CHAPTER IV

REPETITIVE DNA HOMOLOGIES AMONGST THE DNAs OF FOUR VIGNA SPECIES AND CAJANUS CAJAN
INTRODUCTION

Since single copy sequences are known to have evolved essentially by nucleotide substitution (1), interspecies comparison of single copy sequences can provide a measure of evolutionary sequence divergence (1). Repetitive DNA sequences, on the other hand, have evolved in a major way due to various molecular events such as amplification, deletion, translocation and base substitution (2,3). Study of interspecies homology of repeated DNA sequences in closely related phyletic lineages can, therefore, help to identify their origin and also to search for possible ways in which these sequences have accumulated in the genomes (4).

In higher plants, studies on DNA-DNA hybridization have specifically been carried out to understand (i) phylogeny of related species (5-24), (ii) genome donor during evolution of crop plants (13,14), (iii) modes of evolution of repetitive and single copy DNA sequences (15,16,21-24) and (iv) the probable molecular causes/effects of species isolation (9-12, 21,22). Such studies have, hitherto, been carried out amongst plants belonging to families like Gramineae (5-16), Liliaceae (17), Leguminosae (5,18,19), Cucurbitaceae (20-22), Solanaceae (5,20) and Chenopodiaceae (23,24). However, no DNA-DNA hybridization data are available amongst genera Phaseolus
and *Vigna* of family Leguminosae. As mentioned earlier, these two genera have a large number of morphological similarities and include many species with a doubtful generic status. On the basis of karyomorphological and biochemical parameters, some of the species of *Phaseolus* have been transferred to genus *Vigna* (25). It was, therefore, thought necessary to study relatedness of DNA sequences of some of these reclassified *Vigna* species to those of a true *Vigna* species, namely cowpea (*Vigna unguiculata*). The three *Vigna* species selected for this study are mothbean (*V. aconitifolia*), mungbean (*V. radiata*) and urdbean (*V. mungo*). For extra-generic comparison, DNA of pigeonpea (*Cajanus cajan*) was also hybridized to cowpea DNA tracer. Two approaches were used in these studies. In the first approach, labelled, total, sheared cowpea DNA was used as tracer and was hybridized with excess, unlabelled, driver DNAs of cowpea, mothbean, mungbean, urdbean and pigeonpea. The extent of reassociation was monitored in a wide Cot range of $1 \times 10^{-1}$ M.s to $1 \times 10^{3}$ M.s. The main objective of such an approach was to identify kinetically distinct components in heterologous reassociations.

In the second approach isolated, total cowpea repetitive DNA (Cot $2.5 \times 10^{1}$ M.s, Chapter III) was used as tracer and was hybridized with excess of unlabelled driver DNAs of cowpea, mothbean, mungbean, urdbean and pigeonpea, at two Cot values of $2.5 \times 10^{1}$ M.s and $1 \times 10^{2}$ M.s. This approach was
mainly used to ascertain repeated DNA sequence homologies and thermal stabilities of heteroduplexes.

MATERIALS AND METHODS

Germination of seeds

Seeds of all the plants under study were germinated as described in Chapter II.

Extraction, shearing, sizing and reassociation of unlabelled DNA

These experiments were carried out as detailed in Chapters II and III.

Isolation of repetitive DNA of cowpea

Sheared cowpea DNA was incubated to its repetitive Cot value of $2.5 \times 10^4$ M.s. (Chapter III) and the reassociated duplexes were separated on hydroxyapatite column at 62°C, in 0.4 M sodium phosphate buffer, pH 6.8. These DNA duplexes were then used for radiolabelling.

