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Structural Differences of Chromosomes in Diploid *Agave*

Suchitra Banerjee[1] (nee Chattopadhyay) and A. K. Sharma

Centre of Advanced Study in Cell and Chromosome Research,
Department of Botany, University of Calcutta,
Calcutta-700019, India

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The genus *Agave* is well known for its commercial importance and medicinal values (Latorre and Latorre 1977, Blunden *et al.* 1980, Sheldon 1980). It is widely distributed in the tropics and principally cultivated in the dry region of the plains.

The occurrence of polyploidy with a constant basic set of n=30 chromosomes has already been reported in this genus (Doughty 1936, Sato 1938, 1942, Granick 1944, Bhattacharya and Ghosh 1977). Bimodal karyotype with five long and twentyfive short chromosomes in the haploid complement is rather a constant unique characteristic of this genus. The importance of chromosome analysis as a pre-requisite for identification and improvement of genotype is well established.

The present paper deals with a critical cytological analysis of five different species of this genus. In view of the constant basic number and bimodal karyotype in this genus, it was thought worthwhile to study the extent to which these species differ in relation to the details of chromosome morphology. Such an investigation may give an insight into the role of structural alterations in speciation.

Materials and methods

The present investigation deals with the detailed somatic chromosomal analysis of five different species of *Agave*, namely; *Agave filifera*, *A. perigrina*, *A. angustifolia* Haw. var. *marginata* Hort., *A. fourcroydes* and *A. americana*, collected from different parts of India.

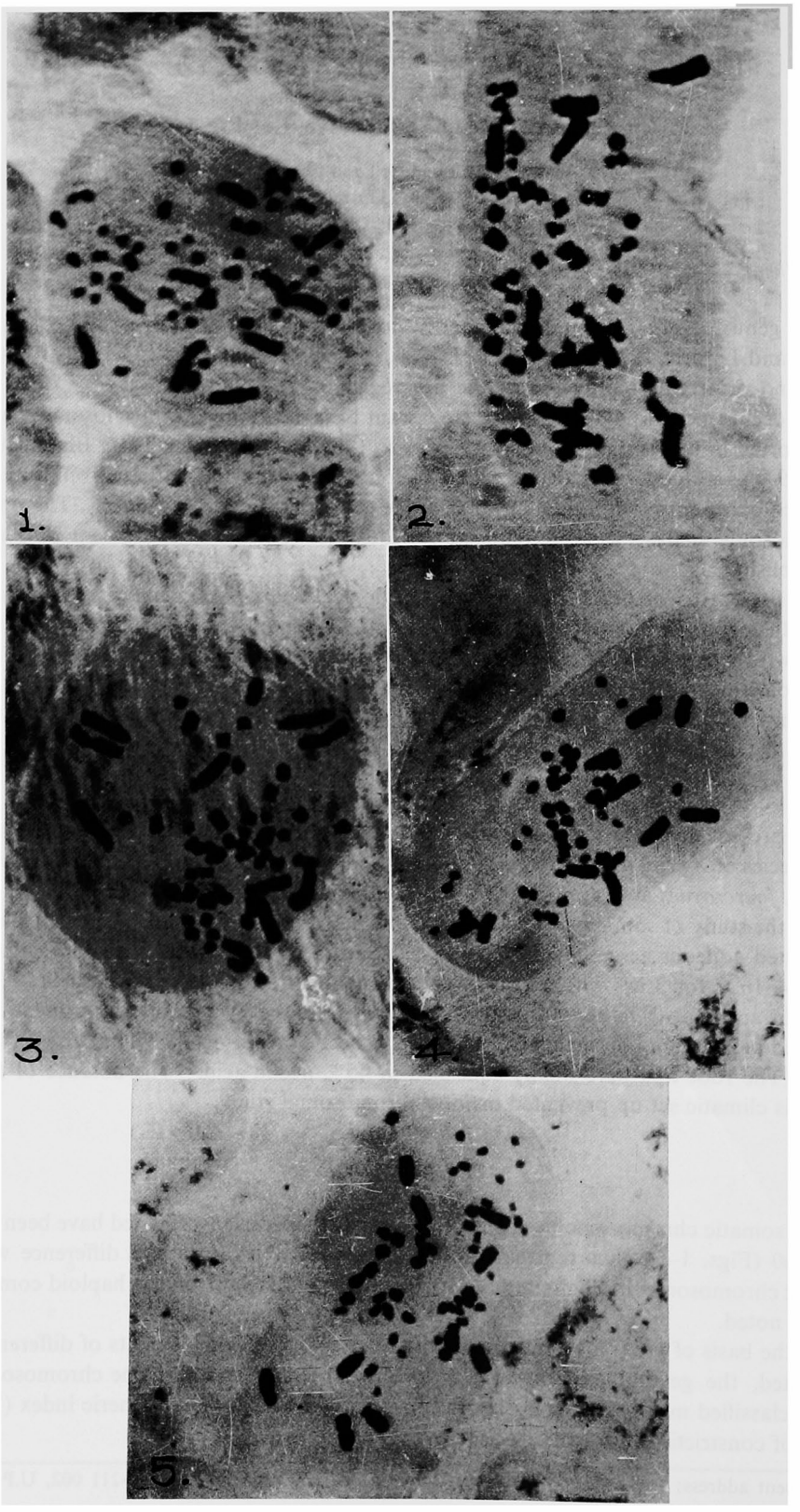
For the study of somatic chromosomes, fresh, healthy root tips were pretreated in a mixture of saturated aqueous aesculin soultion and half saturated aqueous paradichlorobenzene solution at 14–16°C for 3 hrs with an initial shock treatment at 0–5°C for 5 minutes. Overnight fixation in acetic acid—ethanol mixture (1: 3) was followed by 5 minutes treatment with 45% acetic acid and staining by slight heating with 2% acetic orcein—(N) HCl mixture (9: 1) for 2 to 21 hrs. The root tips were finally squashed in 45% acetic acid. The absence of flowering under this climatic set up prevented meiotic chromosomal study.

