CHAPTER III
ANALYSIS OF REPEATED AND SINGLE
COPY DNA SEQUENCES IN TOTAL SUGARCANE DNA
FROM CALLUS AND SHOOT TISSUES
Non-linear regression analysis of reassociation curves of sonicated sugarcane DNAs from two callus cultures, namely CO-740 and CO-7219 and shoots of CO-740 revealed the presence of one fast and one slow DNA component, each reassociating with second order kinetics. A comparison of rate constants of these two DNA components indicated that the fast reassociating DNA fraction consisted of repeated DNA sequences while slow reassociating DNA fraction included mainly single copy DNA sequences. Marked differences were noticed in the amount, kinetic complexity and size distribution of repeated DNA sequences in callus and shoot tissues. In CO-740 callus, the fast DNA comprised 47.5% of the total DNA as against 16.5% in CO-740 shoot and 38.5% in CO-7219 callus. The proportion of the slow component was 25-30% in all the three DNAs. Its kinetic complexity was in the range of $2.5-4.4 \times 10^8$ nucleotide pairs in both the callus cultures and was significantly higher than that in shoot DNA ($6.1 \times 10^6$ nucleotide pairs). The reassociation kinetics data thus revealed that repeated DNA sequences have undergone a significant change during differentiation of callus tissue to shoots in sugarcane.
INTRODUCTION

It is generally accepted that eukaryotic DNA is dynamic and undergoes selective rearrangements and changes during cell differentiation (Nagl and Rucker, 1976; Buiatti, 1977, Verma and Dougall, 1978; Durante et al. 1983; Ssymank, 1983 and Duhrssen et al. 1984). For example, differential replication of satellite DNA and main band DNA has been described during dedifferentiation in Vicia faba (Natali et al. 1986), carrot (Hase et al. 1979) tobacco (Durante et al. 1983) and melon (Grisvard and Anghileri, 1980; Grisvard, 1985). The rDNA, however, has been shown to undergo no gross change during dedifferentiation in carrot and Jerusalem artichoke explants (Hase et al. 1982).

In the tissue culture chapter (chapter I), we have already described the identification of a differentiating sugarcane system. In our efforts to assess the molecular basis of differentiation, we have characterized the total DNA of sugarcane and these data are described in chapter II. In further continuation of this molecular work, we determined the reassociation kinetics of total DNA of the three sugarcane tissues namely CO-740 callus, CO-740 shoot and CO-7219 callus and these data are detailed in the present chapter.
MATERIALS AND METHODS

DNA isolation

The method of isolation of DNA from the three sugarcane tissues has already been described in Chapter II. Only those DNA preparations with an optical density ratio of $A_{280}/A_{260} = 0.55$ and $A_{230}/A_{260} = 0.45$ and an absorbance less than 0.1 at 300 nm were further used.

DNA shearing and sizing

For all the reassociation experiments described in this chapter, sonicated DNA of an average fragment size of 0.5 kbp was used. Before sonication, native sugarcane DNA was dialysed against 0.12 M sodium phosphate buffer (pH 6.8) and was fragmented to a modal length of 0.5 kbp using a Sonic Oscillator (Brownwell Model, Biosonik III, 250 W, 20 KHz) fitted with a 12 mm probe. Approximately 30 ml of DNA solution was sonicated at a maximum intensity for 3 mins with an interval of 10 min between each 1 min of sonication. Care was taken to see that the temperature of the DNA solution did not rise above 4°C.

The average size of DNAs was determined by agarose slab gel electrophoresis using 0.8 to 1% agarose gels in Tris-(hydroxymethyl)-aminomethane (Tris) (40 mM)-acetic acid (20 mM)-EDTA (2 mM) at pH 8.1. Agarose gels were stained with ethidium bromide (5 µg/ml) for 30 min and the DNA bands were photographed using 400 ASA, 35 mm negative.
film after visualization on a transilluminator. Lambda phage DNA digested with Hind III served as a molecular weight marker. The DNA preparations without sonication had a trailing in the range of 10 kbp to 0.5 kbp size while sonicated DNAs had the average size of 0.5 kbp.

DNA reassociation kinetics

The two main steps in a typical DNA reassociation experiment involve DNA strand separation by heat denaturation and reassociation of complementary DNA strands. A proper choice of incubation temperature (62°C, Tm -25°C), salt concentration (0.12 M sodium phosphate buffer, pH 6.8 i.e. 0.18 M Na⁺) and DNA fragment length (0.5 kbp) provides optimum conditions for DNA reassociation. In the present work, two techniques were used to measure the rate of DNA reassociation.