In vitro $^{32}P$ labelling of DNA

Sheared, total DNA as well as isolated repetitive DNA of cowpea were in vitro labelled with $\alpha-^{32}P$ dTTP (Amersham, specific activity $\sim 3000$ Ci/mM) by the nick-translation
reaction of Rigby et al. (26). The reaction mixture generally consisted of 2 - 3 \( \mu g \) of DNA, 20 \( \mu M \) of each of unlabelled nucleotides, 90 - 100 pmoles of \( \alpha^{32}P \) dTTP, 0.05 M Tris-HCl, pH 7.4 and 0.005 M MgCl\(_2\). E. coli DNA polymerase I (BRL, USA) was added to a final concentration of 2 - 3 units/\( \mu g \) of DNA. The reaction was carried out at 15\(^\circ\)C for 90 min and terminated by deproteinization with freshly distilled phenol (saturated with Tris-buffer). The aqueous layer was further deproteinized with chloroform-isoamyl alcohol (24:1, v/v) and adjusted to 0.12 M sodium phosphate buffer, pH 6.8. The labelled DNA duplexes were purified by hydroxyapatite chromatography and the fraction eluted at room temperature in 0.4 M sodium phosphate buffer, pH 6.8 was used as such or after dilution to 0.36 M sodium phosphate buffer, pH 6.8. The specific activity of the probe was of the order of 2.5 to 5 \( \times 10^6 \) cpm/\( \mu g \).

**Sizing of labelled DNA**

The size of the cowpea total DNA and repetitive DNA was determined after in \textit{vitro} nick-translation by agarose gel electrophoresis as described in Chapter III. The labelled DNAs were electrophoresed along with DNA-molecular weight markers. After electrophoresis, the tracks containing labelled DNA were cut into 1 cm pieces. The gel pieces were
dried thoroughly and the radioactivity was measured. Molecular weight of the labelled DNAs (peak radioactivity) was then calculated by comparing their mobility with those of DNAs of known molecular weights.

**Counting of radioactivity**

Throughout the course of DNA-DNA hybridization experiments, counting of $^{32}$P was done in a Beckman LS 100 Liquid Scintillation Counter, as Cerenkov radiations (27). Possible sources of errors due to solution volumes and orientations (28) were taken into consideration in this mode of counting.

**Homologous and heterologous hybridization**

In all hybridization reactions, labelled cowpea DNA was diluted with approximately 5000 fold excess of driver DNA, denatured and allowed to reassociate to desired Cot values. In homologous hybridization reactions, the driver DNA was unlabelled cowpea DNA while in heterologous hybridization reactions, the driver DNA was unlabelled mothbean, mungbean, urdbean and pigeonpea DNA. In a control experiment for estimating self-reassociation of tracer DNA, labelled cowpea DNA alone was reassociated under identical conditions of heterologous reassociations and all the reassociation values of homologous and heterologous reactions were corrected for self-reassociation. In hybridization involving repetitive
DNA as tracer, the melting behaviour of the reassociated duplexes was determined by hydroxyapatite thermal chromatography.

**Use of total, sheared cowpea DNA as tracer**

1) **Homologous hybridization**

Labelled cowpea DNA (≈ 50,000 cpm and 0.01 to 0.02 µg) was diluted with sheared, unlabelled cowpea DNA in approximately 5000 fold excess, denatured in a boiling water bath for 10 min in sealed/stoppered tubes and incubated at 62°C for appropriate lengths of time to obtain Cot values in the range 1 x 10⁻¹ to 1 x 10³ M.s. All reassociations were done in 0.36 M sodium phosphate buffer, pH 6.8 and necessary correction was applied to the rate of reassociation according to Britten et al. (29). Following incubation, the DNA samples were diluted to 0.12 M sodium phosphate buffer, pH 6.8 and loaded onto hydroxyapatite columns equilibrated at 62°C. Unreassociated DNA was eluted in 0.12 M sodium phosphate buffer, pH 6.8 while the reassociated duplexes were eluted in 0.4 M sodium phosphate buffer, pH 6.8. The fraction reassociated was calculated as follows:
Fraction reassociated (\%) = \frac{B}{A+B} \times 100 \text{ where } A \text{ is the total radioactivity of fractions eluted in 0.12 M sodium phosphate buffer, pH 6.8 and } B \text{ is the total radioactivity of fractions eluted in 0.4M sodium phosphate buffer, pH 6.8.}

ii) Heterologous hybridization

Labelled, total, sheared cowpea DNA was diluted with excess unlabelled DNA of mothbean, mungbean, urdbean or pigeonpea and the reassociations were carried out in 0.36 M sodium phosphate buffer, pH 6.8, at 62°C as described above for the homologous reassociation. In each case, reassociation was monitored in the Cot range $1 \times 10^{-1}$ to $1 \times 10^3$ M.s and corrected for self-reassociation of tracer DNA. The homology of the other driver DNAs to that of cowpea at each Cot value was calculated from the reassociation values by normalizing homologous reassociation to 100% homology (9,10).

