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A STUDY OF QUANTITATIVE VARIATIONS OF NUCLEIC ACIDS IN *GOSSYPIUM**

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Abstract—The DNA and RNA content of fifteen diploid species representing the six genomes of *Gossypium* were determined and compared statistically with one another. The DNA content of the species ranged from 59 to 199 μg per gram of seed embryo and followed the genomic pattern C, F, E, B, A, D, with respect to order of decreasing chromosomal size. There was, however, no distinct correlation between RNA content and species or genome grouping. The amphidiploids exhibited a DNA content intermediate between that of the A and D genomes.

INTRODUCTION

ALL OF the thirty diploid species of *Gossypium* recognized by botanists are separated into six genomic classes (A–F) on the basis of their morphological characters, geographic origin and crossing behavior. There are also three amphidiploid species, two cultivated and one wild, that contain chromosomes from both the A and D genomes.

In addition to the extensive taxonomic and cytological investigations that have led to this genomic classification, recent studies of gossypol content,[1] DNA composition,[2] amino and fatty acids,[3] free and protein-bound amino acids,[4] and i.r. spectra of acetone–hexane–water extracts of hexane-defatted meals[5] were also undertaken to either clarify or establish new criteria within this classification. None of these investigations produced results that cast any serious doubt on the original cytotaxonomic groupings of the cotton species. Indeed, the data from analysis of the DNA base ratios,[2] free amino acid patterns,[4] and i.r. spectra[5] were confirmatory.

It was of interest, therefore, to examine both the DNA and RNA content of representative diploid and amphidiploid species of *Gossypium* and to evaluate the results in light of the present genomic classification.

RESULTS AND DISCUSSION

The DNA content of the species investigated in this study varies significantly as seen from the analysis of variance presented in Table 1. To designate the individual or groups of species that differ from one another, the DNA content of each species is tabulated in descending order along with the results of a Duncan multiple range analysis in Table 2. Also included in this table are the corresponding genome classifications for each species.

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[2] D. R. ERGLE, F. R. H. KATTERMAN and T. R. RICHMOND, *Plant Physiol.* **39**, 145 (1964).
[3] F. L. CARTER, A. E. CASTILLO, V. L. FRAMPTON and T. KERR, *Phytochem.* **5**, 1103 (1966).
[4] P. SARVELLA and B. J. STOJANOVIC, *Can. J. Genet. Cytol.* **10**, 362 (1968).
[5] A. S. EL-NOCKRASHY, J. G. SIMMONS and V. L. FRAMPTON, *Phytochem.* **8**, 1949 (1969).

TABLE 1. ANALYSIS OF VARIANCE OF DNA AND RNA CONTENT OF DEFATTED COTTON EMBRYOS FROM EACH OF THE SPECIES

Nucleic acid	Variance
DNA	0·034*
RNA	0·235*

* $P > 0{\cdot}01$.

TABLE 2. SEPARATION OF DNA CONTENT MEANS BY DUNCAN'S MULTIPLE RANGE TEST*

Gossypium species	DNA content†		Genome classification
sturtianum	1·99	a	C_1
bickii	1·98	a b	C
longicalyx	1·54	c	F_1
stocksii	1·52	c d	E_1
tomentosum	1·47	c d e	$(AD)_3$
somalense	1·46	c d e f	E_2
hirsutum	1·45	c d e f g	$(AD)_1$
arboreum	1·32	e f g h	A_2
barbadense	1·20	h i	$(AD)_2$
anomalum	1·12	i j	B_1
lobatum	1·09	i j k	D_7
aridum	1·02	j k l	D_4
gossypioides	0·86	l m	D_6
herbaceum	0·84	m n	A_1
thurberi	0·81	m n o	D_1
klotzschianum	0·78	m n o p	D_3
raimondii	0·67	n o p q	D_5
armourianum	0·59	q r	D_2

* Separations based on $P > 0{\cdot}01$.
† Milligrams of DNA/gm defatted seed embryos.

Apart from the tetraploid AD species, all but one of the diploid species are grouped in genome classes and follow the order C, F, E, A, B, D, with respect to decreasing DNA content. The differences that contribute to the significant variance appear to exist between genome groupings as a whole. Several of the species within the D genome, however, exhibit significant differences on an individual basis.

Upon examination of the RNA content, a significant variance between individual and groups of species was also noted (Tables 1 and 3). In contrast to the DNA data, however, there was no distinct correlation between species or genome grouping and RNA content.

Since there are no prior data on nuclear volume measurements in *Gossypium*, Table 4 presents the relative chromosomal sizes of these genome groupings based upon the best information available.[6] The order of decreasing size with respect to genome groupings is C, E and F, B, A, D; except for the order of the B and A genomes (whose chromosomes are approximately equal in size), our data indicate a definite correlation between DNA content and chromosomal size.

[6] M. S. BROWN, private communication (1969).

