RNA Composition in Cotton[1]

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Summary. Nucleotide compositions of soluble (sRNA), whole-cell, and in one instance particulate and nonparticulate, RNA of embryos, cotyledons (dark and light-grown), leaves, stems, and roots of *Gossypium hirsutum* were compared. Evidence of substantial differences in the overall compositions of the RNAs was not obtained. However, a tendency for some plant parts to differ, particularly in sRNA composition, and that of the cotyledons (including particulate and nonparticulate RNA) to change during the formation of chlorophyll, was indicated by small but statistically significant shifts in certain nucleotide components.

Available data (4, 7, 8) show that the nucleotide composition of whole-cell RNAs from higher plants tend to be similar. Differences noted have generally been small, even between plants of widely different systematic position and origin, and they have been considered to be insignificant. In contrast, recent evidence suggests that the composition of soluble RNA may differ in the embryos of some species of *Gossypium* (4) and may change in mimosa epicotyls (3) with change of growth rate.

Except for the study on mimosa, little or no attention has been given to the question of possible effects of inherent and developmental physiological differences on the RNA composition of higher plants. For example, is the RNA nucleotide composition of the seed the same as that of the green leaf, and does the composition of the latter differ from one without chloroplasts?

In this paper we have attempted to develop information on these questions by reporting data on the RNA composition, whole-cell and soluble alone, in various parts of the cotton plant, including cotyledons from light and dark-grown seedlings. The latter was of special interest because studies on *Euglena gracilis* (6) suggest that specific RNA species are produced during chloroplastic development.

Materials and Methods

Plant Material. Cotton seed (*Gossypium hirsutum* L., var. Paymaster 54B) was germinated and grown in washed river sand (copiously watered with Hoagland's solution) in the greenhouse at 30° for 14 days. Germination and growth of the dark-grown seedlings were carried out under similar cultural conditions except that all light was omitted. Stems (top 5 cm of main stalk), young leaves (one-fourth expanded), mature leaves, and fibrous roots were harvested from plants grown in the greenhouse in the same solution, but aerated, in 20-liter stone jars. The root samples were from 65-day-old plants.

RNA Preparation and Nucleotide Analysis. Procedures for the preparation, hydrolysis, nucleotide separation, and analysis of whole-cell and soluble RNA have been described (4, 5). The soluble RNA of light (green) and dark (etiolated) grown cotyledons was obtained A) as a purified isolate from phenol-extracted defatted tissue (sRNA), as were the other sRNAs and B) as total RNA in the supernatant (100,000 × *g*) from macerated fresh tissue. The latter is known to contain some high molecular weight RNA in addition to sRNA (1). Pseudouridylic acid (PsU), a characteristic minor nucleotide of sRNA, though not determined as such, was included in the uridylic acid values.

Results and Discussion

Green and Etiolated Cotyledons. The nucleotide compositions of whole-cell, supernatant material, and sRNA are given in table I. In order to bring out certain trends and differences not apparent in the over-all compositions, the 6 possible ratio values of the nucleotides were analyzed statistically and the results are given in table II. In whole-cell RNA, only those ratios containing uridylic acid showed a consistent difference between green and etiolated cotyledons, A/U and C/U being significantly higher ($P>0.05$) in the latter. In the supernatant RNA the consistent differences ($P>0.01$) were in the

[1] Abbreviations include: A, adenylic acid; C, cytidylic acid; G, guanylic acid; U, uridylic acid; 6-AM, nucleotides (A,C) with an amino group in 6 position; 6-K, nucleotides (G,U) with a keto group in 6 position.

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Table I. *Nucleotide Compositions of Whole-Cell, Supernatant and sRNA from Green and Etiolated Cotyledons*

Data in moles per 100 moles nucleotide. They represent the mean of 3 preparations of 2 analyses each.

