
SYNOPSIS

Genesis of the problem

Pulses or grain legumes form important and ancient components of the Indian agricultural system. As crops, these are largely grown under rain-fed and low agronomic input conditions. This situation has resulted from the fact that the cereal crops for various reasons have been given priority in cultivation, thereby, utilizing better soils and better irrigation and other agronomic inputs. Thus we find that despite having domesticated several thousand years ago, the grain legumes are grown on marginal lands of poor fertility and their cultivation conditions are not so very different from those in their natural/native habitats. It has, therefore, been suggested that the grain legumes, as domesticated crops, are still being subjected to the natural selection pressures, unlike the cereal crops where extensive cultivation and breeding selection have evolved new plant types. In view of these features of grain legumes, there is an increasing awareness of the need for extensive research with respect to genetics, physiology and agriculture of these cultivated plants. Hence a large number of pulse breeding programmes have been undertaken by a number of agencies and of recent we find that research in molecular genetics of pulses is also gaining importance.

Why study Vigna species ?

In the Indian subcontinent, the main pulse crops are the grain legumes belonging to genera Vigna and to some extent Phaseolus. These pulses are mainly tropical in habitat and show considerable plasticity in their ecological requirements. Amongst pulses, the present work focuses on five plants, namely, cowpea (Vigna unguiculata), mothbean (Vigna aconitifolia), mungbean (Vigna radiata), urdbean (Vigna mungo) and pigeonpea (Cajanus cajan). Of these five species under consideration, information is available on partial characterization of DNAs of mothbean, mungbean and urdbean in terms of thermal denaturation, CsCl centrifugation and reassociation kinetics, while no such data are known for cowpea and pigeonpea DNAs. Ours is probably the first attempt of characterization of nuclear DNA of cowpea and pigeonpea. Moreover, on account of the confused taxonomical status of mothbean, mungbean and urdbean as species of Vigna/Phaseolus, we thought it worthwhile, to compare these three species to a true Vigna species, namely, Vigna unguiculata (cowpea). Interestingly enough, while interspecies genetic hybridization were attempted (and partial success also achieved) amongst mothbean, mungbean and urdbean themselves and with related wild Phaseolus species, no such efforts have been directed towards crossing of cowpea with other cultivated as well as wild Vigna species. Moreover, since cowpea has a gene pool structure distinct from that

of the other three species which have overlapping gene pools, neither natural nor artificial gene mixing 'seems to have taken place amongst these Vigna species.

Specific objectives of the present work

Using cowpea DNA and seed globulins as reference in all the comparisons, we were interested in knowing (i) the extent of similarity that the four Vigna species share and (ii) whether the effect of gene pool barriers could be identified at the molecular level.

Strategies used

The present work is based on integration of two broad-based approaches to comparison, namely, (i) DNA characterization and DNA relatedness and (ii) seed protein comparison. In this way it may be possible to have a close examination of the genome in two perspectives, since the rate of change of DNA during evolution is quite different from the rate of expression of this change in a protein. The present studies have been carried out using the following methods :

- i) Comparison of the base composition of DNAs
- ii) Determination of the content and nature of repetitive DNAs
- iii) In vitro DNA-DNA hybridization for determining DNA relatedness

- iv) Restriction endonuclease cleavage analyses of DNAs and finally
- v) Electrophoretic and immunochemical comparison of seed storage globulins

Organization of thesis (Scope of thesis)

The entire thesis has been organized in the form of seven chapters.

Chapter I describes the current status of plant genome organization and evolutionary theories postulated to describe the present day status of genome organizations. Information given in this chapter is essentially based on published papers and efforts have been made to have an upto date account of the recent achievements in the field of plant genome research.

In chapter II, I have described results of studies on determination of base composition and related physico-chemical properties of DNAs by thermal denaturation and isopycnic CsCl centrifugation. The quantitation of 5-methyl cytosine by HPLC is also detailed in this chapter.

Results of studies on reassociation kinetics of the DNAs of cowpea and pigeonpea are detailed in chapter III, along with the data on mothbean, mungbean and urdbean which have been reanalysed by non-linear regression methods.