(i) Optical reassociation

Since DNA strand separation produces a hyperchromic shift in absorbance which is reversed as base pairs reform, reassociation of DNA may be followed by monitoring the decrease in absorbance as a function of time. Gilford 250 spectrophotometer equipped with a thermoprogrammer (Model 2527), analogue multiplexer (Model 6046) and automatic reference compensator was used for this purpose. DNA samples, 0.5 kpb long, in 0.12 M sodium phosphate buffer (pH 6.8)
were added to thermal cuvettes (0.3 ml capacity) and temperature of the solution was raised to 100°C. The absorbance change occurring during the heating process from 62°C to 100°C was monitored at 260 nm. The hyperchromicity of the DNA sample was calculated using the following formula:

$$H = \frac{A_{260}(100^\circ C) - A_{260}(62^\circ C)}{A_{260}(100^\circ C)}$$

(Zimmerman and Goldberg, 1977; Wimpee and Rawson, 1979)

where $H$ is the hyperchromicity and $A_{260}$ is the absorbance of the DNA sample at 260 nm. The total hyperchromicity was normalized to 100%. The denatured DNA samples were maintained at 100°C for 4-5 min to ensure complete denaturation and were then quickly cooled to 62°C by circulation of cold water. The change in absorbance of the DNA was continuously recorded with time. For the determination of zero time or the start of reassociation, the mid point (80°C) between 100°C and 62°C was considered (Britten et al., 1974). A Cot value at different time intervals is defined as half the product of absorbance of denatured DNA x hours of incubation i.e. $\text{Cot} = \frac{A \times t}{2}$ (Britten and Kohne, 1968; Kohne and Britten, 1971). Thus a Cot value of one is equivalent to incubation of DNA solution at a concentration 83 µg/ml (approximately 2 O.D.) for 1 h. The percentage reassociation at different Cot values was calculated using the formula:
\[ \% R = \frac{A_{260} \text{ of denatured DNA} - A_{260} \text{ at a given time}}{A_{260} \text{ of denatured DNA} - A_{260} \text{ of native DNA at 62°C}} \times 100 \]

The reassociation of DNA was monitored optically in the Cot range of \(1 \times 10^{-3}\) to \(1 \times 10^1\) M.s.

(ii) Hydroxyapatite column chromatography

Hydroxyapatite was prepared essentially according to the procedure of Tiselius et al. (1956) and stored at 4°C with a few drops of chloroform. The hydroxyapatite preparations usually formed columns with good flow characteristics. The extent of DNA recovery from each batch of hydroxyapatite was checked by loading known quantities of DNA and the recovery was always more than 95%. For the preparation of hydroxyapatite columns, hydroxyapatite slurry was poured into a double jacketed glass column (15 x 2.5 cm) to a bed volume of approximately 15 ml. The column was equilibrated with 0.12 M sodium phosphate buffer (pH 6.8) and maintained at 62°C with a thermostatic circulating waterbath. The hydroxyapatite chromatography was essentially according to Kohne and Britten (1971) and Britten et al. (1974). Sonicated DNA samples ranging in concentration from 30 µg/ml for lower Cot values to 300 µg/ml for higher Cot values in 0.12 M sodium phosphate buffer (pH 6.8) were sealed in boiling tubes and denatured by keeping in a water bath at 100°C for 10 min. These tubes were then incubated at specific Cot values at 62°C. The samples were
loaded on columns which were pre-equilibrated with 0.12 M sodium phosphate buffer (pH 6.8) and maintained at 62°C. Single stranded DNA was eluted with 0.12 M sodium phosphate buffer (pH 6.8) while double stranded DNA was eluted with 0.4 M sodium phosphate buffer (pH 6.8). The relative amounts of single and double stranded DNA were determined by measuring absorbance of DNA fractions at 260 nm on a Shimadzu double beam spectrophotometer (Model UV-210A) assuming that one absorbance unit (A$_{260}$) is equal to 50 µg of DNA/ml. The extent of reassociation was calculated using the formula:

$$\% R = \frac{\text{µg of DNA eluted with 0.4 M sodium phosphate buffer (pH 6.8)}}{\text{total µg of DNA eluted from the HAP column}} \times 100$$

In experiments at high Cot values (>1 x 10$^2$ Ms), the DNA samples were incubated in 0.36 M sodium phosphate buffer (pH 6.8) in order to accelerate the rate of reassociation and thus reduce the incubation time. A corresponding correction was applied to the reassociation rate using the factor 4.4778 (Britten et al. 1974). DNA samples reassociated in higher salt concentrations were diluted to 0.12 M sodium phosphate buffer (pH 6.8) with double distilled water prior to loading onto a hydroxyapatite column.

