CHAPTER III
REASSOCIATION KINETICS OF THE DNAS OF VIGNA UNGUICULATA AND CAJANUS CAJAN
Till recently, a large number of plant species have been characterized with respect to their DNA structure and DNA sequence organization (1-9). The basic goals of these studies have been as follows:

i) To understand the structural basis of gene regulatory processes/mechanisms (8-12). It has been suggested that specific types of genome organizations are involved in regulation of transcription as well as in regulating the amounts and types of stored messenger RNAs during development (8-10).

ii) To understand the molecular basis of genome evolution and speciation (13-16). Dover et al. (13) and Peacock et al. (14) have suggested that there may be a causal relationship between genome differentiation and species differentiation and that there is a need to know the principle sources and kinds of changes in genomes in order to understand evolution and speciation.

iii) To obtain fundamental information about the molecular biology of the major crop plants (16-18). As an augmentation of the role of plant breeding in selection of improved crops, it is expected that the understanding
of molecular biology of crop plants will enhance and accelerate successes in genetic manipulations (17).

As mentioned earlier, cowpea is a true *Vigna* species originated and domesticated in Africa. In contrast, mothbean, mungbean, and urdbean were earlier considered to constitute the Asiatic group of *Phaseolus* species with major centre of origin being Indian subcontinent, South East Asia and Burma. These have now been classified as species of *Vigna*. It was, therefore, of interest to compare the content and nature of different DNA sequence classes of the cowpea genome with those of the other three *Vigna* species. Some work on DNA reassociation kinetics on mothbean, mungbean and urdbean has been done earlier in our laboratory (19-21). In the present chapter, the work on these species has been extended to include non-linear least squares analyses of the reassociation kinetics data of short DNA fragments to determine second order components and size distribution of repetitive DNAs. In case of cowpea, the entire data result from the present studies.

Pigeonpea is the most important pulse crop in India which is also the largest producer of this crop. In our laboratory, work has been initiated on molecular biology and genetic engineering of this plant with the ultimate aim of cloning and analysing the genes for seed storage proteins. Since no basic DNA data were available for pigeonpea, we deemed it necessary to characterise its DNA.
This chapter describes the results of our studies on DNA reassociation kinetics of cowpea and pigeonpea and intrageneric comparison of these data in four Vigna species.

MATERIALS AND METHODS

DNA sources and isolation of DNA

The DNAs of all the four Vigna species and of pigeonpea were isolated as described in Chapter II.

Reassociation of DNA

Reannealing of DNA strands is measured as a function of concentration of reassociating DNA strands and time of incubation (defined as Cot). It can be monitored as a hypochromic shift in absorbance of DNA at 260 nm (optical reassociation), by hydroxyapatite column chromatography and by single strand specific SI Nuclease assay (22, 23). The experimental data on reassociation of DNA strands can be modelled into components following second order kinetics using non-linear least squares regression analyses and based on these, curves for best fit and ideal components can be plotted. From such analyses, additional parameters like proportion, rate constants for reassociation, frequency of reiteration and kinetic complexities of the reassociating components can be derived.
The rates of reassociation of DNA strands are dependent on a number of factors such as DNA concentration, duration of reassociation, temperature of reassociation, ionic concentration during reassociation and sizes of the reassociating strands.

In our experiments, the standard criteria for DNA reassociation were as follows:

a) Reassociations were carried out at 62°C (Tm-22°C) which is considered to be an optimum parameter according to Britten et al. (22).

b) Reassociations were carried out in buffers with sodium ion concentration equivalent to 0.18M (usually 0.12M sodium phosphate buffer, pH 6.8). When higher ionic concentration buffers were used to accelerate the rate of reassociation, suitable corrections were applied according to Britten et al. (22).

c) Cot 1 was defined as incubation of denatured DNA (100 µg/ml) at 62°C for 1 hour in 0.12M sodium phosphate buffer, pH 6.8.

