ISOLATION AND CHARACTERIZATION OF t-RNAs AND r-RNAs IN COTTON

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Another fraction of t-RNA from cotton seed meal was isolated in addition to the four (4) already well characterized fractions. This new fraction is different in that it was not isolated by the detergent method but resulted from a physiological saline extraction of the meal. Although its nucleotide composition differs radically from Fractions I and II, it has the same number of methylated nucleotides as does the latter. More characterization studies are now in progress.

With regard to the four (4) well characterized t-RNA fractions, four (4) separate isolations of each of the fractions from a single species of Gossypium was undertaken. It was found that the base ratio composition of three of the fractions, as well as the newly described one, was consistent between isolations. This was not true for Fraction I. This indicates an incomplete release in some instances of the particular set of t-RNAs that compose Fraction II. Therefore, some caution should be taken in interpreting base ratio changes of Fraction I between different species of Gossypium as noted in previous cooperative work with scientists at Texas A & M University.

A technique has been worked out to isolate both chloroplastic and cytoplasmic monomeric ribosomes from 7-10-day-old cotton cotyledons. This technique will be used to study the relative ribosomal contents of both classes in a variegated mutant variety of cotton. This in turn should partially clarify the mechanism involved in the production of this mutant.

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GAMMA IRRADIATION OF POLLEN OF MARKER LINES FOR THE ISOLATION OF PLANTS DEFICIENT FOR SPECIFIC CHROMOSOMES

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One of the major objectives of the research project on the cytogenetics of cotton is the isolation of a monosomic chromosome for each member of the genome in the cultivated tatraploid species. The monosomic chromosomes are ideally suited for the investigation of the genetic factors carried by each chromosome. Presently, eight of the chromosomes of Gossypium hirsutum have been reported to be identified by monosomy. Monosomes for several other chromosomes in the complement have also been isolated, and these are in the process of being studied genetically and cytologically.

The results of a study of the irradiation of cotton pollen to determine the best dosage level for the induction of monosomes in G. hirsutum were published this year. It was determined in this study that gamma-radiation doses of approximately 400r or less are quite effective in inducing whole chromosome deficiencies.

The study reported below was designed to isolate monosomes for specific chromosomes. This can be accomplished by irradiating pollen of a specific type of genetic stock and pollinating other stocks carrying certain alleles of the gene markers in the pollen line.

Listed below are the marker stocks and their genotypes used in the pollen irradiation study reported here.

$gl_1gl_1gl_2gl_2gl_3gl_3$	38-6-A
$Gl_1Gl_1gl_2gl_2Gl_3Gl_3$	Empire 61(WR)
$Gl_1Gl_1Gl_2Gl_2gl_3gl_3$	Empire 61(WR)
SmSm	D2 Smooth #723 AG149
L^SL^S	Supra Okra AG150
RdRd	Red Dwarf AG151

The phenotypic description of the various combinations of the heterozygotes and homozygotes of the glandless alleles is given in Lee's 1962 publication in Genetics 47:131-142. In the present study it was assumed that the hemizygous recessive would behave phenotypically as the homozygous recessive.

Pollen was collected from plants homozygous for the dominant allele of the different dominant markers and placed in small test tubes which were then exposed to 400r of gamma radiation at the rate of 80r per minute. Following the exposure the pollen was then applied to emasculated flowers of plants homozygous for the corresponding recessive mutants. Seeds were later harvested and planted individually in small peat pots. All of the mutant characters can be scored in the seedling stage; therefore, in the earlier seedling stages following germination, the seedlings were scored for the presence or absence (mutants) of the dominant character. All mutants found were later transplanted to the field for cytological analysis of the metaphase I stage. The results of this study are summarized in the following table.

Locus	Total No. Seedlings Scored	No. of Mutant Seedlings	No. of Seedlings Surviving Transplanting	No. of Plants Analyzed Cytologically	Remarks
Gl_1	1900	17	13	12	1 plant = 25 II + I s
Gl_2	3027	50	49	45	3 plants = 25 II + I 1 1 plant = 24II + hetero II + I 1 1 plant = 20II + 2IV + heteroII + I 1
Gl_3	3340	45	40	39	1 plant = 23II + IV + I s
Sm	495	4	4	4	None = 2n-1
L^s	1342	4	4	4	None = 2n-1
Rd	543	3	3	3	None = 2n-1

In addition to the monosomic plants shown in the table, ten additional plants with monosomic chromosome, supposedly independent of those listed in the table, were found and given an Mo number for further study. Most of this latter group, as in the group in the table, carry in addition to the monosome other chromosome aberrations. The additional aberrations generally make it much more difficult to reisolate the monosomic chromosome.

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TESTS FOR THE ASSOCIATION OF EIGHT MONOSOMES AND THE MARKER GOLDEN CROWN (gc)

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In our program to associate the effects of a gene with a specific chromosome in the cultivated cottons, the test for the association of monosomes and the gene marker "golden crown" was completed this year.

A general outline of the procedures employed to determine whether a recessive marker gene and a specific monosomic chromosome is associated is given below:

P_1 haplo-X dominant X P_2 diplo-X recessive.

F_1 haplo-X dominant or recessive + diplo-X dominant.

(a) If the F_1 haplo-X has a recessive phenotype, this indicates that the genetic marker locus and the monosomic chromosome are "linked," i.e., the locus is on that particular chromosome.

(b) On the other hand, if the F_1 haplo-X has the dominant phenotype, then this indicates that the monosomic chromosome and the marker loci are independent, i.e., the marker locus is not located on the monosomic chromosome. However, when working with polyploid forms we cannot always rely on the presence of the dominant phenotype in the F_1 as reliable criterion for concluding that the chromosome and marker locus are independent. It is possible that the mutant allele may be an anti-morph in which case a single dose of the mutant allele would result in the haplo-F_1 having a phenotypic expression similar to the diplo-dominant. The above results are summarized under 1 and 2 on the following page.