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Cytophotometric Estimation of Nuclear DNA in Different Species and Varieties of *Agave*

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Several taxa have recently been subjected to nuclear DNA estimation through cytophotometry (Iyengar and Sen 1978, Ohri *et al.* 1981, Resslar *et al.* 1981, Sharma and Chattophadhyay 1983, Sharma and Mukhopadhyay 1984, Mukhopadhyay and Sharma 1984). Such *in situ* estimation often provides a distinctive parameter for species differentiation (Chooi 1971, Ayonoadu 1974, Bennett *et al.* 1977, Rees *et al.* 1978, Raina and Rees 1983, Seal 1983, Sharma and Mukhopadhyay 1984). However, cases are also on record where the DNA values vary even at an intraspecific level (Miksche 1971, Furuta *et al.* 1975, Narayan and Rees 1976, Price *et al.* 1980, Sharma and Chattopadhyay 1983). In this regard, the role of repetitive fraction has been emphasized by different authors as well (Bullen and Rees 1972, Rees and Jones 1972, Rees *et al.* 1979, Resslar *et al.* 1981).

The nuclear DNA estimation in *Agave*, a steroidal sapogenin yielding plant growing widely in India, was thought desirable in view of the commercial importance of this group as well as indication of cytological races already observed in this genus (Chattopadhyay 1983). Nine different species and varieties of *Agave* were subjected to the present investigation where the amounts of 4C nuclear DNA were estimated through *in situ* cytophotometric method and its correlation, if any, with different cytological parameters was analysed.

Materials and methods

In course of the present investigation, relative amounts of nuclear DNA were quantitatively estimated through *in situ* cytophotometric method in 9 different species and varieties of *Agave*, namely, *Agave filifera*, *A. perigrina*, *A. angustifolia* Haw. var. *marginata* Hort., *A. fourcroydes*, *A. americana* L., *A. tequilana*, *A. americana* L. var. *marginata* Trelease, *A. sisalana*, *A. decipiens*. Cytological studies involving karyotype analysis and determination of total chromosome lengths and volumes were also carried out in these plants.

For karyotype analysis and determination of chromosome lengths and volumes, fresh, healthy root tips were pretreated in saturated paradichlorobenzene-aesculin solution for 3–6 hrs. at 10–16°C. This was followed by overnight fixation in acetic acid: ethanol (1: 3) mixture and standard acetic-orcein: 1N HCl (9: 1) staining procedure. The chromosome volume was determined from metaphase plates, assuming the chromosome as cylindrical structure by the following formula:

$$\text{Chromosome volume } (V) = \pi r^2 h$$

where, r=radius of the chromosome=Breadth/2; h=total length of the chromosome. The total chromosome volume was then calculated by adding the volumes of all the chromosome complements.

The relative amounts of *in situ* nuclear DNA were quantitatively estimated in arbitrary units through single wave length method from 4C nuclei of the somatic cells. Fresh and

healthy root tips were fixed in acetic acid-ethanol mixture (1 : 2) at 12–15°C for 2 hours, washed with distilled water and hydrolysed in 1N HCl at 60°C for 10 minutes. After washing thoroughly in distilled water, the root tips were treated with 45% acetic acid for 5 minutes and finally stained in standard Feulgen staining solution (Schiff's reagent) in a dark and cool place for $1^1/_2$ hrs. The tip portions of the roots were finally squashed in 45% acetic acid and the transmittance values of 40 different cells of each species were recorded in a Reichert Zetopan microspectrophotometer through single wave length method (550 nm) (Sharma and Sharma 1980), after adjusting the blank at 100. The radius of the aperture for the transmittance of light was kept constant at '6' for all the species studied. For confirmation, fixation of the root tips in neutral formalin (40%) was also carried out under the same experimental set up. The relative absorbances were calculated on the basis of optical density to indicate the relative amounts of DNA in terms of arbitrary units.

Observations

The somatic chromosome numbers of 9 different species and varieties of *Agave* were found to range from 2n=60 to 180. Five of the nine species revealed 2n=60 chromosomes representing a diploid series. Amongst the rest, 2n=90, 120, 150 and 180 chromosomes were recorded in one each, which represented the triploid, tetraploid, pentaploid and hexaploid levels respectively. Each species has its own distinct karyotype (Table 1). The karyotype formulae of the different species revealed extensive structural alterations of chromosomes, specially involving the ones bearing secondary constrictions (Fig. 1). Inspite of such wide structural changes, the basic proportionality in the bimodal karyotype of 5 long: 25 short chromosomes of the haploid complement was maintained upto the highest ploidy level.