Reassociation of *E. coli* (control DNA), cowpea and pigeon pea DNAs were carried out using the above criteria.

i) Shearing of DNA

Size of DNA fragments in reassociation reactions is an important parameter that needs to be well defined and
controlled. For studying reassociation kinetics over a wide Cot range, generally small sized DNA fragments are used as they reassociate with reproducible rates and do not form large networks or aggregates (24). Shearing of DNA to fragment sizes of 0.4 to 0.6 kilobase pairs was achieved using a sonicator (Bronwill model Biosonik III, 250W, 20 KHz) fitted with a 1.25 cm diameter probe. Approximately 30 ml of DNA solution in 0.12M sodium phosphate buffer, pH 6.8 was sonicated for 3 min in 1 min pulses separated by 10 min intervals. The DNA solution was kept immersed in an ice-bath during sonication and care was taken to ensure that its temperature did not rise beyond 6 - 8°C.

ii) Sizing of DNA fragments

The size of the native as well as sonicated DNA fragments was measured by agarose gel electrophoresis in 1X TAE buffer (0.04M Tris-acetate 0.002M, disodium EDTA, pH 7.8 - 8.0). The DNA solution was loaded in submerged agarose slab gels and the electrophoresis was carried out at a constant current (20 mA) for 6 hours. Following electrophoresis, the gels were stained in ethidium bromide (1 μg/ml) in 1X TAE and visualized on a 302 nm long wavelength U.V. transilluminator (Ultra Violet Products, San Gabriel, USA). HindIII digest of λ DNA
and HaeIII digest of Φ X 174 RF DNA were used as the DNA size markers (Figure III.1) and the photographs were taken using the Minolta X 700 (35 mm) camera with red filter.

iii) Optical reassociation of sonicated DNAs

The hyperchromic shift in U.V. absorbance of DNA during strand separation (melting) is reversed as reannealing of DNA takes place. This decrease in absorbance (hypochnoric shift) can be monitored as a function of time. The extent of reassociation of DNA at a given time can be calculated by normalizing the total hypochromic shift to 100% reassociation. Gilford 250 UV-visible double beam spectrophotometer was used to measure the optical reassociation of DNA. The sheared DNAs (average 0.48 kbp long) were first denatured at 100°C and the temperature was maintained for 10 min to ensure complete denaturation. After denaturation, the samples were rapidly cooled to 62°C and incubation was continued for various lengths of time to obtain the desired Cot values. During cooling, absorbance was constantly monitored and "Zero time" or reference time for start of reassociation was taken at as 80°C which is approximately a mid point of the temperature range from 62°C to 100°C (22). Percent reassociation of DNA (R) was calculated according to the formula:
Figure III.1: Determination of the size distribution of unsheared and sheared DNAs of cowpea

Electrophoresis was carried out in neutral 1.0% agarose gels in TAE (1 X) for 3-5 μg of the DNA sample. The sizes were determined by comparison with mobility of the marker DNA bands.

Lane 1: DNA digested with HindIII. The bands correspond to fragments of sizes 23.1, 9.4, 6.4, 4.2, 2.2 and 2.0 kb.

Lane 2: Unsheared DNA of cowpea

Lane 3: Sheared DNA of cowpea

Lane 4: ϕ X 174 RF DNA digested with MaelIII. The bands correspond to fragments of sizes 1.5, 1.08, 0.87, 0.61 and 0.31 kb.
\[ R = \frac{A_{260}\text{ of denatured DNA} - A_{260}\text{ of DNA at a given time}}{A_{260}\text{ of denatured DNA} - A_{260}\text{ of native DNA at } 62^\circ C} \]

The Cot value at a given time was calculated from the concentration of DNA at the start of reassociation and the time of incubation from zero time.

iv) Measurement of DNA reassociation by hydroxyapatite column chromatography

This was carried out essentially according to Britten et al. (22). Sheared DNAs were denatured by heating in a boiling water bath for 10 min and then incubated at 62^\circ C for specific time intervals to achieve the desired Cot values. Following incubation, the DNA solution was loaded onto a hydroxyapatite column pre-equilibrated at 62^\circ C with 0.12 M sodium phosphate buffer, pH 6.8, the unreassociated DNA was washed off in 0.12 M sodium phosphate buffer and the reassociated DNA was eluted with 0.4M sodium phosphate buffer, pH 6.8. The optical densities of these fractions (both reassociated as well as unreassociated DNAs) were measured at 260 nm in a Shimadzu U.V. visible double beam spectrophotometer (model U.V. 210 A). The percent reassociation of DNA (R) was calculated as follows:

\[ R = \frac{B}{A + B} \times 100 \text{ where } A \text{ and } B \text{ are the amounts in} \]
micrograms of DNA eluted with 0.12 M and 0.4 M sodium phosphate buffer, pH 6.8, respectively (25). All the reassociations were carried out in the Cot range of $1 \times 10^{-1}$ to $5 \times 10^{2}$ M.s. for *E. coli* DNA and $1 \times 10^{-1}$ to $1 \times 10^{4}$ M.s. for the plant DNAs using approximately 500 μg of DNA in each experiment. For Cot values greater than $1 \times 10^{2}$ M.s., the reassociations were generally carried out in 0.36 M or 0.4 M sodium phosphate buffer, pH 6.8, at 62°C, in sealed ampoules and the correction factors of 4.4778 and 4.9049 respectively were applied to the rates of reassociations (22). Prior to loading these DNAs onto hydroxyapatite columns, they were diluted to 0.12 M sodium phosphate buffer pH 6.8 with distilled water at 62°C. Unreassociated and reassociated DNAs were eluted as described above.

The reassociation values were plotted on a semi-logarithmic graph paper with the corresponding Cot values on the logarithmic axis.

v) Computer analyses of DNA reassociation kinetics data

Eukaryotic DNAs reassociate over a Cot range of 7-8 orders of magnitude. Since the individual components of the reaction are not always kinetically distinct, computer based programmes have been used to define second-order reassociating components by non-linear least squares regression analyses methods. The programmes are
generally based on minimization of error (26-29) in fitting of experimental data by direct search procedures. The second order reaction for reassociation, for which the data are fitted, is described by the equation:

\[
\frac{C}{C_0} = \frac{1}{1 + K \text{Cot}}
\]

where \( C_0 \) is the initial concentration of single stranded DNA, \( C \) is the concentration of single stranded DNA after "t" seconds of reassociation and 'K' is the rate constant of the reactions (29). In the present investigation, least squares analyses of experimental data were carried out using a ICL 1900 series computer (Regional Computer Centre, University of Poona, Pune 411 007), employing a standard optimization subroutine STEPIT (written by J.P. Chandler and distributed by Quantum Chemistry Programmes Exchange, Indiana University, Bloomington, Indiana, USA). The RMS error (29) was given by the equation:

\[
\text{RMS error} = \sqrt{\left( \frac{C}{C_0} \text{ experimental} - \frac{C}{C_0} \text{ fitted} \right)^2}
\]

In the equation for second order reaction, the form of \( \frac{C}{C_0} \) was assumed to be \( \frac{C}{C_0} = T + \frac{F_i}{1 + K_i \text{Cot}} \) where \( T \) is the fraction remaining unreassociated at a given Cot value, \( T \) is the fraction of DNA that fails to reassociate at the maximum Cot values and \( F_i \) and \( K_i \) are the fraction reassociated and rate constants for
the 1\textsuperscript{st} component respectively (29). Least RMS errors were obtained when parameters T, F\textsubscript{i} and K\textsubscript{i} were allowed to free float.

Isolation and sizing of repetitive DNA duplexes

Native, unsheared DNAs were reassociated to respective limit repetitive Cot values of all the five plants in 0.18 M sodium chloride buffered with 0.006 M PIPES (1,4-Piperazinediethanesulfonic acid, Sigma Chemicals Co., USA) at 62°C. Following reassociation, the DNA samples were adjusted to 0.25 M sodium acetate, pH 4.5, 0.0001 M zinc sulfate, 0.025 M 2-mercaptoethanol. SI nuclease (from Boehringer-Mannheim, FRG or Bethesda Research Laboratory, USA) digestion with 1 unit enzyme/\textmu g of DNA was carried out for 30 min at 37°C. The reaction was terminated by chilling and by adjusting the DNA sample to 0.12 M sodium phosphate buffer, pH 6.8. The SI Nuclease resistant DNA duplexes were then separated by chromatography on hydroxyapatite and the eluted fractions were dialysed to 0.12 M sodium phosphate buffer, pH 6.8. The size distribution of these duplexes was determined by gel filtration on Agarose gels (Biogel A -50m, BioRad, USA) packed in 90 cm x 1.2 cm column using 0.4 cm diameter solid glass beads. The column was washed with 0.12 M sodium phosphate buffer, pH 6.8 before loading 70-80 \textmu g of SI resistant duplexes in the same buffer. The elution was in 0.12 M sodium phosphate buffer, pH 6.8 and the absorbance
of the effluent was continuously monitored at 253 nm on a LKB UVICORD (model No. 2138). The exclusion limit of this column was determined to be >1.5 kbp, using sheared as well as unsheared calf thymus DNA of known fragment lengths.