Studies on DNA-DNA hybridization in all the five plant species using cowpea total as well as repetitive DNA as tracer were carried out to determine intrageneric (amongst the Vigna species) and intergeneric (between Vigna and Cajanus) DNA sequence homologies and these results are presented in chapter IV.

In chapter V, I describe the results of studies on restriction endonuclease cleavage analyses of all the five plant DNAs and the identification of a discretely organized repeat family in pigeonpea.

Comparative studies on seed globulins of the five plants, immunochemical characterization of the seed globulins of cowpea and relatedness of other seed globulins to those of cowpea are described in chapter VI.

Chapter VII details an overview of all the results obtained during the course of the present studies (chapters II to VI) with an emphasis on the interpretations of these results for identifying the effects of gene pools, centres of origin and domestication on the gross properties of DNA and seed globulins of the Vigna species.

Summary of the thesis

The present thesis describes the use of an integrated approach of studying DNA characteristics as well as seed storage globulins in four Vigna species and in Cajanus cajan (pigeonpea). To our knowledge, such a combined approach involving simultaneous broad-based comparison of DNA as well as proteins has not been used before atleast in plants. The salient features of the results obtained in the case of all the five plants are summarized below.

The melting temperature (T_m) and buoyant density (ρ) of the DNAs are in the range 81.6 to 85.1°C and 1.690 to 1.694 g/cm³. The G + C content of the DNAs ranges from 30.08 to 38.55% while the m⁵C amounts are found to be 7.85 to 11.11 mole percent by HPLC. The G + C contents determined from T_m are more than those determined from ρ suggesting the presence of large proportions of m⁵C. When m⁵C amounts are used to predict ρ , it is found that in cowpea, mothbean and urdbean DNAs, practically no m⁵C is present in the mainband DNA, while in mungbean all and in pigeonpea some of m⁵C is present in mainband DNA itself.

When the high resolution thermal denaturation (HRTD) profiles are compared qualitatively in case of all the plants, it is seen that these are polyphasic and typically like those of eukaryotic DNAs. Furthermore, an intrageneric comparison within the genus Vigna has revealed a substantial similarity

of these DNAs as is evident from the fact that even for a small temperature range (0°C) of comparison, as many as 8 peaks are shared among the four Vigna species.

The reassociation kinetics data of short DNA fragments (average ^{length}/ 0.48 kbp) can be modelled into a two component system only. The repetitive DNA amounts determined from these studies are 25.0 to 40.0% of total DNAs and the rate constant and reiteration frequency of the repetitive DNAs fall in the range of 1.219 to 212.76 and 192 to 13490 copies respectively. The size distribution of S1 nuclease resistant repetitive duplexes isolated from long, unsheared DNA fragments reveals two major size classes of repeats ($\gg 1.5$ kbp and $\ll 0.5$ kbp). The reassociation kinetics data in the four Vigna species have been compared along with the published data for other Leguminous species to ascertain if the quantitative changes in DNA contents of these species can be correlated to specific changes in the repetitive or non-repetitive DNA fractions. These correlations indicated a uniform extent of increase in repetitive DNA with every one picogram increase in non-repetitive DNA being accompanied by an increase of 2.15 pg in the repetitive DNA.

The studies on homology of total as well as isolated repetitive DNA of cowpea (tracer DNA) to that of the other Vigna plant DNAs reveal average homologies of 29 to 77% and 25 to 83% respectively. The moderately high homologies of these Vigna DNAs to that of cowpea may have resulted from the

differences in centres of origin and gene pools of these plants. Furthermore, the repetitive heteroduplexes are seen to exhibit very low nucleotide sequence divergence. Based on the above data, it may be suggested that both sequence amplification as well as sequence divergence have played only a minor role in the evolution of these repeats.