After completing the DNA reassociation experiments at various Cot values, a graph of percent reassociation
versus Cot was plotted on a semilogarithmic paper. The data points obtained optically and by hydroxyapatite column chromatography were fitted together for the construction of the Cot curve. Reassociation kinetics of *E. coli* DNA and mouse liver DNA, studied under identical conditions, served as internal standards.

**Non-linear least squares regression analysis of reassociation kinetics data**

Eukaryotic DNAs generally reassociate over a range of Cot values spanning up to eight orders of magnitude or even more. Since the individual DNA components of the reaction are not usually well separated, a computer programme was used to generate a set of Cot curves whose sum fits the experimental data. The individual curves represent single kinetic components and are fitted to the second order equation by least square analysis of the data. These components may not exist as discrete entities in the genome but represent a simplified mathematical description of the data. The programme is usually run on the basis of finding the minimum number of curves required to fit the data (Pearson *et al.* 1977).

Since DNA reassociation follows second order kinetics, it can be defined using the equation \( \frac{dC}{dt} = -kC^2 \) where \( C \) is the concentration of single stranded sequences and \( t \) is the incubation time (Britten *et al.* 1974). This yields
the equation \( \frac{C}{Co} = \frac{1}{1 + KCot} \) where \( Co \) is the initial concentration of single stranded DNA in moles nucleotides per litre i.e. \( C = Co \) when \( t = 0 \). Least squares analysis of the experimental data was carried out by employing a standard optimization subroutine STEPIT (written by J.P. Chandler and distributed by QCPE, Indiana University, Bloomington, Indiana, USA). The computer fitted curves are drawn as a solid line through the data points and the dotted lines represent kinetics of the individual components if they existed alone.

The RMS error is given by the equation:

\[
\text{Error} = \sum \left[ \frac{(C)}{(Co)_{\text{expt.}}} - \left( \frac{C}{Co} \right)_{\text{fit}} \right]
\]

--- Equation I

and was minimized by a direction search procedure.

The form for \( \frac{C}{Co} \) was assumed to be

\[
\frac{C}{Co} = T + \sum \frac{Fi}{1 + Ki \cdot Cot}
\]

--- Equation II

where \( \frac{C}{Co} \) is the fraction denatured,

- \( T \) the fraction of the genome that fails to reassociate
- \( Fi \) the fraction of the genome reassociating in the \( i^{th} \) component
- \( Ki \) the reassociation rate for the \( i^{th} \) component

The parameters \( T, Fi \) and \( Ki \) are allowed to free float to minimize the error in Equation I. The computer used for such curve fitting procedures was ICL 1900 series.
RESULTS

Precautions taken during reassociation kinetics studies

The 230/260 and 280/260 ratios of the isolated sugarcane DNAs were often high. This could be due to presence of proteins and/or carbohydrates. Purification of such DNA preparations by hydroxyapatite column chromatography helped to obtain DNA preparations with desired 230/260 and 280/260 ratios. In this purification procedure, the isolated DNA preparations were equilibrated with 0.12 M sodium phosphate buffer (pH 6.8) and then loaded on HAP columns which were also pre-equilibrated with 0.12 M sodium phosphate buffer (pH 6.8) at room temperature. Single stranded DNA was eluted with 0.12 M sodium phosphate buffer (pH 6.8) while double stranded DNA was eluted with 0.4 M sodium phosphate buffer (pH 6.8).

Measurement of reassociation of DNA by hydroxyapatite column chromatography can lead to an overestimate of double stranded DNA due to the presence of single stranded tails and hyperpolymers. The use of very small fragments of DNA, therefore, can minimise this problem and provide a better estimate of the extent of reassociation at a given Cot value. Hence in the present work, DNA fragments with an average size of 0.5 kbp were used. Recovery of DNA from hydroxyapatite at this fragment length was nearly 100% indicating the absence of hyperpolymer formation.
Finally, the rate of reassociation is dependent on the concentration of the complementary DNA strands in solution, temperature of incubation and salt concentration. Extreme care was taken to maintain the proper conditions of reassociation. Typically, DNA concentrations were kept between 30-300 µg/ml to avoid viscosity effects on the rate of reassociation (Pay and Ronsse, 1975) and the temperature and salt concentration were 62°C and 0.18 M Na⁺, respectively.