**RESULTS**

Nonlinear least squares regression analyses of DNA reassociation kinetics data

The experimental data and the computer fitted curves for *E. coli*, cowpea and pigeonpea are depicted in Figure III.2a-c while the computer fitted curves alone in case of mothbean, mungbean and urdbean are shown in Figure III.2d. In the case of the latter three plants, the actual experimental data are already published elsewhere (19-21). These data, however, were not analysed by least squares methods and hence the computer analyses is included in this chapter for a more meaningful comparison with the cowpea data. Table 1 gives a detailed analysis of the data in Figures III.2b-d. *E. coli* DNA was found to reassociate as a single second order component with a rate constant of 0.2096 and Cot 1/2 of 4.77 x 10^6 M.s. These values are found to agree with the reported values (33,35,36). The genome size of *E. coli* was assumed to be 4.5 x 10^6 nucleotide pairs (34,35). The genome size and Cot 1/2 of *E. coli* were used in calculations of kinetic parameters in the case of *Vigna* and pigeonpea DNAs.
Figure III.2: Reassociation curves of average 0.48 kb long DNA of *E. coli*, cowpea, pigeonpea and the three *Vigna* species, mothbean, mungbean and urdbean. In the case of former three DNAs, experimental data obtained by both, optical as well as hydroxyapatite column method have been fitted together and are depicted along with the fitted curve and individual components. In case of mothbean, mungbean and urdbean, only the computer fitted curves are depicted.

In a, b and c, are the reassociation data points. The solid line through the data points is the computer fitted curve while the dashed lines indicate reassociation of ideal components.

a: Reassociation curve for *E. coli* DNA (RMS = 0.052)
b: Reassociation curve for cowpea DNA (RMS = 0.0492)
c: Reassociation curve for pigeonpea DNA (RMS = 0.0468)
d: Reassociation curves for mothbean DNA (........, RMS = 0.0209), mungbean DNA (- --------, RMS = 0.0250) and urdbean DNA (-.-.-.-.-., RMS = 0.0120)
Table 1: REASSOCIATION KINETICS OF SHEARED (AVERAGE 0.48 kb long) DNAs of FOUR VIGNA
SPECIES AND PIGEONPEA

<table>
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<tr>
<th>PLANT SPECIES</th>
<th>DNA COMPONENT (Cot RANGE, M.s.)</th>
<th>FRACTION OF TOTAL DNA</th>
<th>Cot 1/2 OBSERVED (M.s.)</th>
<th>K OBSERVED</th>
<th>Cot 1/2 PURE (M.s.)</th>
<th>K PURE</th>
<th>KINETIC COMPLEXITY (NUCLEOTIDE PAIRS)</th>
<th>COPY NUMBER</th>
<th>1C DNA CONTENT BY FEWLEGEN (pg)</th>
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<td>V. UNGUICULATA</td>
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<td>(COWPEA)</td>
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<td></td>
<td>II: 4.3 x 10^-3 TO</td>
<td>0.225</td>
<td>2.1 x 10^2</td>
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<td>2.5 x 10^1</td>
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<td>III: 2.5 x 10^1 TO</td>
<td>0.505</td>
<td>2.83 x 10^2</td>
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<td>1.0 x 10^4</td>
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<td>IV: Cot &gt; 1.0 x 10^4</td>
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<td>V. ACONITIFOLIA</td>
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<td>(MOTHBEAN)</td>
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<td>II: 5.6 x 10^-3 TO</td>
<td>0.295</td>
<td>2.78 x 10^0</td>
<td>0.3595</td>
<td>8.201 x 10^-1</td>
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<td>5.0 x 10^1</td>
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<td>III: 5.0 x 10^1 TO</td>
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<td>IV: Cot &gt; 1.0 x 10^4</td>
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<td>V. RADIATA (MUNGBEAN)</td>
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<td>1.194x10^-1</td>
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<td>1.5x10^1</td>
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<td>3.45x10^2</td>
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<td>5.0x10^3</td>
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<td>IV: Cot&gt;5.0x10^3</td>
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<td>V. MUNGO (URD BEAN)</td>
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<td>5.0x10^1</td>
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<td>III: 5.0x10^1 TO</td>
<td>0.460</td>
<td>9.09x10^2</td>
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<td>C. CAJAN (PIGEONPEA)</td>
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</table>