Use of isolated repetitive DNA of cowpea as tracer

i) Homologous reassociation

Labelled cowpea repetitive DNA was diluted with approximately 5000 fold excess of unlabelled cowpea DNA and reassociated to Cot $2.5 \times 10^1$ M.s and $1 \times 10^2$ M.s.
These two Cot values were selected for two reasons
(a) Cot $2.5 \times 10^1 \text{ M.s}$ is the "limit repetitive" Cot value of cowpea DNA and (b) the heterologous hybridization curves in the Cot range $1 \times 10^1$ to $1 \times 10^3 \text{ M.s}$ (Fig. IV.1) indicate a change in reassociation rates of duplexes at or around Cot $1 \times 10^2 \text{ M.s}$.

The reassociations were carried out in 0.36 M sodium phosphate buffer, pH 6.8 with an appropriate correction to the rate of reassociation (29). Reassociated duplexes were eluted from hydroxyapatite columns by raising the column temperature in $5^\circ\text{C}$ increments from $62^\circ\text{C}$ onwards and eluting DNA at each temperature with 0.12 M sodium phosphate buffer, pH 6.8. This was followed by a final elution at $95^\circ\text{C}$ with 0.4 M sodium phosphate buffer, pH 6.8. Counts eluting at $62^\circ\text{C}$ in 0.12 M sodium phosphate buffer, pH 6.8 were due to unreassociated DNA, while the sum total of counts eluted with 0.12 M sodium phosphate buffer, pH 6.8 at each temperature increment and finally with 0.4 M sodium phosphate buffer, pH 6.8 represented the reassociated DNA. The fraction reassociated (R) was, therefore, scored as
(Counts in the fractions eluted at each temperature increment in 0.12 M sodium phosphate buffer, pH 6.8 + counts in the fraction eluted in 0.4 M sodium phosphate buffer, pH 6.8 at 95°C) x 100

\[ Z_R = \frac{\text{Sum total of counts in the fraction eluted at 62°C in 0.12 M sodium phosphate buffer, pH 6.8 + counts in the fraction eluted at each temperature increment as above}}{\text{The temperature at which 50% of total duplex counts were eluted was taken as the melting temperature (Tm) of heteroduplexes. All reassociation values were corrected for self-reassociation of tracer DNA.}} \]

ii) Heterologous reassociations

Labelled cowpea repetitive DNA was diluted with excess unlabelled DNA of mothbean, mungbean, urdbean or pigeonpea and was reassociated to Cot 2.5 x 10^1 and 1 x 10^2 M.s in each case, as described above for the homologous reassociation. The reassociated repetitive duplexes were eluted by thermal chromatography and percent reassociation and Tm were determined as stated above. All reassociation values were corrected for self-reassociation of tracer DNA.
RESULTS

Self-reassociation of tracer DNA

In all the hybridization reactions, unlabelled DNA was used in 5000 fold excess as compared to the labelled DNA to ensure the maximum hybridization of labelled DNA with unlabelled DNA. The tracer DNA, however, may still undergo self-reassociation and this was found to be the case in the present studies. The reassociation values of tracer DNA in the Cot range $1 \times 10^{-1}$ to $1 \times 10^3$ M.s were in the range 2 to 10.5%. In view of this, the total reassociation values at corresponding Cot values were corrected for self-reassociation of tracer DNA to obtain true reassociation values.

Smith and Flavell (9) have reported tracer-tracer DNA reassociation of 18.7% in case of labelled wheat DNA at Cot $5 \times 10^1$ M.s. Similar range of tracer-tracer DNA reassociation values have been obtained in sponge gourd DNA (21,22) and pearl millet and barnyard millet DNAs (15,16).