Using the above parameters for reassociation, reassociation kinetics of *E. coli* and mouse liver DNA were determined and these were used as controls.

**Reassociation kinetics of sugarcane DNAs**

Non-linear least squares regression analysis of the reassociation data obtained by optical measurements and hydroxyapatite column chromatography helped in determining the number of components reassociating with second order kinetics in the three sugarcane DNAs. The results of such analysis are depicted in Fig. 3.1 and are summarized in Table 3.1. From these data, it can be seen that one fast and one slow DNA component, each reassociating with second order kinetics is present in all the three sugarcane tissues studied. From the Cot ½ of fast and slow DNA fractions, it is clear that the fast DNA reassociates 6 to 726 times faster than the slow DNA. This indicates that the fast reassociating DNA fraction consists of repeated DNA
FIGURE 3.1 REASSOCIATION KINETICS OF SONICATED
SUGARCANE DNAs (0.5 kbp)
Reassociation curves of 0.5 kbp long DNA
of CO-740 callus, CO-740 shoot and CO-7219 callus
using optical spectrophotometry as well as hydroxy-
apatite column chromatography. The solid line through
the data points represents the least squares fit for the
components allowing all parameters to free float. The lower
dashed curves represent the reassociation kinetics of pure
components
Mouse DNA (0-0-0) and E. coli DNA ( — ) were used
as control
TABLE 3.1 REASSOCIATION KINETICS OF SUGARCANE DNAs

<table>
<thead>
<tr>
<th>DNA fraction</th>
<th>Fraction of DNA</th>
<th>Cot ( h/2 ) (^a) observed M.s.</th>
<th>Frequency of repetition (^b)</th>
<th>Cot ( h/2 ) (^c) pure M.s.</th>
<th>Kinetic complexity (^d) nucleotide pairs</th>
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<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
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<tr>
<td>CO-740 Callus</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Very fast reassociating DNA</td>
<td>0.14</td>
<td>-</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>(Cot &lt; 10(^{-2}) M.s.)</td>
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<tr>
<td>Fast reassociating DNA</td>
<td>0.47</td>
<td>6.40</td>
<td>2.09 ( \times 10^2 )</td>
<td>3.04</td>
<td>2.28 ( \times 10^6 )</td>
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<tr>
<td>(Cot 1 ( \times 10^{-2} ) to 1 ( \times 10^2 ) M.s.)</td>
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<tr>
<td>Slow reassociating DNA</td>
<td>0.25</td>
<td>1.34 ( \times 10^3 )</td>
<td>1</td>
<td>3.43 ( \times 10^2 )</td>
<td>2.55 ( \times 10^8 )</td>
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<td>(Cot 1 ( \times 10^2 ) to 5 ( \times 10^3 ) M.s.)</td>
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<td>Unreassociated DNA</td>
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<td>-</td>
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<td>(Cot &gt; 5 ( \times 10^3 ) M.s.)</td>
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<td>CO-740 shoot</td>
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<tr>
<td>Very fast reassociating DNA</td>
<td>0.16</td>
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<tr>
<td>(Cot &lt; 5 ( \times 10^{-3} ) M.s.)</td>
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<tr>
<td>Fast reassociating DNA</td>
<td>0.16</td>
<td>4.56</td>
<td>5.80 ( \times 10^1 )</td>
<td>7.63 ( \times 10^{-2} )</td>
<td>5.70 ( \times 10^4 )</td>
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<td>(Cot 5 ( \times 10^{-3} ) to 2.5 M.s.)</td>
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<tr>
<td>Slow reassociating DNA</td>
<td>0.30</td>
<td>2.71 ( \times 10^1 )</td>
<td>1</td>
<td>8.16</td>
<td>6.12 ( \times 10^6 )</td>
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<tr>
<td>(Cot 2.5 to 2 ( \times 10^3 ) M.s.)</td>
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<tr>
<td>Unreassociated DNA</td>
<td>0.37</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>(Cot &gt; 2 ( \times 10^3 ) M.s.)</td>
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### TABLE 3.1 (continued)