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

Name of the species	Somatic chromosome number (2n)	Karyotype formula
Agave filifera	2n=60	$A_2B_2D_6E_2F_2H_{44}I_2$
Agave perigrina	2n=60	$B_4C_2'D_4G_2H_{46}I_2$
Agave angustifolia Haw. var. *marginata* Hort.	2n=60	$C_2C_2'D_6F_2F_2'H_{32}I_{14}$
Agave fourcroydes	2n=60	$B_4C_2'D_4H_{50}$
Agave americana	2n=60	$A_2B_2C_2D_4F_2H_{26}I_{22}$
Agave tequilana	2n=90	$A_2''C_3C_3'D_{1(3)+2(2)}E_4'F_2'H_{5(3)+3(2)}I_{10(3)+9(2)}$
Agave americana L. var. *marginata* Trelease	2n=120	$A_4C_4C_2'C_2''D_8G_2H_{72}I_{26}$
Agave sisalana	2n=150	$A_2B_2C_5'D_{2(5)+3(2)}F_5'G_2'H_{3(5)+20(2)}I_{4(5)+9(2)}$
Agave decipiens	2n=180	$A_2A_2'B_6C_4'C_2''D_{14}E_2'F_4H_{108}I_{36}$

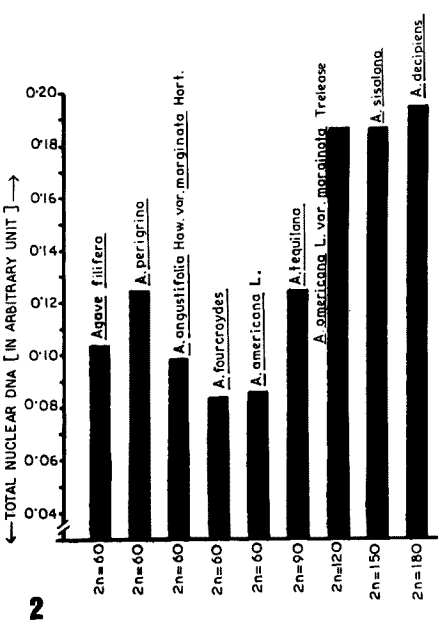
In the total chromosome length, even the five diploid species showed considerable variations among themselves, the minimum and maximum values being 99.08 μm and 129.89 μm in *A. perigrina* and *A. angustifolia* respectively. The total chromosome lengths of the triploid, tetraploid, pentaploid and hexaploid species were not the exact multiples of the diploid set (Table 2).

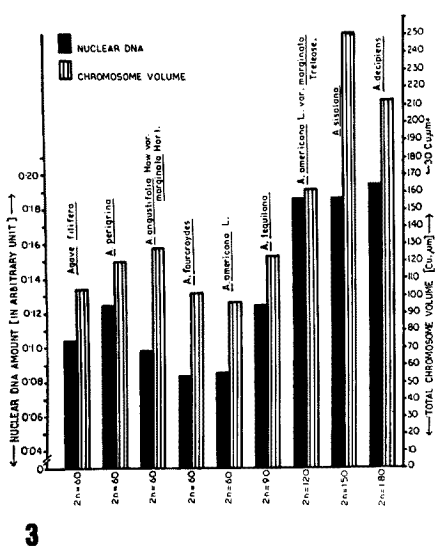
Remarkable variations in total chromosome volume were noted among these species. In certain cases, however, species with higher chromosome numbers revealed lesser volumes than those having lesser number of chromosomes. For example, in *A. tequilana*, where the 2n num-

Fig. 1. Diagrammatic representation of common chromosome type present in different species and varieties of *Agave*.

Table 2. The amount of nuclear DNA of different species of *Agave* along with the values of other cytological parameters

Name of the species	Somatic chromosome number (2n)	Total chromosome length (μm)	Total chromosome volume (cu. μm)	Nuclear DNA amount (in arbitrary units)
Agave filifera	2n=60	121.90±0.19	104.83±0.22	0.1042±0.0023
Agave perigrina	2n=60	99.08±0.15	120.59±0.28	0.1250±0.0020
Agave angustifolia Haw. var. *marginata* Hort.	2n=60	129.98±0.20	127.32±0.30	0.0993±0.0024
Agave fourcroydes	2n=60	103.72±0.13	102.26±0.25	0.0843±0.0016
Agave americana	2n=60	113.20±0.16	97.07±0.24	0.0863±0.0016
Agave tequilana	2n=90	158.80±0.11	123.82±0.13	0.1245±0.0023
Agave americana L. var. *marginata* Trelease	2n=120	225.26±0.12	162.40±0.15	0.1865±0.0027
Agave sisalana	2n=150	282.14±0.12	251.77±0.15	0.1870±0.0026
Agave decipiens	2n=180	319.82±0.10	213.42±0.10	0.1945±0.0027





Figs. 2–3. 2, bar diagram showing the amount of nuclear DNA in several species and varieties of *Agave*. 3, comparative bar diagram representing the amount of nuclear DNA and total chromosome volume in various species and varieties of *Agave*.

ber was 90, the total chromosome volume was 123.82 cu. μm, whereas in *A. angustifolia* Haw. var. *marginata* Hort. with 2n=60 chromosomes, the value was 127.32 cu. μm. Again in *A. decipiens* with 2n=180 chromosomes, the total chromosome volume was 213.42 cu. μm whereas in *A. sisalana* with 2n=150 chromosomes, the value was 251.77 cu. μm (Table 2 and Fig. 3).