Use of labelled total cowpea DNA as tracer

$^{32}$P labelled cowpea DNA was hybridized with excess of (a) unlabelled cowpea DNA (homologous hybridization) and (b) unlabelled mothbean, mungbean, urdbean and pigeonpea DNAs (heterologous hybridization), over a Cot range of $1 \times 10^{-1}$ to $1 \times 10^3$ M.s. Reassociation curves for these hybridizations
are depicted in Figure IV.1. In Table 1, homologies of the driver DNAs to cowpea DNA in the Cot range $1 \times 10^{-1}$ to $1 \times 10^{3}$ M.s. and average homologies for the whole range of Cot values are listed. From this table, it is clear that the three *Vigna* DNAs show greater than 50% homology to cowpea DNA while pigeonpea DNA shows only 29% average homology in the Cot range $1 \times 10^{-1}$ to $1 \times 10^{3}$ M.s. Furthermore, at Cot $1 \times 10^{2}$ M.s. there is a maximum homology of the three *Vigna* DNAs to that of cowpea. Urdbean DNA shows the highest average homology (73%) to cowpea DNA, while mothbean and mungbean DNAs are homologous to cowpea DNA to a similar extent (50,57%).

In order to estimate the homology of repeated DNA sequences of mothbean, mungbean, urdbean and pigeonpea to those of cowpea DNA, the homologous reassociation of cowpea DNA at the repetitive Cot value of the driver DNAs was normalized to 100% homology and the heterologous reassociation values were then recalculated as percentage homology. These data are presented in Table 2. It is clear from the table that repetitive DNAs of the three *Vigna* species are more homologous to cowpea DNA (53 - 83% than that of pigeonpea DNA which is only 25% homologous to cowpea DNA. Among the three Asian *Vigna* species urdbean repetitive DNA shows the highest homology to cowpea repetitive DNA.
Figure IV.1: Curves for homologous and heterologous reassociation when total DNA of cowpea are used as tracer.

*Cowpea DNA - Cowpea DNA (●——●)
*Cowpea DNA - Mothbean DNA (▲——▲)
*Cowpea DNA - Mungbean DNA (□——□)
*Cowpea DNA - Urdbean DNA (▼——▼)
*Cowpea DNA - Pigeonpea DNA (○——○)
*Cowpea DNA : Labelled cowpea DNA
Table 1: HOMOLOGY OF MOTHBEAN, MUNGBEAN, URDBEAN AND PIGEONPEA DNAs to total DNA of Cowpea in the Cot range $1.0 \times 10^{-1}$ to $1.0 \times 10^3$ M.s.

<table>
<thead>
<tr>
<th>Cot (M.s.)</th>
<th>Labelled cowpea total DNA - unlabelled cowpea DNA reassociation$^a$ (%)</th>
<th>Homology of labelled cowpea DNA to unlabelled DNAs of Mothbean$^b$ (%)</th>
<th>Mungbean$^b$ (%)</th>
<th>Urdbean$^b$ (%)</th>
<th>Pigeonpea$^b$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.0 \times 10^{-1}$</td>
<td>08.54</td>
<td>32.6</td>
<td>44.5</td>
<td>22.7</td>
<td>08.2</td>
</tr>
<tr>
<td>$1.0 \times 10^0$</td>
<td>15.11</td>
<td>14.3</td>
<td>17.3</td>
<td>62.5</td>
<td>22.6</td>
</tr>
<tr>
<td>$1.0 \times 10^1$</td>
<td>15.41</td>
<td>47.8</td>
<td>59.8</td>
<td>98.0</td>
<td>30.0</td>
</tr>
<tr>
<td>$5.0 \times 10^1$</td>
<td>22.96</td>
<td>53.4</td>
<td>61.7</td>
<td>83.0</td>
<td>20.3</td>
</tr>
<tr>
<td>$1.0 \times 10^2$</td>
<td>27.05</td>
<td>71.3</td>
<td>79.3</td>
<td>100.0</td>
<td>22.5</td>
</tr>
<tr>
<td>$1.0 \times 10^3$</td>
<td>60.15</td>
<td>62.4</td>
<td>64.6</td>
<td>69.0</td>
<td>48.3</td>
</tr>
</tbody>
</table>

Average homology in the Cot range $1.0 \times 10^{-1}$ to $1.0 \times 10^3$ M.s. (\%) = 50.3 57.3 73.7 29.4

$^a$: Reassociation value is corrected for self-reassociation of tracer DNA.