Fraction	Green					Etiolated				
	A	C	G	U	6-AM/6-K	A	C	G	U	6-AM/6-K
Whole-cell	24.2	24.7	30.6	20.6	0.96	24.3	25.3	31.0	19.2	0.99
Supernatant	21.1	28.4	29.6	20.9	0.98	19.5	27.6	34.2	18.6	0.89
sRNA	21.8	27.4	31.6	19.1	0.97	20.3	28.9	31.8	19.1	0.97

Table II. *Nucleotide Ratios of Whole-Cell, Supernatant, and sRNA from Green and Etiolated Cotyledons*

Fraction	Green						Etiolated					
	A/U	C/A	G/A	C/U	G/C	G/U	A/U	C/A	G/A	C/U	G/C	G/U
Whole-cell	1.17	1.02	1.27	1.20	1.24	1.49	1.27*	1.04	1.27	1.32*	1.22	1.61
Supernatant	1.01	1.34	1.40	1.36	1.04	1.42	1.05	1.41	1.75**	1.48	1.24**	1.83**
sRNA	1.14	1.26	1.45	1.43	1.15	1.65	1.06*	1.43**	1.57	1.52	1.10	1.67

* $p > 0.05$.
** $p > 0.01$.

Table III. *Nucleotide Composition of RNA from Whole-Cell and Subcellular Fractions of Green and Etiolated Cotyledons*

Data in moles per 100 moles nucleotide. They represent the mean of 3 preparations of 2 analyses each.

Fraction	Green					Etiolated				
	A	C	G	U	6-AM/6-K	A	C	G	U	6-AM/6-K
Nuclei	24.3	24.6	30.5	20.6	0.96	24.3	25.6	30.8	19.4	0.94
Plastids	25.0	25.0	30.2	19.8	1.00	24.7	25.4	30.5	19.4	1.00
Mitochondria	24.6	25.3	29.7	20.4	1.00	25.1	25.4	30.0	19.4	1.02
Microsomes	24.4	24.8	30.2	20.5	0.97	25.1	25.3	29.7	19.8	1.02
Whole-cell	24.2	24.7	30.6	20.6	0.96	24.3	25.3	31.0	19.2	0.99

guanylic acid containing ratios (G/A,G/C,G/U). With sRNA, even though 2 of the nucleotide ratios (A/U,C/A) differed significantly in the 2 tissues, there was not the consistency exhibited by whole-cell and supernatant RNA. Therefore, since neither supernatant nor sRNA nucleotide ratio-changes followed the same pattern as whole-cell RNA, the involvement of only particulate RNA was implied.

In order to determine which of the particulate RNAs were responsible for the forementioned changes in whole-cell RNA, green and etiolated cotyledons were fractionated into nuclei, plastids, mitochondria, microsomes (5), and their nucleotide compositions compared. The results (table III) showed that A) the RNA composition of each particulate was quite similar to that of the corresponding whole-cell and B) only the nucleotide ratios containing uridylic acid (A/U,C/U,G/U) indicated any difference between the particulate RNA of green and etiolated cotyledons (table IV). In each of the particulates, as in whole-cell RNA (table II), the 3 ratio values were significantly higher in the etiolated cotyledons.

Returning to table I, it will be noted that all of the amino-keto ratios are near unity, except that all of the etiolated supernatant which has an unusually high guanylic acid content. Generally, a poor ratio of either supernatant or sRNA indicates the presence of minor nucleotides (6) which can usually be resolved with multiple development in the first dimension of a 2-dimensional chromatogram (1). This technique neither improved the ratio nor indicated the presence of any additional minor nucleotides. Thus, the supernatant RNA of the etiolated cotyledons appeared to contain a component, possibly a guanine-rich polynucleotide, not present in that of the green cotyledons.

RNA Composition of Plant Parts. The nucleotide composition of the RNA of whole-cell and sRNA is given in table V. The failure to include sRNA data on the stems and mature leaves is that the preparations from these parts were analytically unsatisfactory (6-AM/6-K too far from unity).

Although the over-all composition of whole-cell RNA appears to be much the same throughout, statistical analysis of the 6 possible nucleotide ratios indicated 0.01 significance for C/A. Separation of the means, by Duncan's multiple range test (table VI), showed that the C/A values of stems and roots, as a group, differed significantly from those of the remaining parts and tissues. In the latter, the suc-

Table IV. *Analysis of Variance of Uracil Ratios in Particulate RNA from Green and Etiolated Cotyledons*

Ratios	Source	Variance
C/U	Green vs etiolated	0.0379*
A/U	Green vs etiolated	0.0155*
G/U	Green vs etiolated	0.0600*

* $p > 0.01$.

cessive ratio decreases in embryos, cotyledons, young and mature leaves were not all significant, as between A) cotyledons and young leaves and B) young and mature leaves. However, it is of some interest that the decrements in C/A values correlate partially with chronological sequences in leaf development and growth and may signify a small change in RNA composition during leaf development.