Observations

The somatic chromosome numbers of the different species investigated have been found to be 2n=60 (Figs. 1–5), thus representing a diploid series. Abrupt size difference with long and short chromosomes in a definite proportion of 5 long: 25 short in the haploid complement, has been noted.

On the basis of gross morphological similarities between complements of different species investigated, the general chromosome types have been described. The chromosome types (Fig. 6), classified mainly on the basis of centromeric position or centromeric index (F%) and number of constrictions present, are as follows:

[1] Present address: Department of Botany, University of Allahabad, Allahabad-211 002, U.P., India.


1.
2.
3.
4.
5.

Type A: Long chromosome with two constrictions located at the same side of the longer arm. The distal segment is smaller than the middle one.

Type A′: Long chromosome with two constrictions, almost similar to type A, only the distal and the middle segments are of equal sizes.

Type A″ Long chromosome with two constrictions located at the same side of the longer arm, the middle segment being smaller than the end one.

Type B: Long chromosome with two constrictions located at the two opposite ends of the chromosome. The middle segment is much larger than the distal ones, which are of equal size.

Type C: Long chromosome with two constrictions located at the two opposite ends of the chromosome. The middle segment is longer than the other two, one of which is slightly larger than the other one.

Type C′: It is similar to chromosome type-C, only the size difference of the two distal segments is more marked. An end segment is distinctly larger than the other one, though the middle segment is still larger than the two distal ones.

Type C″: Long chromosome with two constrictions. Distal segment is smaller than the two other arms which are more or less of equal size.

Type D: Long chromosome with nearly subterminal primary constriction.

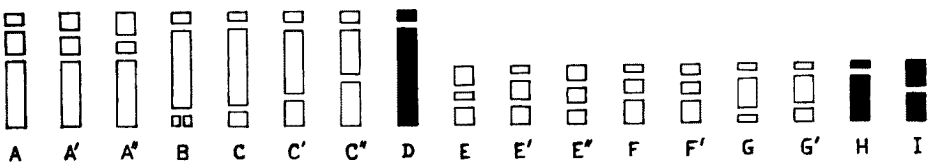


Fig. 6. Diagrammatic representation of common chromosome types present in different species and varieties of *Agave*. ×3300 approx.

Type E: Short chromosome with two constrictions. The middle segment is smaller than the distal ones which are of equal size.

Type E′: Short chromosome with two constrictions. Distal segment is smaller and the two other arms are equal in size.

Type E″: Short chromosome with two constrictions; all the three segments are of equal size.

Type F: Short chromosome with two constrictions located on the same side of the long arm. One end segment is smaller than the middle one.

Type F′: Short chromosome with two constrictions located at the same side of the long arm. The distal and the middle segments are of equal size.

Type G: Short chromosome with two constrictions located at the two opposite ends of the chromosome. The middle segment is much longer than the end ones, which are equal in size and very small.

Type G′: Short chromosome, similar to type G. Only the two distal segments differ in size, one being slightly larger than the other.

Type H: Short chromosome with nearly submedian primary constriction.

Type I: Short chromosome with median to nearly median primary constriction.