(a): Component I represents the very rapidly reassociating sequences, II represents the total repetitive sequences, III represents the single copy sequences and IV represents the fraction of sequences remaining unassociated at the maximum Cot value, in all plants. (b): Values obtained from reassociation curves (Fig.III.2). (c): Cot 1/2 values observed x fraction of the genome. (d): K pure = 1 + Cot 1/2 pure. (e): Values obtained from standard relationship for E. coli DNA where Cot 1/2 is 4.77 x 10^6 M.s. (under our experimental conditions) and genomic complexity is 4.5 x 10^6 nucleotide pairs. (f): Cot 1/2 of component III + Cot 1/2 of component II. (g): From the standard relationship 1 pg = 9.12 x 10^8 nucleotide pairs. (h): Values are reported elsewhere in literature.
It is clear from Fig. III.2b-d that all the plant DNAs exhibit only two kinetic components, namely fast and slow. A fraction of the total DNA (2.5 to 11.0%) reassociates too rapidly to be resolved under the present experimental conditions and 5 to 24.5% of the total DNA does not reassociate at the maximum Cot value. In all the five plant genomes, the fast component accounts for 25-40% of the total DNA, reassociates with rate constants of 0.3 to 47 and has an average frequency of reiteration in the range of 192 to 13,490. The fast component is, therefore, considered to consist of repeated DNA sequences and the Cot value at which all of the fast component reassociates (the limit repetitive Cot values) is $2.5 \times 10^1$ M.s. in cowpea, $5.0 \times 10^1$ M.s. in mothbean, $1.5 \times 10^1$ M.s. mungbean, $5.0 \times 10^1$ M.s. in urdbean and $5.0 \times 10^0$ M.s. in pigeonpea. A close examination of the data reveals that cowpea DNA is significantly different from those of the rest of the Vigna species and pigeonpea with respect to rate constant and copy number.

The slow component is considered to include mainly single copy DNA sequences and is 30, 172, 37, 88 and 58 times more complex than E. coli genome in case of cowpea, mothbean, mungbean, urdbean and pigeonpea respectively. Based on the rate constants of reassociation of single copy components, the kinetic estimates of haploid genome sizes were in the range of 0.145 pg to 0.846 pg in the five plant species.
Estimation of repetitive DNA content using SI nuclease

Another approach of estimating repetitive DNA content is to reassociate high molecular weight total DNA to its limit repetitive Cot value and then subject it to SI nuclease treatment followed by isolation of total repetitive DNA duplexes by hydroxyapatite column chromatography. Using this procedure the amount of repetitive DNA was found to be 22.8% in cowpea and 26.4% in pigeonpea.

Size distribution of SI nuclease resistant repetitive DNA duplexes

The agarose $A_{0.50}$ gel filtration profiles of the SI nuclease resistant repetitive DNA duplexes and of a mixture of unsheared and sheared ($\approx 0.5$ kbp) calf thymus DNAs are depicted in Fig. III.3a-f. All the plant DNAs exhibit substantial proportions of both long ($\approx 1.5$ kbp) and short ($\approx 0.5$ kbp) repeats (Table 2). These elution profiles also indicate the presence of intermediately sized repetitive duplexes (in the continuum from 0.5 to 1.5 kbp). The size distribution of repeated DNA sequences has been studied in Pea (37), French bean (20) and Soybean (38). In the pea genome short repeats (average 0.3 kbp) predominate while in French bean the size is of an intermediate average length
Figure III.3: Sizing of repetitive DNA duplexes
(S1 nuclease resistant) by gel filtration through Agarose A-50 column.

a: Thick curve for Calf thymus DNA
   (unsheared + 0.5 kbp long)
b: Cowpea DNA
c: Mothbean DNA
d: Mungbean DNA
e: Urdbean DNA
f: Pigeonpea
Table 2: SIZE DISTRIBUTION OF S1 NUCLEASE RESISTANT REPETITIVE DUPLEXES BY AGAROSE A 50 GEL FILTRATION