In the plant part sRNAs (table V), successive decreases in adenylic acid, with corresponding increases in cytidylic acid contents, were found in embryos, cotyledons, and young leaves. The nucleotide ratios involved in these changes are summarized in figure 1. As shown both A/U and G/C approached unity while C/A increased from embryos to young leaves. Excepting G/U, the changes depicted by the graph for the other ratios were significant (table VII). Inasmuch as G/U did not change significantly, the C/A value tends to be an enhanced reflection of the changes in A/U and G/C.

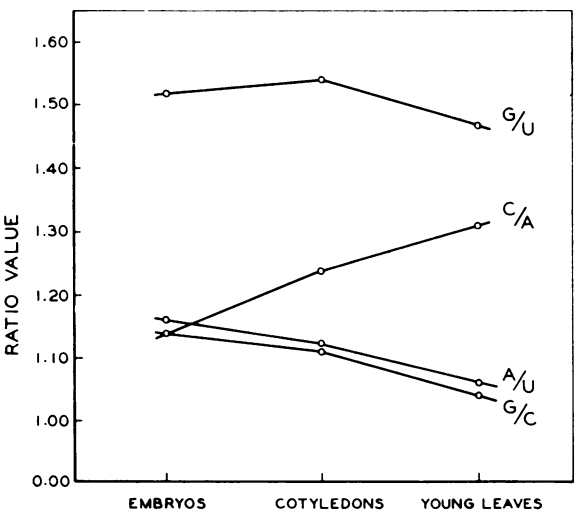

FIG. 1. Differences in sRNA composition of cotton embryos, cotyledons, and young leaves as indicated by nucleotide ratios. A, adenylic acid; C, cytidylic acid; G, guanylic acid; U, uridylic acid.

Table VI. *Separation of C/A Ratio Means of Whole-Cell RNA from Each Plant Part by Duncan's Multiple Range Test*

Tissues	C/A Ratios
Stems	1.13 a
Fibrous roots	1.10 a** b*
Embryos	1.06 c
Cotyledons	1.03 c* d
Young leaves	1.02 d* e
Mature leaves	0.99 e f

* $p > 0.05$.
** $p > 0.01$.

Table VII. *Analysis of Variance of Nucleotide Ratios in sRNA from Embryos, Cotyledons, and Leaves*

Ratios	Source	Variance
A/U	Tissues	0.0580*
G/C	Tissues	0.0520**
C/A	Tissues	0.1624**
G/U	Tissues	0.0224

* $p > 0.05$.
** $p > 0.01$.

Another aspect of sRNA composition, to which some attention was given, concerned the identity of the minor nucleotides, a characteristic part of all sRNAs. Two-dimensional chromatography of the hydrolyzed sRNAs (5) effected separation of the major nucleotides from several minor ones. Chromatograms of cotton root sRNA (fig 2), for example, showed the usual 4 major nucleotides (A, C, G, U) and pseudouridylic acid (PsU) plus 6 additional small spots (0.2–0.8 moles per 100 moles nucleotide).

Table V. *Whole-Cell and sRNA Composition of Parts of the Cotton Plant*
Data in moles per 100 moles nucleotide

	Whole-cell RNA*					sRNA**				
Plant parts	A	C	G	U	6-AM/6-K	A	C	G	U	6-AM/6-K
Embryos	24.0	25.5	30.2	20.3	0.98	23.0	26.3	30.5	20.2	0.97
Cotyledons	24.1	24.8	30.6	20.5	0.96	22.1	27.3	30.6	20.0	0.98
Stems	23.4	26.5	29.2	20.9	1.00	...	...	...	...	...
Young leaves	24.5	24.9	29.0	21.5	0.98	21.4	28.0	29.9	20.6	0.98
Mature leaves	24.7	24.5	30.9	19.9	0.97	...	...	...	...	...
Fibrous roots	23.7	25.9	30.9	19.5	0.98	21.6	28.3	29.3	20.8	1.00

* Mean of four preparations.
** Mean of 11, 9, 12, and 2 preparations from embryos, cotyledons, young leaves, and roots, respectively.