The karyotype formula for each species has been derived on the basis of the number of different chromosomal types that are present. The detailed cytological characters of these five

Figs. 1–5. Somatic metaphases showing 2n=60 chromosomes. 1, *A. filifera*. 2, *A. perigrina*. 3, *A. angustifolia* Haw. var. *marginata* Hort. 4, *A. fourcroydes*. 5, *A. americana*. ×1580.

species investigated are represented in Tables 1 and 2.

The karyograms (Fig. 7) of the different species differ from each other, despite the fact that these species represent the same diploid number, thus showing a wide range of structural alterations at the interspecific level.

Numerical alterations of the nucleolar chromosomes have also been noted. Amongst these species, *A. fourcroydes* has six nucleolar chromosomes while the rest have eight.

In respect of the total chromosome length and total chromosome volume as well, these species differ to a certain extent (Table 2). The total F% values (TF%) are more or less similar in all the species studied. Variation from the normal somatic chromosome number has been noted in very low frequency in all the species studied (Table 1), which has been recorded in other vegetatively reproducing species as well.

Table 1. Somatic chromosome number, variation number and karyotype formula in species and varieties of *Agave*

Name of the species	Somatic chromosome number (2n)	No. of chromosomes bearing secondary constriction	Somatic variation nuclei (2n)	Percentage of variant nuclei (%)	Karyotype formula
Agave filifera	2n=60	8	2n=36, 48, 58	2	$A_2B_2D_6E_2F_2H_{44}I_2$
Agave perigrina	2n=60	8	2n=36, 40, 120	4	$B_4C'_2D_4G_2H_{46}I_2$
Agave angustifolia Haw. var. *marginata* Hort.	2n=60	8	2n=45, 26, 36	1.5	$C_2C'_2D_6F_2F'_2H_{32}I_{14}$
Agave fourcroydes	2n=60	6	2n=65	0.5	$B_4C'_2D_4H_{50}$
Agave americana	2n=60	8	Not found	—	$A_2B_2C_2D_4F_2H_{26}I_{22}$

Table 2. The values of different cytological parameters in species and varieties of *Agave*

Name of the species	Somatic chromosome number (2n)	Total chromosome length (μm)	Range of chromosome length (μm)	TF% value	Total chromosome volume (cu. μm)	Range of chromosome volume (cu. μm)
Agave filifera	2n=60	121.90±0.19	0.91–5.45	24.87	104.83±0.22	0.21–6.94
Agave perigrina	2n=60	99.08±0.15	0.68–4.55	23.09	120.59±0.28	0.17–7.66
Agave angustifolia Haw. var. *marginata* Hort.	2n=60	129.98±0.20	1.09–5.91	29.05	127.32±0.30	0.25–8.64
Agave fourcroydes	2n=60	103.72±0.13	0.91–4.55	22.29	102.26±0.25	0.32–7.09
Agave americana	2n=60	113.20±0.16	1.00–5.23	30.05	97.07±0.24	0.20–7.44

Discussion

The present investigation of five different species of *Agave* reveals diploidy with 2n=60 chromosomes. As far as the previous records go, the range of chromosome number in species of *Agave* varies from 2n=60 to 180 (Doughty 1936, Granick 1944, Bhattacharya and Ghosh 1977). Occasional aneuploids have also been reported (Cave 1964).

The critical karyotype analysis shows differences between the different species in minute details of the chromosome structure. Such differences, as the analysis shows, involved mostly chromosomes with secondary constrictions—both long and short ones. It is clearly manifested in the karyotype formulae (Table 1, Fig. 7) as well. However, in addition to these detectable changes, there might have been cryptic alterations which could not be fully resolved. Despite

the application of refined techniques, the small size of the chromosomes often did not permit the identification of chromosome segments to the extent it is desired. The application of suitable banding technique may bring out further details.