$^b$: The homologies are estimated as follows: (i) Cowpea-cowpea homologous reassociation at the specific Cot value is normalized to 100% homology, (ii) The heterologous reassociation value is corrected for self-reassociation of tracer DNA and (iii) The corrected values of heterologous reassociation are compared to the corrected homologous reassociation values.
Table 2: HOMOLOGIES OF MOTHBEAN, MUNGBEAN, URDBEAN AND PIGEONPEA DNAS TO COWPEA DNA AT RESPECTIVE LIMIT REPETITIVE COT VALUES

<table>
<thead>
<tr>
<th>Labelled total DNA of cowpea</th>
<th>Limit repetitive Cot value of driver DNA&lt;sup&gt;a&lt;/sup&gt; (M.s.)</th>
<th>Fraction of total DNA at limit repetitive Cot</th>
<th>Homology at limit repetitive Cot&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowpea</td>
<td>2.5 x 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.25</td>
<td>100.0</td>
</tr>
<tr>
<td>Mothbean</td>
<td>5.0 x 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.38</td>
<td>53.0</td>
</tr>
<tr>
<td>Mungbean</td>
<td>1.5 x 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.33</td>
<td>65.0</td>
</tr>
<tr>
<td>Urdbean</td>
<td>5.0 x 10&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.40</td>
<td>83.0</td>
</tr>
<tr>
<td>Pigeonpea</td>
<td>5.0 x 10&lt;sup&gt;0&lt;/sup&gt;</td>
<td>0.26</td>
<td>25.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values described in chapter III.

<sup>b</sup> From Fig. IV.1 and Table 1.
Use of labelled, isolated repetitive DNA of cowpea as tracer

i) Reassociation and homologies of repetitive duplexes

Homologies of labelled cowpea repetitive DNA to that of mothbean, mungbean, urdbean and pigeonpea DNA at Cot $2.5 \times 10^1$ and $1 \times 10^2$ M.s are listed in Table 3. The table includes homologies of cowpea DNA to that of other driver DNAs at these two Cot values of $2.5 \times 10^1$ and $1 \times 10^2$ M.s as computed from Figure IV.1 and Table 1, for comparison. From this table, the following trends emerge:

a) At Cot $2.5 \times 10^1$ M.s. the homologies of all the driver DNAs to cowpea repetitive DNA are higher than when total DNA of cowpea was used as tracer, except for urdbean DNA, which shows similar homologies in both cases.

b) At Cot $1 \times 10^2$ M.s. however, in all cases except pigeonpea, the driver DNAs show similar homologies when, both isolated repetitive as well as total DNAs of cowpea are used as tracer. Pigeonpea DNA shows a higher homology to cowpea repetitive DNA than to total cowpea DNA.
<table>
<thead>
<tr>
<th>Labelled cowpea repetitive DNA Vs Driver DNA of</th>
<th>Cot 2.5 x 10^1 M.s.</th>
<th>Cot 1 x 10^2 M.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reassociation (%) a</td>
<td>Homology (%) b</td>
</tr>
<tr>
<td>Cowpea</td>
<td>25.1</td>
<td>100.0</td>
</tr>
<tr>
<td>Mothbean</td>
<td>29.9</td>
<td>100.0</td>
</tr>
<tr>
<td>Mungbean</td>
<td>22.6</td>
<td>90.0</td>
</tr>
<tr>
<td>Urdbean</td>
<td>19.3</td>
<td>76.0</td>
</tr>
<tr>
<td>Pigeonpea</td>
<td>9.6</td>
<td>38.0</td>
</tr>
</tbody>
</table>