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<td>CO-7219 Callus</td>
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</tr>
<tr>
<td>Very fast reassociating DNA (Cot &lt; 10⁻² M.s.)</td>
<td>0.14</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
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<tr>
<td>Fast reassociating DNA (Cot 1 x 10⁻² to 1 x 10² M.s.)</td>
<td>0.38</td>
<td>3.11</td>
<td>7.26 x 10²</td>
<td>1.19</td>
<td>8.99 x 10⁵</td>
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<td>2.26 x 10³</td>
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<td>5.84 x 10²</td>
<td>4.38 x 10⁸</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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*Values obtained from the respective reassociation curves (Fig. 3.1)*

*Values obtained by comparing the Cot 1/2 of E. coli DNA under our experimental conditions and assuming the genome size of E. coli as 4.5 x 10⁶ base pairs (Cairns, 1963).*

*Cot 1/2 values of unique DNA divided by Cot ½ values of the given fraction.*

*Cot 1/2 values observed x fraction of genome.*
sequences while the slow reassociating DNA fraction is made up of mainly single copy DNA sequences. Marked differences are, however, noticed among the three systems in the proportion, frequency of reiteration and kinetic complexity of the fast reassociating DNA component. In CO-740 callus, for example, the fast DNA reassociating between Cot $1 \times 10^{-2}$ to $1 \times 10^2$ Ms comprises 47.5% of the total DNA. In CO-740 shoot and CO-7219 callus on the other hand the fast DNA reassociates in the range of Cot $5 \times 10^{-3}$ to 2.5 Ms and Cot $1 \times 10^{-2}$ to $1 \times 10^2$ Ms respectively and represents 16.5% in CO-740 shoot and 38.5% in CO-7219 callus. The frequency of repetition and kinetic complexity of the fast DNA sequences are also high in callus cultures as compared to those in shoots. The proportion of the slow component is 25-30% in all three tissue systems. Its kinetic complexity is in the range of $2.5 - 4.4 \times 10^8$ nucleotide pairs in both the types of callus cultures and is significantly higher than that of shoot DNA ($6.1 \times 10^6$ np).

Apart from the fast and slow reassociating DNA components, 14-16% of the total DNA forms duplexes very rapidly at Cot $< 10^{-2}$ Ms in all the three sugarcane tissues. These are instantaneously reassociating sequences and may consist of highly repeated DNA and/or fold back sequences (Walbot and Dure, 1976). There is 12-37% of the total
sugarcane DNA which fails to reassociate at $\text{Cot} > 10^3 \text{ Ms.}$

This may be because of degradation of DNA during long incubation periods, failure of DNA to form stable duplexes and/or the presence of some sequences requiring a longer time to reassociate (Cullis, 1981, Pearson et al. 1978).
DISCUSSION

With the advent of recombinant DNA technology, it has been possible to clone specific genes and characterize them in details with respect to their coding regions and regulatory sequences in the 5' upstream and 3' downstream regions. DNA reassociation kinetics, although relatively an old experimental approach, gives a general guidance to genome structure and also an overall information about the content of repetitive DNA and about average estimates of its copy number and frequency of repetition. These studies are a prerequisite for further work such as DNA sequence organization and interspecies DNA sequence homologies.