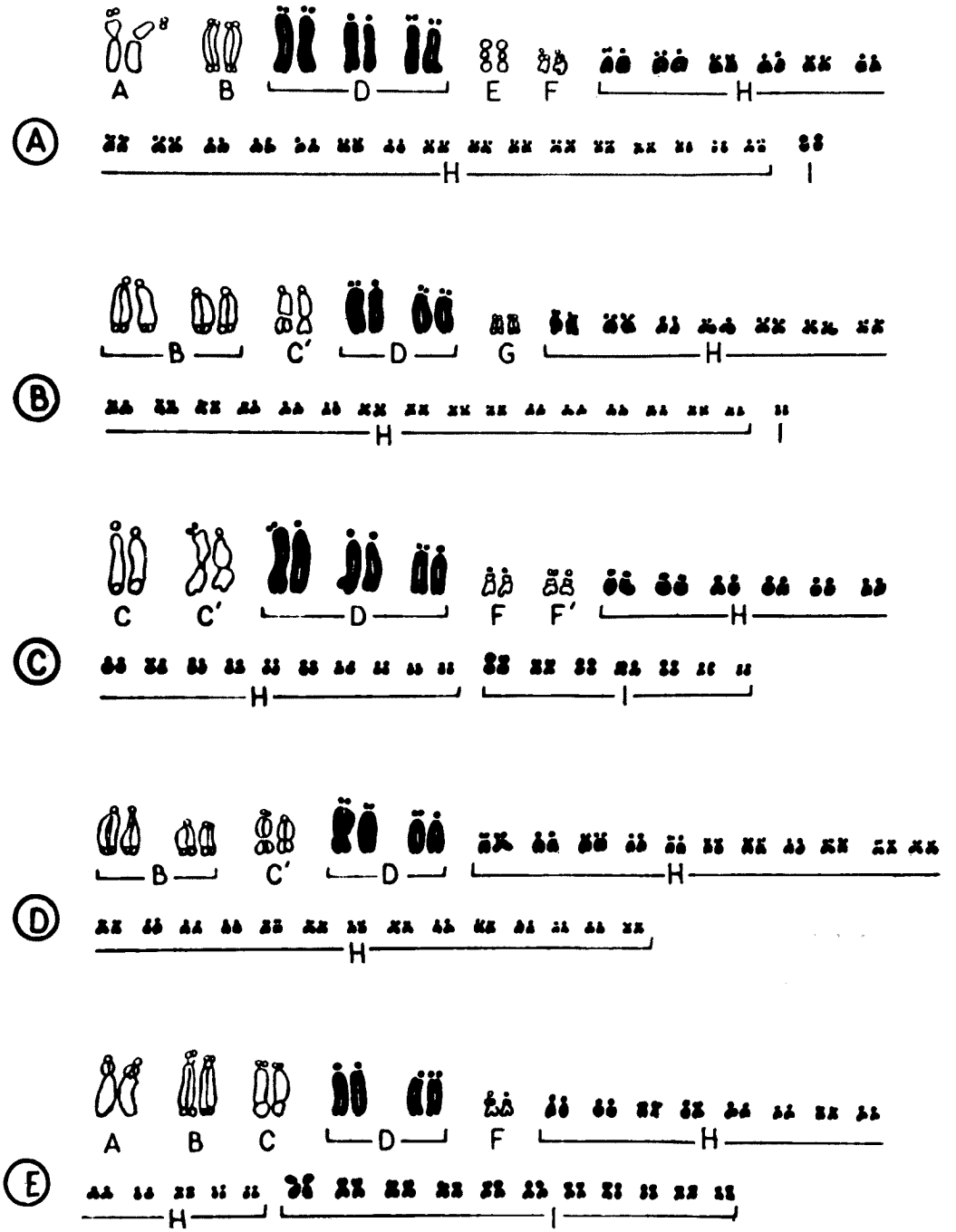


Fig. 7. Comparative karyograms of different species and varieties of *Agave*. A, *A. filifera* (2n=60). B, *A. perigrina* (2n=60). C, *A. angustifolia* Haw. var. *marginata* Hort. (2n=60). D, *A. fourcroydes* (2n=60). E, *A. americana* (2n=60). ×1830.

Inspite of the same chromosome number in all the five species studied, the alteration in karyotype formulae at the interspecific level is a clear index of the role of structural alterations in evolution of species in this genus (vide Sharma and Bhattacharyya 1962). The extent to which such structural alterations have attained homozygosity in evolution is difficult to state in view of the absence of flowering under this climatic set up. However, it is quite likely that they have been retained in view of the presence of basikaryotype (5: 25 ratio in haploid complement) and vegetative reproduction of the species. Somatic mosaicism has been recorded in other species as well and it is not unlikely that the entrance of variant nuclei in the daughter shoots has been responsible for the origin of new genotypes.

Summary

Cytological studies have been carried out on five different species of *Agave*, collected from different parts of India. All of them revealed diploidy having $2n=60$ chromosomes. A distinct bimodal karyotype with very long and very short chromosomes in a definite proportion of 5: 25 ratio is the most significant characteristic of this genus. However, the karyotype formulae, as brought out in the present study differ quite distinctly amongst the species investigated. Such variations principally involve the nucleolar chromosomes of both long and short types. The total chromosome lengths and volumes also differ at the interspecific level. Structural alterations along with differential degrees of coiling of chromosomes are the suggested factors principally responsible for such variations.

Acknowledgements

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