# SYNOPSIS

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The family Gramineae comprises the most important group of plants as they are the principal source of food for humans and feed for domesticated animals. Cereal genomes which include wheat, rye, barley and oats have been well investigated with respect to the structure, organisation, chromosomal location, divergence and evolution of their repeated DNA sequences. Great millet which has been the focus in the present work ranks next to rice, wheat and maize in the world market. In India, great millet and pearl millet are important next to rice and wheat and are grown extensively in central and southern parts of the country.

Repeat DNA families have been identified and characterized in a number of plant species such as Zea mays, Secale species, Allium cepa, Vicia faba, Cucurbita species, Arabidopsis thaliana and Lupinus luteus. However, very little is known about these families in millets. In the present work, the DNAs of great millet and five other related millets namely pearl millet, fox tail millet, little millet, barn yard millet and finger millet have been characterized using a few molecular approaches that have been well standardised in our laboratory. Apart from studying the general behaviour of these millet DNAs towards restriction enzymes and getting information about the methylation status of the total DNAs, specific repeat families such as EcoRI and XbaI have been identified and characterized to some extent in the great millet genome. Similarly, AluI repeat family has been identified in all the millets under consideration and its interspecies homology has been assessed. Such

characterisation of the repeat sequences of the millets could serve as a base to explore the possibilities of using them in a manner analogous to the way in which the repeat sequences from other plants have been used in crop plant improvement. Some of the important findings of the present work are summarised below.

**I. Base compositional heterogeneity among the millet DNAs**

The high resolution thermal denaturation profiles of millet DNAs show several maxima and shoulders and are skewed towards the GC rich side indicating a greater base compositional heterogeneity in GC rich sequences. Linear regression analysis shows that no correlation exists between the repetitive DNA content/nuclear DNA content and the number of peaks, while a very good correlation exists between $\Delta T$ and repetitive DNA content/nuclear DNA content indicating that increase in the repetitive DNA content has resulted in a greater sequence heterogeneity in these DNAs. When a stringent temperature interval of 0.1°C is allowed, very few peaks are shared among these millet DNAs, suggesting that such peaks are probably species-specific. In all the millets, a peak in the temperature range of 89.4-89.9°C appears to be conserved though there is a variation in its proportion and position (within 0.5°C). An attempt has also been made to quantitatively determine the maximum extent of base compositional similarity between any two DNAs. By such analysis, these millets can be classified into two groups, wherein the members of each group show greater than 30% similarity with each other, while the similarity between members of the two groups is less than 7%.

**II. Nature of repeat families in the six millets**

The total DNAs of great millet, little millet, fox tail millet, barn yard millet and finger millet were reassociated to Cot 10 Ms at 55°C, 62°C, 69°C and 75°C. In all these millets, the fraction of DNA scored as repetitive decreases with an increase in incubation temperature. In little millet, barn yard millet, finger millet and great millet, the copy number of repeats decreases as the temperature of reassociation is increased suggesting that the repeat families in their genomes are predominantly heterogeneous. In fox tail millet, no significant change is observed in the copy number over the temperature range studied. However, optical reassociation studies of total repeats isolated at 55°C and 75°C indicate that repeat families in this plant are homogeneous. In great millet, almost one third of the sequences that behave as single copy at standard conditions are actually fossil repeats. Such fossil repeats are not a prominent feature of the genomes of the other four millets. The ratios of sequence complexities of repeats isolated at 75°C to those isolated at 55°C are 2.2, 3.5, 81 and 0.3 in case of little millet, finger millet, fox tail millet and great millet respectively. These three observations suggest that among these millets, the rate of turnover of the genome of fox tail millet is the slowest while that of great millet is the fastest.

**III. Methylation status of DNAs in six millets**

HPLC analysis of six millet DNAs indicates that the 5-methyl cytosine content varies from 3% in barn yard millet to 9.6% in great

millet. The fraction of cytosine residues methylated varies between 14% in little millet to 31% in pearl millet. These values are higher than those observed for animal cells and thus show a trend complying with the literature data for other plants.

The digestion patterns of millet DNAs with MspI and HpaII suggest a predominance of $^{m}$CpG methylation in great millet and of $^{m}$CpC methylation in the other five millets. The restriction enzyme data using MboI, Sau3AI and Dpn I indicates that many of the $^{5'}$GATC$^{3'}$ sequences are methylated at adenine and/or cytosine residues except in little millet where adenine methylation is insignificant and there is a predominance of cytosine methylation in these $^{5'}$GATC$^{3'}$ sequences. The presence of a 23 kbp fragment in MboI digests of great millet and finger millet and its absence in Sau3AI digests suggest the occurrence of several $^{5'}$GATC$^{3'}$ sites methylated at adenine residues in this fragment. The absence of this fragment in the MboI digest of the great millet embryo DNA indicates methylation of some adenine residues during a change from the embryo to the seedling stage.

**IV. Identification of repeat families in six millet DNAs and characterization of a 0.55 kbp AluI repeat family in great millet**

When millet DNAs are digested with as many as fifteen different restriction enzymes, bands are observed in most cases with most of the enzymes, indicating the presence of a wide variety of restriction sites in these DNAs. The 0.55 kbp AluI repeat family has been partially characterized with respect to its genomic distribution and homology with AluI repeats of the other five millets. Digestions of great millet

DNA using increasing amount of the enzyme shows that the AluI repeat family in great millet is probably tandemly arranged. This is confirmed by probing the above digests with the 0.55 kbp AluI band. Hybridization of this band with the AluI digests of the other millet DNAs at 60°C reveals a weak homology among the AluI repeats of these millets.

**V. Characterisation of two species-specific repeat families in great millet**

The 1.3 kbp EcoRI and 1.4 kbp XbaI repeat families in great millet have been partially characterized with respect to their genomic distribution and their homology with the EcoRI and XbaI families in the other five millet DNAs. The digestion of great millet DNA using increasing amounts of EcoRI and XbaI shows that these two repeat families are disperse in nature. Hybridization of these two families to the digests of great millet DNA indicates that they are arranged in a clustered and scrambled manner. Similarly, hybridization with the EcoRI and XbaI digests of the five other millet DNAs reveals the species-specific nature of these two repeat families. The later also hybridise to the total repetitive fraction of great millet DNA isolated at a highly stringent temperature of 75°C suggesting that the members of these two families are probably largely homogeneous.