The DNA values of these species along with other cytological parameters are represented in Table 2 and Figs. 2, 3.

At the diploid level, the variation in the amount of nuclear DNA was not much prominent, the minimum and maximum values being 0.0843 and 0.1250 in arbitrary units.

In the triploid species *A. tequilana*, the amount of nuclear DNA was 0.1245 units, which was lesser than that of the diploid species *A. perigrina* where the value was 0.1250 units.

In comparison to the difference in chromosome number, the difference in the amount of nuclear DNA was remarkably insignificant between *A. americana* L. var. *marginata* Trelease and *A. sisalana* having 2n=120 and 150 chromosomes respectively.

So far as the amounts of nuclear DNA and the cytological parameters were concerned, no definite correlation could be established between total chromosome lengths, volumes and the amounts of nuclear DNA. This could be exemplified by the fact, that in the diploid species *A. perigrina*, the total chromosome length was 99.08 μm, the total chromosome volume was 120.59 cu. μm and the amount of nuclear DNA was 0.1250 units, whereas in the triploid species *A. tequilana*, the values were 158.80 μm, 123.82 cu. μm and 0.1245 units respectively. parative histogram would also reveal the same fact in the other species under consideration as The comwell (Fig. 3).

Discussion

Of the 9 different taxa studied, 60 chromosomes have been obtained in five, whereas 90, 120, 150 and 180 chromosomes have been found in the rest. It has been shown that along with polyploidy there have been extensive structural alterations of the chromosomes principally involving those with secondary constrictions (Table 1). Structural changes in other chromosomes too, have not been ruled out. Inspite of a gross homogeneity in the bimodal karyotype, each one of them has distinct karyotype of its own, differing from the rest in minute details.

Chromosome length, volume, karyotype and the nuclear DNA amount

The total chromosome length varies even between diploids and in polyploids. It does not necessarily show an exact multiple. The same fact holds good for total chromosome volume as well (Table 2). All these factors clearly indicate that there have been extensive structural alterations along with differential degree of coiling of chromosomes amongst diploid and polyploids—a process which is under genetic control.

At the diploid level the range of the amount of nuclear DNA is not very high, varying between 0.0843 to 0.1250 in arbitrary units (Table 2). The amount of DNA is not necessarily correlated with the chromosome length. This is indicated by the fact that the total chromosome length in *A. fourcroydes* and *A. angustifolia* is 103.72 μm and 129.98 μm as compared to *A. perigrina* where it is 99.08 μm. On the other hand, the amount of nuclear DNA is highest in the latter (0.1280) as compared to 0.0843 and 0.0993 of the formers (Table 2). Evidently, in such cases the chromosome length is affected by genetically controlled mechanism of spiralisation. It is, however, remarkable that the nuclear DNA amount is quite distinct for the species as observations were carried out on a large number of individuals of each clone. The correlated structural alterations of chromosomes, as recorded in karyotypes, may have a bearing on this issue. As far as the four other taxa are concerned having triploid, tetraploid, pentaploid and hexaploid numbers, the amounts of DNA present a very interesting feature. For example,

in the species with 120 and 150 chromosomes the amount of nuclear DNA is more or less identical, indicating once more the importance of structural changes of chromosomes in evolution. This is the reason why the nuclear DNA does not show an exact multiple in its amount from diploid to higher degrees of polyploidy. The present study on the nuclear DNA content in different species of *Agave* has clearly brought out the distinctive value of DNA, even amongst taxa of same level of ploidy and the influence of structural alterations in affecting its amount from diploid to hexaploid level.

Summary

A detailed cytological and cytochemical investigation involving determination of somatic chromosome number, karyotype, total chromosome length, volume and estimation of 4C nuclear DNA were carried out in 9 different species and varieties of *Agave*. Of the nine species studied, five belonged to diploid level with 2n=60 chromosomes and the rest were triploid, tetraploid, pentaploid and hexaploid having 2n=90, 120, 150 and 180 chromosomes respectively. Inspite of a distinct bimodal karyotype with very long and very short chromosomes in the 5: 25 ratio and a constant base number of n=30 chromosomes featured in the detailed karyotype analysis, each species has got its own distinct karyotype, differing from the rest in minute details. The total chromosome lengths and volumes differed both within and outside the same ploidy level and the volume did not necessarily increase with the increase in chromosome number.

The amount of 4C nuclear DNA, estimated through Feulgen microspectrophotometry and expressed in arbitrary unit of relative absorbances, did not show much variations in the diploid species, while the triploid species revealed slightly lesser value than that of one specific diploid species. The difference in the amounts of nuclear DNA was insignificant amongst the pentaploid and hexaploid species as compared to the difference of their chromosome numbers. No direct correlation could, however, be established between the cytological parameters and the amounts of nuclear DNA. On the basis of overall analysis, the influence of structural alterations in affecting the amounts of nuclear DNA as well as other cytological characters from diploid to hexaploid level has been suggested.

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