Restriction endonuclease cleavage analyses reveal the presence of a distinct family of repeats with MboI/Sau3AI in pigeonpea and Southern hybridization experiments have further shown this family to be homologous to a highly repetitive probe, namely, $Cot\ 1.0 \times 10^{-1}$ M.s. sequences. Unlike in the case of lily and rye DNAs where long and short repeats are identified respectively in restriction endonuclease digests, the pigeonpea repeats, at a primary level atleast, are found to be organised as both long fragment and short fragment clusters.

The pattern of digestion of total DNAs with restriction endonucleases reveals the occurrence of site-specific methylation and, in general, a low frequency of occurrence of m^5C at CpG dinucleotides. In the case of Vigna DNAs, digestions with MboI and Sau3AI indicate the presence of methyladenine also. The presence of this base modification gains indirect support from HPLC analyses where extra peaks are eluted in the DNA hydrolysates of the four Vigna species alone.

The SDS - PAGE patterns of seed globulins of cultivated Vigna species when compared to those of two wild Vigna species reveal that the former have apparently acquired/diversified new seed proteins. When the total seed globulins of cowpea are used to generate antibodies in rabbits, unusually, only one abundant subunit (approximate molecular weight 52 Kd) from among the many is maximally antigenic. Furthermore, the comparative immunochemical studies with seed globulins of all Vigna species with respect to cowpea globulin antibodies reveal substantial homologies amongst these species. These studies on seed globulins carried out using electrophoretic as well as immunochemical techniques indicate considerable similarities amongst the Vigna species, thereby reaffirming their taxonomic status as species belonging to genus Vigna.

Apart from these general results, it is possible to identify cowpea as being distinct from the other three Vigna species because (i) it has a distinct heavy satellite, (ii) reassociation kinetics of its DNA reveal a repetitive DNA with a copy number as high as 13490, (iii) using cowpea total as well as repetitive DNA as tracer, total and repetitive DNA homologies for other Vigna species are not very high and (iv) the Jaccard index of similarity for the electrophoretic patterns of seed globulins also is not as high as expected for closely related species. On the basis of the above differences in DNA characteristics and seed globulins of

cowpea with respect to the other three Vigna species, and in view of the known differences in gene pools and centre of origin and domestication in these species, it has been inferred that the effects of the latter (gene pools etc.) are reflected in some of the molecular parameters in the Vigna species.

The achievements of the present studies

The present studies on the four Vigna species in particular provide a strong substantiation to their taxonomic status. Since three of the present four species, namely, mothbean, mungbean and urdbean have been reclassified as Vigna from their earlier genus Phaseolus, it is important to establish their affinities to Vigna using a true Vigna species for reference, which in the present studies is cowpea (Vigna unguiculata). While the earlier studies on karyomorphological parameters did ratify the taxonomic revision of Asiatic Phaseolus species to Vigna, the present studies provide additional and a more strong substantiation of the similarities at the genomic level also.

Among the other salient results ensuing from the present studies, of notable interest are the observations that (i) cowpea nuclear DNA exhibits a distinct heavy satellite, (ii) all the Vigna DNAs have methylated adenine also as a modified base, (iii) the pigeonpea genome has a distinct repeat family of MboI elements which belongs to the highly

repetitive sequence group and (iv) the antigenicity studies of cowpea globulins enabled us to use sensitive quantitative immunoassays to screen different varieties and developing stages of cowpea, suggesting these assays as a major biotechnological application of screening germplasm for desirable traits.

COWPEA

Vigna unguiculata (L.) Walp. syn. Vigna sesquipedalis Fruhw.,
Vigna sinensis (L.) Savi ex Hassk.

2n = 2x = 22.

Common names : cowpea, blackeye bean, blackeye pea, cowgram

Cowpea is an ancient food crop. Though probably originated in Africa, it is widely distributed through tropical and subtropical regions at present. It is one of the major food legumes and is grown as a hot-weather, rain-fed or semi-arid crop. The plant is intolerant to frost and is generally regarded as drought resistant.

