7Sm	10,11,12
					19,21,25	13,14,15,16,
$0.76 \le Y \le 1.0$	5.33	3m	0	3	14,15,16,17,18,	17,18,19,20,
$0.51 \le Y \le 0.75$	7.33	6Sm	18,22,24,26	10	20,23,22,24,26	21,22,23,
$Y \le 0.50$	0.66	1St	0	1	13	24,25,26
$0.76 \le Y \le 1.0$	1.33	-	30	1	28	27,28,
$0.51 \le Y \le 0.75$	1.66	-	29	1	27	29,30
$Y \le 0.50$	0	-	0	0	0	
	29.96	20	10	30		

The standard karyotype proposed for two species of *Agave* was derived on the basis of centromeric formula, range and average chromatin length. Data regarding on chromosome morphology, i.e. length, arm ratio, relative length, TCL%, TF%, position and chromosome types are given in Table 3.

Longest chromosome pair (2.32 µm) was observed in *A. americana* and shortest (1.04 µm) in *A. striata*. Maximum chromatin length of haploid complement (49.81 µm) was found in *A. striata* and minimum (48.37 µm) in *A. americana*. However, Granick (1944) made karyotype analysis in

32 taxa of *Agave* and reported similarities and dissimilarities in chromosome morphology. In the present observation chromosome lengths ranged from 2.3 to 1.12µm in *A. americana* and 2.28 to 1.04 µm in *A. striata*.

The decrease in the total chromatin length can be one of the most important factors in the evolutionary trend (Stebbins1950). *A. americana* having minimum chromatin length (48.37 µm) indicates its primative character. However, this species shows also advance ness because of its maximum short and relatively short chromosomes. TF% is considered also for indicating the primitive and advanced nature of plant species. *A. americana* with TF% (41.51) indicates its primitive nature and *A. striata* having TF% (39.75) indicates the advanced nature.

Table 3. Analysis of unidentified chromosomes in two species of *Agave*.

Characters	V	VII	X	XI	XIII	XIV	XVI	XVII	XVIII	XX	XXIII	XXIV	XXVI	XXVIII	XXIX
***Agave americana* L. TF% = 41.51, total chromatin length = 48.37**															
Long arm length (µm)	-	1.19	1.07	0.99	1.23	0.93	0.93	0.96	-	0.84	-	-	-	0.67	-
Short arm length (µm)	-	0.63	0.68	0.72	0.89	0.68	0.68	0.64	-	0.63	-	-	-	0.57	-
Total arm length (µm)	-	1.82	1.75	1.74	1.62	1.61	1.60	1.60	-	1.53	-	-	-	1.24	-
Arm ratio	-	0.56	0.65	0.62	0.61	0.73	0.76	0.66	-	0.77	-	-	-	0.83	-
Relative chromosome length (µm)	-	78.44	75.43	75	69.82	69.39	68.96	68.96	-	65.95	-	-	-	53.45	-
TCL%	-	3.76	3.62	3.59	3.35	3.33	3.30	3.30	-	3.16	-	-	-	2.56	-
Centromeric position	-	Sm	Sm	Sm	Sm	Sm	m	Sm	-	m	-	-	-	m	-
***Agave striata* Zucc. TF% = 39.75, total chromatin length = 49.81**															
Long arm length (µm)	1.09	-	1.09	1.02	-	-	-	0.95	0.92	-	0.99	0.84	0.77	-	0.70
Short arm length (µm)	0.81	-	0.72	0.75	-	-	-	0.68	0.68	-	0.55	0.62	0.58	-	0.42
Total arm length (µm)	1.97	-	1.81	1.75	-	-	-	1.63	1.60	-	1.54	1.45	1.35	-	1.12
Arm ratio	0.63	-	0.66	0.73	-	-	-	0.71	0.75	-	0.54	0.73	0.75	-	0.58
Relative chromosome length (µm)	86.40	-	79.38	76.75	-	-	-	71.49	70.18	-	67.54	63.59	59.21	-	49.12
TCL%	3.95	-	3.63	5.51	-	-	-	3.27	3.21	-	3.09	2.91	2.71	-	2.25
Centromeric position	Sm	-	Sm	Sm	-	-	-	Sm	Sm	-	Sm	Sm	Sm	-	Sm

Findings in the present study indicate that if any structural changes played a role in evolution of the two species of *Agave*, this did not change the karyotype in any significant manner. However, the proposed karyotype in the present study indicates that the evolution in the two species might have occurred due to change in the chromosome structure minutely.

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