<table>
<thead>
<tr>
<th>DNA OF</th>
<th>Proportion of repeats (%)</th>
<th>Long ($\geq 1.5$ kb)</th>
<th>Intermediate (0.5 - 1.5 kb)</th>
<th>Short ($\leq 0.5$ kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea (2.5 x 10^1 M.s.)</td>
<td>25</td>
<td>21</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Mothbean (5.0 x 10^1 M.s.)</td>
<td>36</td>
<td>32</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Mungbean (1.5 x 10^1 M.s.)</td>
<td>51</td>
<td>11</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Urdbean (5.0 x 10^1 M.s.)</td>
<td>29</td>
<td>23</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Pigeonpea (5.0 x 10^0 M.s.)</td>
<td>50</td>
<td>7</td>
<td>43</td>
<td></td>
</tr>
</tbody>
</table>

Figures in parenthesis are the limit repetitive Cot values.
It has been suggested that speciation involves certain quantitative changes in DNA also (16). However, it cannot be stated precisely as to whether these changes are restricted to repetitive or unique DNA sequences alone. Studies carried out to date in a number of plant and animal species belonging to same taxa have revealed a non-random nature of the quantitative changes in both repetitive as well as non-repetitive DNA fractions. In order to ascertain the nature of these non-random DNA changes, Hutchinson et al. (16) determined correlations of the amounts of repetitive and non-repetitive DNAs with total DNA contents and were able to calculate the ratio of increase in repetitive DNA to increase in non-repetitive DNA. This ratio was 5.53 and 3.91 in species belonging to genera Lolium and Lathyrus respectively (16). Such ratios were also observed in species belonging to families Gramineae (16,39,40), Compositae (16,41) and to genera like Anemone (16,42), Allium (16,43) and a wide range of higher plants (16,44,45).

The changes in amounts of repetitive and non-repetitive DNA sequences have been correlated to some of the cytological
parameters such as interphase nuclear structure, chromosome packaging, amounts of condensed chromatin and changes in DNA associated with euchromatin and heterochromatin (16,44,46). According to Narayan (46), in Lathyrus at least, genome evolutionary processes are envisaged to have occurred as a succession of spasmodic changes rather than a continuum of progressive changes.

The almost general observation of the above correlations have been argued by Hutchinson et al. (16) to be an evidence/indication for ordered genomic changes during speciation.

To ascertain whether the present 4 Vigna species show continuous or discontinuous DNA changes, a similar comparison was, therefore, attempted in the above species which differed in their haploid DNA in the range 0.5 to 2.0 pg. From Fig. III.4a, it is clear that the comparison of DNA content with proportions of repetitive and single copy DNA in the Vigna species results in non-significant correlations. The comparison of the slopes of the two regressions (Fig.III.4a), nevertheless reveals that the ratio of repetitive to non-repetitive DNA is 0.667. This situation is somewhat similar to that in case of rodents (47) and molluscs (48) where the lack of correlation is attributed to a very small number of species, which is true in the case of Vigna species also. That this is indeed so, is evident from the comparison of 23
Figure III.4: Linear regressions for the correlation between amounts of repetitive DNA (---) and single copy DNA (-----) with haploid (1 C) nuclear DNA content.

a: Correlations and regression in case of four Vigna species, namely, cowpea, mothbean, mungbean and urdbean.

b: Correlations and regression in case of twenty three legume species, including the above four Vigna species.
species (including the present 4 *Vigna* species and pigeonpea) belonging to family Leguminosae, for variations in repetitive and non-repetitive DNA contents (Fig. III.4b). In this case, highly significant correlations are obtained with approximately 2.15 fold increase in repetitive DNA for every 1 pg increment in non-repetitive DNA as a result of increasing haploid nuclear DNA contents. In view of the observation that a significant correlation is obtained when a larger number of species are compared, it may be suggested that the low amounts of repetitive DNA (25-40%) in the 4 *Vigna* species and pigeonpea (26%) can be attributed to their low haploid DNA contents (0.5 to 2.0 pg and 0.9 pg respectively).

The size distribution of repetitive DNAs suggest that all the five plant DNAs will have a mixed pattern of inter-persson. However, amongst the five plants and in particular amongst the four *Vigna* species, the relative proportion of short repetitive sequences may be considered to be indicative of turn-over rates. Since it has been suggested that a fast rate of genome turnover results in a large proportion of short repeats (49) our data in the present species can be considered to indicate that cowpea DNA has a relatively faster turn over rate (Table 2) while mungbean and mothbean genomes are turning over slowly. These preliminary indications in case of mungbean agree with the reported slow turnover of mungbean genome (50).
REFERENCES