The plant is an annual herb, often flowering indeterminately. The habit of the plant varies from cultivar to cultivar and ranges from trailing to erect. The inflorescence is an unbranched axillary raceme with conspicuous and self-pollinated flowers. The flowers are in alternate pairs and may be white, dirty yellow, pale blue, pink or violet in colour. The pods are linear, crescent shaped or coiled and may be yellow, brown, purple, white, creamy white or black in colour. The number of seeds in the pods ranges from 8 - 20. Like most legumes, the seed proteins are deficient in sulfur containing amino acids, eventhough the protein content is 23 - 24%.

Affinities/Ancestry : To an African wild species Vigna dekindtiana.

MOTHBEAN

Vigna aconitifolia (Jacq.) Marechal syn. Phaseolus aconitifolius Jacq.

$2n = 2x = 22$.

Common names : mothbean, dewgram, matbean.

Mothbean is widespread in India, Burma and Pakistan and grows wild in these regions. It is believed to have originated in these regions. It is grown as a drought resistant, hot weather crop and is capable of growing as a rain-fed crop also. However, it cannot withstand water-logging and is also extremely sensitive to frost.

The plant is a perennial or annual creeping herb and grows in a profusely branched manner along the soil over a very short height. The inflorescence is axillary with small, bright yellow, self-pollinated flowers. The pods are small, square or ovate and heavy. There are 4 - 8 small, square or ovate and smooth seeds in each pod. The seeds contain 22 - 23% proteins which are deficient in the sulfur containing amino acids.

Affinities/Ancestry : Since the plant is reported to grow wild in India, Burma and Pakistan, the cultivated forms are probably derived from them.

MUNGBEAN

Vigna radiata (L.) Wilczek syn. Phaseolus aureus Roxb.,
Phaseolus radiatus (L.)

2n = 2x = 22.

Common names : mungbean, greengram, goldengram.

Mungbean is a popular food legume in South East Asia, India, Burma, Thailand, Indonesia and the Philippines and has probably originated in these regions. Although the crop tolerates wide temperature ranges and drought, it does not withstand water-logging and frost.

The plant is an annual, erect to suberect herb, showing considerable variations in its form and adaptation to climate. Yellow or greenish yellow, self-pollinated and self-fertilized flowers are produced in clusters, axillary or terminal racemes. The pods may be straight or curved, subcylindrical, grey, greyish, olivegreen, brown or buff coloured. Each pod contains about 10 - 20 seeds. The overall morphology of mungbean plant is very similar to that of urdbean plant. The seeds contain 19 - 25% proteins which are deficient in methionine.

Affinities/Ancestry : To wild species of Vigna, namely,
Vigna sublobata.

URDBEAN

Vigna mungo (L.) Hepper syn. Phaseolus mungo L.

2n = 2x = 22.

Common names : urdbean, blackgram.

Urdbean is an ancient legume believed to have originated in South East Asian subcontinent. It shares considerable similarities with mungbean and is intolerant to frost and water-logging.

It is a bushy, hairy, sometimes spreading or trailing annual herb. The inflorescence is axillary with terminal clusters of small, pale yellow, lemon yellow or bright golden yellow, self-pollinated flowers. The pods are slender, cylindrical, erect or suberect with a short hooked beak and may be buff, light or dark brown coloured. Each pod contains 4 - 10 small seeds. The seeds contain 24% proteins which are deficient in methionine.

Affinities/Ancestry : Like mungbean, to a wild species,

Vigna sublobata.

PIGEONPEA

Cajanus cajan (L.) Millsp. syn. Cajanus indicus Spreng.

2n = 2x = 22.

Common names : Pigeonpea, redgram, congobean.

Pigeonpea is produced maximally in India as a pulse crop and is also believed to have originated here. The crop is drought resistant and is extremely sensitive to frost and water-logging.

It is an erect, woody, short lived perennial shrub. The inflorescence is short, axillary or terminal raceme. The flowers may be pale yellow to orange and purple or reddish in colour. The pods are flattened, downy with oblique walls and end in a beak. The colour of the pods may be green, purple or maroon or green with splotched purple or maroon. The pods contain a few seeds. The seeds contain 14 - 28% proteins which are deficient in sulfur containing amino acids.

Affinities/Ancestry : To species of Atylosia, particularly Atylosia lineata.