a: Values estimated as described in Materials and Methods
b: The values are calculated as follows:
   i) Reassociation of cowpea-cowpea duplexes at the specific Cot value is normalised to 100% homology
   ii) The heterologous reassociation value is corrected for self-reassociation of tracer and
   iii) The corrected heterologous reassociation value is then compared to that of homologous reassociation in (i).
Thermal elution profiles and melting curves for hetero-
duplexes between labelled cowpea repetitive DNA and
unlabelled DNAs of cowpea, mothbean, mungbean, urdbean
and pigeonpea at Cot $2.5 \times 10^1$ and $1 \times 10^2$ M.s. are
depicted in Figures IV.2 and IV.3 respectively. All
the elution profiles, except for cowpea-mothbean
heteroduplexes (Fig. IV.3) at Cot $1 \times 10^2$ M.s. are
biphasic, showing a minor low melting fraction (LMF)
and a major high melting fraction (HMF). The cowpea-
mothbean heteroduplexes at Cot $1 \times 10^2$ M.s. show a smooth
melting and elution profile with a Tm of 81°C. At Cot
$2.5 \times 10^1$ M.s. the proportion of LMF varies in the range
19 to 28% with Tm values from 66.5 to 67.5°C in all
the cases (Table 4). The extent of base mismatch in
these LMFs as compared to labelled cowpea-unlabelled
cowpea duplexes is of the order of 0.5 to 1.0%, indicating
that well matched duplexes are formed. At Cot $1 \times 10^2$ M.s.
the proportion of LMF varies from 17 to 43% with a
relatively wider Tm range of 67 - 71°C. The nucleotide
sequence divergence at Cot $1 \times 10^2$ M.s. is also low
(Table 4). It is further evident from the table that
excepting for pigeonpea DNA, the other DNAs show an
increase in the proportions of LMF at Cot $1 \times 10^2$ M.s.
than at Cot $2.5 \times 10^1$ M.s. indicating thereby that

ii) Thermal stabilities of repetitive duplexes

Thermal elution profiles and melting curves for hetero-
duplexes between labelled cowpea repetitive DNA and
unlabelled DNAs of cowpea, mothbean, mungbean, urdbean
and pigeonpea at Cot $2.5 \times 10^1$ and $1 \times 10^2$ M.s. are
depicted in Figures IV.2 and IV.3 respectively. All
the elution profiles, except for cowpea-mothbean
heteroduplexes (Fig. IV.3) at Cot $1 \times 10^2$ M.s. are
biphasic, showing a minor low melting fraction (LMF)
and a major high melting fraction (HMF). The cowpea-
mothbean heteroduplexes at Cot $1 \times 10^2$ M.s. show a smooth
melting and elution profile with a Tm of 81°C. At Cot
$2.5 \times 10^1$ M.s. the proportion of LMF varies in the range
19 to 28% with Tm values from 66.5 to 67.5°C in all
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these LMFs as compared to labelled cowpea-unlabelled
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increase in the proportions of LMF at Cot $1 \times 10^2$ M.s.
than at Cot $2.5 \times 10^1$ M.s. indicating thereby that
Figure IV.2: Melting and thermal elution profiles of homologous and heterologous repetitive duplexes at Cot $2.5 \times 10^1$ M.s.

- Melting curve
- Thermal elution profile

a: *Cowpea - Cowpea DNAs
b: *Cowpea - Mothbean DNAs
c: *Cowpea - Mungbean DNAs
d: *Cowpea - Urdbean DNAs
e: *Cowpea - Pigeonpea DNAs
f: *Cowpea - *Cowpea DNAs
Figure IV.3: Melting and thermal elution profiles of homologous and heterologous repetitive duplexes at Cot $1.0 \times 10^2$ M.s.

- Melting curve
- Thermal elution profile

a: *Cowpea - Cowpea DNAs
b: *Cowpea - Mothbean DNAs
c: *Cowpea - Mungbean DNAs
d: *Cowpea - Urdbean DNAs
e: *Cowpea - Pigeonpea DNAs
f: *Cowpea - *Cowpea DNAs
Table 4: CHARACTERIZATION OF LOW MELTING FRACTIONS (LMF) OF REPETITIVE HETERODUPLEXES

