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1. Characterization of chloroplast and nuclear DNA of maize.

The base composition of chloroplast and nuclear DNA was determined for two corn hybrids by two techniques, acid hydrolysis and melting point profile. The corn hybrids were Coker X210 and X210V, which are single cross hybrids differing only in that X210 contains normal cytoplasm while X210V has the Texas cytoplasm (Tcms). To the best of our knowledge, the base composition of chloroplast DNA (cDNA) has not been reported previously. Base composition of total cellular DNA has been determined by others (1, 2, 3, and 4) although their determinations were not always in complete agreement. In this study the cell organelles, nuclei and chloroplasts were first isolated and then DNA was isolated from the different organelles. Since total cellular DNA is comprised primarily of nuclear DNA (nDNA), a difference in base composition between total cellular and nDNA is unlikely.

Chloroplasts and nuclei were isolated from green leaves taken from plants 1-2 months of age. Leaves were ground in a Waring blender with buffered sucrose. Chloroplasts and nuclei were isolated by differential centrifugation in a sucrose gradient first and later in a discontinuous glycerol gradient. The chloroplast fraction was further purified by selective solubilization with Triton X-100. Nuclear DNA was isolated from nuclei by a modification of the Marmur method (5). The same method was employed for isolating cDNA from chloroplast except that some alcohol precipitation steps were omitted. Further purification of c and nDNA's was obtained by preparative CsCl density centrifugation (6). DNA was pelleted from CsCl solutions by centrifugation at 50,000 RPM for 18 hours at 25°C in the Beckman L2-65 ultracentrifuge (Type 65 rotor).

The thermal denaturation temperature (T_m) and the mole percentage of guanine plus cytosine were determined by the methods of Marmur and Doty (7). T_m measurements were made on a Gilford 2000 multiple sample absorbance recorder coupled with a Beckman DU spectrophotometer equipped with a temperature controlled cuvette chamber. DNA was melted in 1 SSC (.15 M NaCl, .015 M trisodium citrate). An acid hydrolysis technique (8)

was used for determining base composition. The molar percent GC was determined by "Differential Extinction Technique".

The means of the molar percentage of guanine and cytosine are given in Table 1 for the c and nDNA's of the two hybrids as determined by the two methods. An analysis of variance was used to test for significance. Determination of GC percentage by the acid hydrolysis technique (8) or the thermal denaturation (T_m) technique (7) gave results which were not significantly different. Nuclear DNA from X210 and X210V had similar GC percentages as expected. Chloroplast DNA from X210 and X210V also had similar GC percentages. This result was of interest because X210 carries the normal cytoplasm while X210V has the Texas type. Texas cytoplasm differs from normal in at least three factors: male sterility and resistance to two leaf diseases.

Table 1
Molar percentage of guanine and cytosine of maize DNA's
from two hybrids determined by two methods

	nDNA		cDNA	
	T_m method	Acid hydrolysis method	T_m method	Acid hydrolysis method
X210 (normal cytoplasm)	42.9	43.0	40.9	38.2
X210V (Texas cytoplasm)	43.2	44.5	40.1	38.8

The overall mean GC percentage for nDNA is 43.3 as compared with 39.5 for cDNA. This difference is significant at the 1% level. The 43.3% GC for nDNA compares favorably with the result of 42% found by Rinehart and Sansing (1). No value is available for comparison of the GC percentage of cDNA. However, Kirk (9) contends that the cDNA of typical higher plants has a GC content of about 37-38%, which compares favorably with our estimate of 39.5% for maize.

The acid hydrolysis technique also allowed us to check for the presence of 5-methyl cytosine. None was found in cDNA, but 6.9% of the nDNA

was found to be 5-methyl cytosine. No data are apparently available on the amount of this base in maize DNA's. Our results are consistent with other higher plant DNA's since 5 to 6% 5-methyl cytosine is found in several other nDNA's of grasses and little or no methyl cytosine is found in the cDNA's of higher plants.

The GC content of total cellular DNA was determined by the thermal denaturation method for the two hybrids. A mean value of 43.7% GC was obtained which is not significantly different from 43.3% found for nDNA. Therefore, the organelle DNA's are either not present in sufficient quantities to influence the GC content or are identical. The former is known to be true for cDNA.

References:

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