COMMONWEALTH SCIENTIFIC AND INDUSTRIAL
RESEARCH ORGANIZATION
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Characterization and distribution of GC-rich sequences in the DNA of maize

Satellite DNAs generated by Actinomycin D binding in CsCl density gradients were reported previously (MNL 47:28-30). The DNA from all corn lines examined apparently contained the same satellites but in different amounts. Subsequent work measuring the degree of hybridization of one of these satellites to total DNA from different lines confirmed this observation. It seems likely that the content of a particular satellite is positively correlated with the hetero-chromatin content of the particular line. Thus, in our continuing study of maize DNA it was important to characterize thoroughly the DNA from plants with the basic genome. Wilburs Flint Knobless has been used for these studies.

As a first step, thermal denaturation profiles were examined. These profiles were heterogeneous, indicating the presence of DNA species with a Tm about 10 C higher than the bulk of the DNA--i.e., DNA species with greater than 60% GC content compared to 42% for the bulk of the DNA. Reports of GC-rich sequences in maize DNA have been made previously (Brooks & Mans, Biochim. Biophys. Acta 312: 14, 1973; Pivec et al., Biochim. Biophys. Acta 340:199, 1974). In fact, this report describes GC-rich sequences that in all likelihood correspond to the same sequences first described by Brooks and Mans.

The GC-rich sequences are distributed throughout the genome. This was demonstrated by a comparison (3 methods) of unsheared and sheared DNA. Since GC-rich sequences have a higher melting temperature, under partial melting conditions these sequences will remain in a native double stranded condition, relative to the bulk of the DNA.

Method 1: If sheared (~500 bp) and unsheared (~30,000 bp) DNA molecules are partially denatured by heating and allowed to reassociate by cooling, the double stranded GC-rich regions will provide nucleation sites for the complete re-naturation of those molecules containing GC-rich sequences. In this regard we have confirmed the observations of Brooks and Mans. About 70% of the hypo-chromicity was regained in the unsheared molecules compared to less than 10% for sheared DNA. Thus, the GC-rich sequences are distributed throughout the genome--i.e., 70% of unsheared molecules contain a GC-rich sequence.

Method 2: Thermal chromatography on hydroxyapatite (HAP). Under appropriate conditions double stranded DNA is bound to HAP, while single stranded denatured DNA may be eluted. By raising the temperature of a HAP column, DNA will be eluted unless it is covalently linked to a native double stranded region. Most sheared DNA was eluted at temperatures lower than were necessary to elute unsheared DNA, again demonstrating that most unsheared DNA molecules contained regions of higher thermal stability.

Method 3: Sheared and unsheared DNA were passed over a HAP column under partially denaturing conditions. Only those molecules containing native double stranded regions will be selectively retained on the column, and they can be recovered without further denaturation by elution with high salt. Under these conditions 70% of unsheared DNA was retained compared to 10% of the sheared DNA.

This third method was used to isolate preparatively DNA enriched for the GC-rich sequences. Further purification was achieved by denaturing and renaturing the DNA. Unpaired regions and single stranded tails were removed by digestion (to completion) with a single strand specific nuclease (S_1). The resultant double stranded DNA molecules had a size of about 200 b.p. (as judged by comparison with known-size fragments of ϕ80 phage DNA on agarose gel electrophoresis). A high GC content was confirmed by the Tm measured in thermal denaturation and the density

in neutral CsCl density gradients. A $Cot_{\frac{1}{2}}$ of 4×10^{-2} $M \cdot s \cdot l^{-1}$ was measured from renaturation kinetics. This value indicated the presence of repeated sequences in the GC-rich fraction. However, this rate is too slow for a single repeating unit of 200 bases. Thus, the GC-rich fraction represents either a family of repeated sequences or fragments of a larger, less-repeated sequence. Studies are continuing to distinguish these possibilities and to determine the length and distribution of these sequences.

Tony Pryor

Glucosidase in maize seedlings

(a) Inheritance of electrophoretic variants. Glucosidase bands in starch gels (pH7.8 Tris-citrate) were developed using a 6 bromo-2 naphthyl β-D glucoside and Fast Garnet GBC salt. Most inbred lines contain a single isozyme band, but in 2 of the 40 lines assayed (P10 and P11) a faster band was observed. Hybrids between variants have 3 isozyme bands, the additional band being a hybrid isozyme of intermediate migration. The segregation of the isozyme variants in F_2 and BC progeny is consistent with the hypothesis that the isozymes are determined by alleles (Table 1). We propose to call this gene glucosidase and the alleles Glu-A and Glu-C. A third variant, Glu-B, has recently been observed.

Table 1. Inheritance of glucosidase isozyme variants. C and A represent lines breeding true for the isozyme variants. χ^2 was calculated on the Null hypothesis that C and A are determined by alleles.

	Number of Progeny					
Cross	C	C/A	A	Σ	χ^2	P
C/A self	5	10	6	21	.04	>.05
C/A x C	9	10	-	19	.05	>.05
C/A x A	-	9	8	17	.05	>.05

(b) Null mutants at the Glu locus. Glucosidase activity has been implicated in the mechanism of plant disease resistance by mediation of the release of pathogen-toxic materials from inactive glycosidic precursors (Pridham, J. B., 1960, Phenolics in Plants in Health and Disease, Pergamon Press, New York). For the glucosidase determined by the gene Glu this supposed role can be tested by determining whether resistance is still expressed in a plant homozygous for a null mutation of the Glu gene.

Mutants were obtained by EMS treatment of seed homozygous for Glu-A, Gdh-F and Adh-S. During the latter half of the study seedling roots were immersed in water for 12 hours prior to electrophoresis to induce the Adh2 gene and allow scoring at this locus. By using electrophoretically recognizable pollen (Glu-C Gdh-N Adh-F) we should have been able to recognize a variety of mutant types at the 4 loci. However, only one class of apparent mutation was observed (Table 2). These ears carried sectors of seed containing only the pollen parent isozyme for glucosidase C. Plants from 17 of the 33 apparent mutants were selfed. In all cases the F_2 progeny segregated, having either the pollen parent isozyme or no glucosidase activity (Table 3), and in most cases where numbers were sufficient the segregation ratio did not differ significantly from a 3:1 ratio. Thus, absence of the female parent isozyme segregates as an allele of the glucosidase gene. Similarly in crosses Glu-A/Glu-C x Glu-C/mutant, progeny occurred in the ratio 1A:1AC:2C. The homozygous nulls obtained from the F_2 progeny breed true.