<table>
<thead>
<tr>
<th>Labelled cowpea repeated DNA Vs Driver DNAs</th>
<th>LMF, Cot $2.5 \times 10^1$ M.s.</th>
<th>LMF, Cot $1.0 \times 10^2$ M.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion $^a$ (%)</td>
<td>$Tm^a$ $^\circ C$</td>
</tr>
<tr>
<td>Cowpea</td>
<td>19.0</td>
<td>67.5</td>
</tr>
<tr>
<td>Mothbean</td>
<td>20.0</td>
<td>66.5</td>
</tr>
<tr>
<td>Mungbean</td>
<td>20.0</td>
<td>66.5</td>
</tr>
<tr>
<td>Urdbean</td>
<td>23.0</td>
<td>67.0</td>
</tr>
<tr>
<td>Pigeonpea</td>
<td>28.0</td>
<td>67.0</td>
</tr>
</tbody>
</table>

$^a$: Values estimated from Fig. IV.2
$^b$: Values estimated from Fig. IV.3
$^c$: $Tm$ of heteroduplexes is subtracted from the $Tm$ of homologous duplexes
$^d$: Calculated as $1^\circ C$ $\Delta Tm$ corresponding to 1\% base mismatch (29).
Table 5: CHARACTERIZATION OF HIGH MELTING FRACTION (HMF) OF HETERODUPLEXES

<table>
<thead>
<tr>
<th>Labelled cowpea repetitive DNA Vs Driver DNAs</th>
<th>HMF, Cot $2.5 \times 10^4$ M.s.</th>
<th>HMF, Cot $1.0 \times 10^2$ M.s.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proportion&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Tm&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cowpea</td>
<td>81.0</td>
<td>88.0</td>
</tr>
<tr>
<td>Mothbean</td>
<td>80.0</td>
<td>86.0</td>
</tr>
<tr>
<td>Mungbean</td>
<td>80.0</td>
<td>87.5</td>
</tr>
<tr>
<td>Urdbean</td>
<td>77.0</td>
<td>86.0</td>
</tr>
<tr>
<td>Pigeonpea</td>
<td>72.0</td>
<td>87.5</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Values estimated from Fig. IV.2
<sup>b</sup>: Values estimated from Fig. IV.3
<sup>c</sup>: Tm of heterologous duplexes is subtracted from Tm of homologous duplexes
<sup>d</sup>: Calculated as $1^\circ C \ \Delta$Tm corresponding to 1% base mismatch (29)
relatively less G + C rich sequences are forming duplexes between Cot $2.5 \times 10^1$ and $1 \times 10^2$ M.s.

The HMFs of heteroduplexes, on the other hand, show relatively greater variations in proportion, Tm and mismatch at both Cot $2.5 \times 10^1$ and $1 \times 10^2$ M.s. (Table 5). With an increase in Cot value from $2.5 \times 10^1$ M.s. to $1 \times 10^2$ M.s. there is a decrease in the proportions of HMFs in all cases except pigeonpea. The base mismatch also varies from 0.5 to 2.0% at Cot $2.5 \times 10^1$ M.s. to 0 to 4.0% at Cot $1 \times 10^2$ M.s. These values, in turn, are higher than those for LMFs indicating that HMFs in these duplexes are more diverged.

DISCUSSION

In studies on relatedness of DNA sequences, total DNAs as well as isolated repetitive and single copy DNA fractions have been commonly used as tracers (19,24,30-33). In the present studies, which report the homology of repeated DNA sequences in four plant species belong to the genus Vigna for the first time, both total cowpea DNA as well as isolated cowpea repetitive DNA have been used as tracers. The two most important features of the data are moderately high homology of repetitive DNA sequences (53-83%) and very low nucleotide sequence divergence (0-4%) in all the heteroduplexes. These data suggest that nucleotide substitution has played a very limited role in the evolution of repeated DNA sequences.
Furthermore, the differences in the centres of origin and gene pools (34,35) of these plant species may have resulted in moderately high homology of the repeated DNA sequences as against the expected very high homology observed in the two *Luffa* species (32). In the case of pigeonpea, on the other hand, the low average homology of 29% is explained by the fact that both cowpea and pigeonpea belong to the same tribe *Phaseolae* of the family Leguminosae (Fabaceae).
REFERENCES