Three of these, 2, 3, and 6, were tentatively identified as 1-methylguanylic acid, 2-methyladenylic acid, and ribose thymidylic acid (5). Of the 3 remaining unidentified spots, number 1 is thought to be diphosphoguanosine. Similar chromatography of embryo, cotyledonary, and young leaf sRNA, however, consistently showed only 2 of the 6 spots that were found in root sRNA. These were quite small (0.09–0.2 moles per 100 moles nucleotide) and coincided with spots 1 (diphosphoguanosine) and 3 (unidentified component) of figure 2.

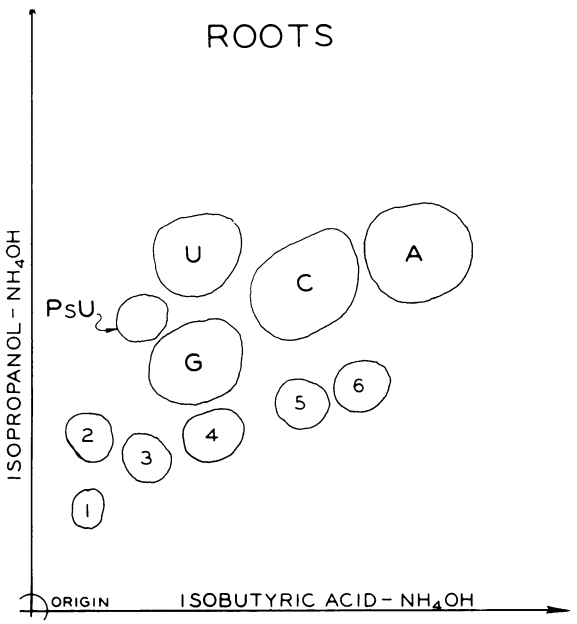

FIG. 2. sRNA roots: 2-dimensional chromatogram of an alkaline hydrolysate. A, adenylic acid; C, cytidylic acid; G, guanylic acid; U, uridylic acid; PsU, pseudouridylic acid; 1, diphosphoguanylic acid; 2, 1-methylguanylic acid; 3, 5, unidentified components; 4,2-methyladenylic acid; 6, ribose thymidylic acid.

Whether the sRNAs of this study possessed amino acid transfer activity was not determined so other characteristics were considered. The nucleotide compositions of transfer RNAs from a variety of sources (mostly animal and microorganisms) are A) high in guanylic and cytidylic acids and include several minor nucleotides (pseudouridylic acid and methylated bases), B) have a secondary or helical structure as shown by near unity relationship for A/U and G/C, and C) show a narrow range (0.6–0.7) for A + U + PsU/G + C (2). Of the cotton sRNA isolates, only that from the roots, with its relatively large complement of minor nucleotides, seemed to have all of the foregoing properties. The sRNA of embryos and cotyledons, while containing pseudouridylic acid and a 0.7 A + U + PsU/G + C value, had a higher than unity value for A/U and G/C and each appeared deficient in methylated bases; leaf sRNA appeared deficient in only the latter.

While most of the active preparations of sRNAs from different sources have shown similarity in nucleotide composition, it is recognized that preparations by the commonly used phenol-extraction procedure may also contain other components without transfer activity (2). Among these are small amounts of messenger, degraded ribosomal, and low-molecular weight RNAs. At present we cannot say to what extent, if any, the depicted differences in the composition of cotton sRNA reflect RNA from these sources.

Generally, the results of this study show that neither whole-cell nor sRNA alone provides striking evidence that the overall RNA composition in 1 part or organ of the cotton plant differs substantially from that of another. Neither does chlorophyll formation, at least in cotyledonary tissue, seem to be accompanied by any marked changes in the proportion of nucleotide components, including those of particulate and nonparticulate RNA. Nevertheless, we are inclined to attach some significance to the small but statistically significant shifts noted in some of the nucleotides. These shifts, observed more often in sRNA than in whole-cell, could signify the occurence of pronounced change in specific types of RNA which remain largely masked due to the relatively greater abundance of unchanged forms.

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