Although the genomes of several plant species have been characterized with respect to their kinetic complexity and content of repetitive DNA, no such information was available in sugarcane when this work was undertaken. The present study has shown for the first time that the content of total repetitive DNA in sugarcane shoot genome is of the order of 33%. In the family Gramineae, cereals with high DNA content (1 C>5 pg) such as wheat, oat, rye, barely and maize have been extensively characterized with respect to their genome organization (Flavell and Smith, 1976; Hake and Walbot, 1980; Rimpau et al. 1978; 1980; Smith and Flavell, 1977). In these species, repeated DNA sequences constitute a major proportion (60-80%) of the total DNA (Table 3.2)
<table>
<thead>
<tr>
<th>Species</th>
<th>Genome size IC</th>
<th>Total repetitive DNA %</th>
<th>Unique DNA %</th>
<th>Reference</th>
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<tr>
<td><em>Eleusine coracana</em></td>
<td>1.6</td>
<td>49.0</td>
<td>51.1</td>
<td>Deshpande and Ranjekar (1980)</td>
</tr>
<tr>
<td>(Finger millet)</td>
<td></td>
<td>0.78</td>
<td>0.81</td>
<td></td>
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<tr>
<td><em>Echinochloa frumentacea</em></td>
<td>2.70</td>
<td>42.0</td>
<td>62.0</td>
<td>SivaRaman and Ranjekar (1984)</td>
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<td>(Barn yard millet)</td>
<td></td>
<td>1.13</td>
<td>1.67</td>
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<td><em>Hordeum vulgare</em></td>
<td>5.4</td>
<td>78.0</td>
<td>22.0</td>
<td>Ranjekar et al. (1978)</td>
</tr>
<tr>
<td>(Barley)</td>
<td></td>
<td>4.25</td>
<td>1.19</td>
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<td><em>Oryza sativa</em></td>
<td>0.6</td>
<td>52.0</td>
<td>48.0</td>
<td>Deshpande and Ranjekar (1980)</td>
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<tr>
<td>(Rice)</td>
<td></td>
<td>0.31</td>
<td>0.28</td>
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<td><em>Panicum miliare</em></td>
<td>1.0</td>
<td>38.0</td>
<td>62.0</td>
<td>ShivaRaman and Ranjekar (1984)</td>
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<td>(Little millet)</td>
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<td>0.38</td>
<td>0.62</td>
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<td><em>Pennisetum americanum</em></td>
<td>2.4</td>
<td>54.0</td>
<td>46.0</td>
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<td>(Pearl millet)</td>
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<td>1.32</td>
<td>1.12</td>
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<td><em>Saccharum officinarum</em></td>
<td>4.0</td>
<td>33.0</td>
<td>67.0</td>
<td>Present study</td>
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<td>(Sugarcane)</td>
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<td>2.68</td>
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<td>30.0</td>
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<td>73.5</td>
<td>26.5</td>
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<td>5.85</td>
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<td>48.0</td>
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<td>80.0</td>
<td>20.0</td>
<td>Ranjekar et al. (1976)</td>
</tr>
<tr>
<td>(Wheat)</td>
<td></td>
<td>13.84</td>
<td>3.46</td>
<td></td>
</tr>
<tr>
<td><em>Zea mays</em></td>
<td>7.7</td>
<td>58.0</td>
<td>42.0</td>
<td>Hake and Walbot (1980)</td>
</tr>
<tr>
<td>(Maize)</td>
<td></td>
<td>4.46</td>
<td>3.23</td>
<td></td>
</tr>
</tbody>
</table>
The work on millet DNAs (1C : 0.8-2.8 pg) has revealed several distinguishing features such as low nuclear DNA content and low proportion of repetitive DNA (26-42%) (Shivarajan and Rajekar, 1984). The 1C DNA content of Saccharum officinarum has been reported to be 4 g (Bennett and Smith, 1976). From the data on the repetitive DNA content in sugarcane and the data available in other Gramineae species, it is possible to comment on the relationship between the amount of repetitive DNA and nuclear DNA content in Gramineae. It can be seen from Fig.3.2 that the proportion of repetitive DNA varies linearly with DNA content. This is true for single copy DNA also. However, the slopes of two lines are different. Above a nuclear DNA content of 6 pg, the content of single copy DNA remains more or less constant while that of repetitive DNA continues to increase. Hence it appears that repeated DNA contributes significantly to the genome size in Gramineae.

Our data on DNA reassociation kinetics of the three sugarcane DNAs have revealed both similarities and specific differences in DNA during differentiation. The proportions of the slow reassociating DNA components and very rapidly reassociating DNA components are similar in all the three DNAs. Co-740 callus and CO-7219 callus DNAs, however, differ from CO-740 shoot DNA in the
FIGURE 3.2 RELATIONSHIP BETWEEN NUCLEAR DNA CONTENT
AND REPETITIVE DNA AND SINGLE COPY DNA
CONTENTS IN GRAMINEAE SPECIES

The data points used are from Table 3.2
FIG. 3-2.
proportion of fast reassociating DNA component. The latter accounts for 16% CO-740 shoot and its frequency of reiteration and kinetic complexity are 58 and $5.70 \times 10^4$ nucleotide pairs respectively. The proportion of fast reassociating DNA is in the range of 38-47% of the total DNA for both the callus tissues. The kinetic complexity and the frequency of reiteration of this DNA are in the range of $8.99 \times 10^5$ to $2.28 \times 10^6$ nucleotide pairs and 209 to 726 respectively. Due to a significant change in the proportion of fast reassociating DNA, the proportion of total repetitive DNA in CO-740 shoot is 32% as against 62% in CO-740 callus. Since fast DNA includes mainly repeated DNA sequences, it can be concluded that repeated DNA sequences undergo a considerable change in their proportion and kinetic complexity during the transition from callus tissue to shoot tissue in sugarcane.
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