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5-METHYL CYTOSINE CONTENT IN THE DNA OF COLCHICINE AND SPONTANEOUSLY INDUCED POLYHAPLOIDS OF *GOSSYPIUM**

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Abstract—A small but significant decrease in 5-methylcytosine content is noted for the DNA composition of colchicine and spontaneously doubled polyhaploids of both *Gossypium hirsutum* and *G barbadense* as compared to the parental forms of these doubled haploids

INTRODUCTION

THE NATURAL occurrence of haploids[1] in angiosperms has been known for some time [2–4] Their first occurrence in *Gossypium* was noted by Harland in 1920 [2] Later, Beasley[3] successfully developed several pure lines of cotton by doubling the chromosome number of the haploids with colchicine. Many of these lines possess most of the agronomic qualities present in the normal cultivated varieties [4] In addition to the colchicine-doubled haploids, the occurrence of spontaneous double haploids in *Gossypium* has also been reported.[5]

A study of the DNA composition of *Gossypium*[6] revealed that the methylated base content of the DNA from the colchicine double haploid varieties was significantly lower than that of the parent tetraploid strains Colchicine is known to interact directly with DNA[7] and possibly mask some of the cytosine residues normally available to the DNA methylating enzymes during the initial growth phases of the cotton seedlings It was of interest, therefore, to examine the spontaneously doubled haploids and determine whether the DNA of these plants also exhibit a decrease of the methylated base

RESULTS AND DISCUSSION

The 5-methylcytosine (5-MC) base content of the DNA from the colchicine and the spontaneously doubled haploids for both the *barbadense* and *hirsutum* species of *Gossypium* is listed in Table 1 along with that of their respective parental strains Both colchicine and spontaneous doubled haploid DNAs are significantly lower in 5-MC content than their parental counterparts Apparently, colchicine has no effect on the decrease of the methylated

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[1] In this report, haploid and diploid refer to the chromosome number of the species discussed Since *Gossypium* is a tetraploid the doubled series are polyhaploid

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[4] MEYER, J R and JUSTUS, M (1961) *Crop Sci* **1**, 462
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bases in DNA since both types of double haploid DNAs exhibit the same degree of 5-MC loss This decrease is probably a reflection of another event that accompanies chromosome doubling by either induced or spontaneous ploidy

TABLE 1 5-METHYLCYTOSINE (5-MC) CONTENT OF ISOLATED DNAS FROM DOUBLED HAPLOIDS OF *Gossypium barbadense* AND *G hirsutum*

Species and type	mol 5-MC/100 mol nucleotide*	Species and type	mol 5-MC/100 mol nucleotide*
Barbadense (Pima S-1)		*Hirsutum* (Deltapine 15)	
Parental	4 6	Parental	4 6
Colchicine doubled haploid	4 3‡	Colchicine doubled haploid	4 4†
Spontaneous doubled haploid	4 4‡	Spontaneous doubled haploid	4 3‡

* Average mean of three separate determinations
† Mean difference probability greater than 0 05 as determined by the *t*-test
‡ Mean difference probability greater than 0 01 as determined by the *t*-test

It has been shown that the DNA content does not parallel the increase in chromosome number For example, in mulberry, the DNA content per cell is 2-fold in tetraploids and only 4 times with an 11-fold increase in chromosome number [8] In cotton, the total DNA increased only 1 5-fold in the (AD) tetraploid state as compared to the average additive values of the A and D genome diploid species [9] Some mechanism must exist to delete extra genetic information from the genome during ploidy Recent investigations on the DNA content of diploid and tetraploid species of Nicotiana[10] presented evidence that ribosomal RNA cistrons may be some of those genes that are preferentially discarded during chromosome doubling

Buoyant density determinations of nuclear DNA from some higher plants indicate that sections of DNA coding for ribosomal RNA separate as a distinct band from nuclear DNA [11] Investigations on the base content of this nuclear DNA satellite from mouse tissue[12] showed that this type of DNA contained more than twice the molar concentration of 5-MC than the main body of nuclear DNA Thus, the preferential loss of ribosomal RNA genes during ploidy would probably be reflected as a slight decrease in overall 5 MC content of DNA This proposition can be demonstrated to a certain degree by considering the data gathered for the nucleic acids of *Gossypium* (a) A separate satellite region for ribosomal DNA in *Gossypium* does exist [13] (b) The total amount as well as the 5-MC content of the DNA in tetraploids is lower than the additive values for the DNAs of the diploid A and D genomes[9,14] known to be the component parts of the tetraploid forms [15]

Haploids occur as one or both members of twin embryo seeds [16] Presumably the DNA of

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the haploid plant would have the same 5-MC content as that of the parent tetraploid plant since the amount of DNA in the haploid state is the expected value of half of that observed in the parental form [17] (Haploids do not produce seed so no 5-MC analysis can be performed) Upon transformation to the double haploid state, whether by induced or natural means, the slight loss of 5-MC in the DNA of these plants would probably be a reflection of still more ribosomal DNA being eliminated from the total AD genome Note, however, that the decrease of 5-MC is much less than that observed for the original ploidy of the diploid A and D genomes to the AD tetraploid state, i e an average of 4 6 mol 5-MC/100 mol nucleotide for the tetraploids as opposed to an average additive value of 8 9 mol 5-MC/100 mol nucleotide for the A and D diploid species [14] Thus, a very small amount of ribosomal DNA is probably lost during chromosomal doubling from the haploid to the double haploid state

EXPERIMENTAL

DNA was isolated, purified and analyzed for 5-MC from seed material in both the colchicine and spontaneously induced double haploid lines of *hirsutum* and *barbadense* as described previously [6] The DNA from the corresponding parental lines was also analyzed in the same manner Dormant seed material was chosen because the 5-MC content of the isolated DNA was less variable than that of the DNA obtained from germinating seedlings (unpublished observations) The colchicine and spontaneously induced lines of *hirsutum* as well as the parental lines (Deltapine 15) were obtained from R Kohel of Texas A & M University, College Station, Texas, while those of *barbadense* (Pima S-1) were obtained from E Turcotte of the USDA Experiment Station in Phoenix, Arizona

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