TABLE 3. SEPARATION OF RNA CONTENT MEANS BY DUNCAN'S MULTIPLE RANGE TEST*

Gossypium species	RNA content†		Genome classification
lobatum	14·79	a	D_7
somalense	14·36	a b	E_2
bickii	13·94	a b c	C
barbadense	13·89	a b c d	$(AD)_2$
thurberi	13·71	a b c d e	D_1
gossypioides	13·65	a b c d e f	D_6
stocksii	13·47	a b c d e f g	E_1
sturtianum	13·29	b c d e f g h	C_1
tomentosum	13·02	b c d e f g h i	$(AD)_3$
aridum	12·77	c d e f g h i j	D_4
hirsutum	12·72	c d e f g h i j k	$(AD)_1$
anomalum	12·70	c d e f g h i j k l	B_1
arboreum	12·08	h i j k l m	A_2
klotzschianum	11·65	j k l m n	D_3
raimondii	11·56	j k l m n o	D_5
armourianum	11·54	j k l m n o p	D_2
longicalyx	11·54	j k l m n o p q	F_1
herbaceum	11·48	j k l m n o p q r	A_1

* Separations based on $P > 0{\cdot}01$.
† Milligrams of RNA/g defatted seed embryos.

TABLE 4

Relative chromosome size in *Gossypium*

C species—very large
E and F species—large—slightly larger than A genome
B species—large—some slightly larger than A genome
A species—moderately large
D species—small

Compared to some species (e.g. the Liliaceae) *Gossypium* chromosomes are not large; that is, the largest are only two or three times the size of the smallest.

This relationship is reflected quite well in the tetraploid AD species. Although the DNA content of both tetraploid forms differ significantly from one another (probably because only two of the North American cultivated varieties were sampled), the average amount of DNA from these species, however, does fall somewhat near the median value of the diploid array. This is due to the amount of DNA contributed by the twenty-six large A and twenty-six small D chromosomes that make up the tetraploid.

Earlier as well as recent studies on other angiosperms[7–9] have revealed that nuclear volume and DNA content are directly proportional to one another; i.e. closely related species exhibit little to no significant variation in both of these parameters while distantly related species vary consistently. Of interest also is whether the larger nuclei of *Gossypium*

[7] J. MCLEISH and N. SUNDERLAND, *Exptl. Cell Res.* **24**, 527 (1961).
[8] A. H. SPARROW and J. P. MIKSCHE, *Science* **134**, 282 (1961).
[9] H. REESE, F. M. CAMERON, M. H. HARAZIKA and G. H. JONES, *Nature* **211**, 828 (1966).

have resulted from a lengthwise duplication or by an increased condition of strandedness (polynemy) of the chromosomal segments. If lengthwise duplication were the principal contribution to nuclear size, one would expect to observe chromosomal loops at pachytene in a hybrid strand.[9] However, Brown[10,11] reported that in hybrids between species of *Gossypium* with large and small chromosomes, the pachytene chromosomes were closely paired and equal in length with no evidence in loops. It is possible that the relative proportions of eu- and hetero-chromatin might contribute to different sizes of the genome, but this would clearly be untenable should they prove to be simply different physical states (see, e.g. Brown).[12] Therefore, it appears most likely that, apart from the AD species which are polyploid, the larger nuclei result from a process of polynemy.

EXPERIMENTAL

Four out of the eighteen species used are cultivated varieties. The diploids, *Gossypium herbaceum* and *G. arboreum*, originated in South Central Asia and are referred to as Old World cottons. The other two cultivated cottons, *G. hirsutum* and *G. barbadense*, are tetraploids and represent upland varieties native to North America.

Freshly decorticated embryos of the *Gossypium* species were Soxhlet extracted with petroleum ether and then with acetone until free of oil and soluble pigments. The oven-dried (70°) residue was ground to 80 mesh fineness preparatory to the extraction of the nucleic acids.

RNA and DNA were extracted and separated by the procedure of Schmidt and Thannhauser[13] as modified by Bonner and Zeevaart.[14] The RNA and DNA in the extracts from 200-mg embryo samples were determined as follows: RNA content was determined by referring the 260 and 290 nm absorption differential to a standard curve prepared with yeast RNA. DNA was determined by the diphenylamine procedure[15] using purified cotton DNA[16] as the standard.

[10] M. S. BROWN, *Genetics* **39**, 962 (1954).
[11] M. S. BROWN, *Am. J. Botany*, **48**, 532 (1961).
[12] S. W. BROWN, *Science* **151**, 417 (1966).
[13] B. SCHMIDT and S. J. THANNHAUSER, *J. Biol. Chem.* **161**, 83 (1945).
[14] J. BONNER and J. A. D. ZEEVAART, *Plant Physiol.* **3**, 43 (1962).
[15] K. BURTON, *Biochem. J.* **62**, 315 (1955).
[16] D. R. ERGLE and F. R. H. KATTERMAN, *Plant Physiol.* **36**, 811 (1961).