CHAPTER I
INTRODUCTION

Sugarcane, belonging to the genus Saccharum and family Gramineae, is a major source of sugar and other sweeteners—gur and khandsari. As one of the two sources of white sugar (the other being sugar beet), sugarcane is grown in over seventy countries of the world. Cultivation of this crop in India is very ancient dating back to the Vedic period (2500 B.C.). Now India is the largest cultivator of sugarcane with an area of 3.5 million hectares yielding 227 million tonnes of cane. This is a multipurpose crop providing food, fuel and fibre. Sugarcane is next only to cotton as a crop of importance to agro-based industries.

Genesis of hybrid production in sugarcane came from the observation of viable seed set in sugarcane by Soltwedel in 1856 (Stevenson, 1956). Improvement in sugarcane is largely through interspecific hybridization and almost all the present day sugarcane varieties were products of hybridization and progressive synthesis involving basic species of Saccharum viz., S.officinarum, S.spontaneum, S.barberi, S.sinense and S.robustum, the first four being greater contributors.

In spite of tremendous success in sugarcane breeding through hybridization and selection producing high yielding and sucrose rich commercial hybrids, little is known on the genetic architecture of the genus or the basic pattern inheritance of economic traits (Heinz, 1987). Detailed linkage maps form the fundamental tool in understanding the organization of plant genomes (Tanksley, 1983).
Traditionally morphological characters were used for the purpose, which required a large number of segregating population, as only a limited number of loci segregated in each cross. But the genus *Saccharum* with a relatively longer crop duration is highly polyploid, heterozygous and outbreeding, readily crossable with the members of *Saccharum* complex and with functioning of reduced and unreduced eggs in interspecific hybridization. DNA content in 2C nuclei of *Saccharum* was above 6 pg (*S. officinarum* - 5.28 to 7.48 pg, *S. robustum* - 6.53 pg, *S. barberi* - 6.54 pg and *S. sinense* - 8.67 pg) compared to 0.30 pg in *Arabidopsis*, 0.88 pg in *Oryza sativa* and 1.88 pg in tomato (Arumuganathan and Earle, 1991). A combination of these factors are limitations to detect such markers and this explains the poor state of development of sugarcane genetics. Added to it is the very high number of relatively small chromosomes causing karyotype analysis extremely cumbersome (Price, 1965). Origin of different *Saccharum* species has been speculated by many workers based on morphological, cytological and ethnological parameters, but evidence on finer details of phylogeny is still inconclusive.

With the discovery of a new set of a molecular markers like isozymes, RFLP, RAPD and microsatellites, it is now feasible to reexamine the pedigree of many crop plants and to make use of these markers in varietal identification. Isozyme markers are the oldest among these and for over three decades, plant breeders have had the opportunity to incorporate isozyme techniques into their research. Among the merits of isozymes, consistency in their expression irrespective of environment thereby eliminating the environmental interaction on the phenotype makes isozyme technique the right choice
for plant breeders. However, this technique is handicapped with the limited number of isozyme systems available and their tissue specific expressivity, which limit their wider adoption.

Methods have been devised in the past two decades that allow the detection of polymorphism in DNA. Polymorphisms in DNA can be used as molecular markers and along with protein markers are making a significant impact in applied plant breeding. These are discrete characters on the phenotypes and provide more objective descriptors. Restriction fragment length polymorphisms or RFLPs are the first in this set of DNA markers. RFLPs are observable as number of variable length of DNA fragments (Grodzicker et al., 1974). Their advantages over isozymes are their potentially unlimited number and uniformity in expression within an organism. Construction of genetic map using RFLP has been done in a few major crop species such as tomato (Bernatzky and Tanksley, 1986), maize (Helentjaris et al., 1986), lettuce (Landry et al., 1987), potato (Bonierbale et al., 1988) and rice (McCouch et al., 1988).

A technique based on polymerase chain reaction known as randomly amplified polymorphic DNA (RAPD) has been used to detect polymorphism in plants, animals and bacterial systems ever since that was brought to light by Williams et al. (1990). Though the applications of RFLP and RAPD are more or less similar, unlike RFLP, RAPDs are dominant markers, requiring only nanogram quantity of DNA and generated from any species without DNA sequence information, permitting the construction of a highly saturated genetic linkage map in a short span of time. The most outstanding examples are the linkage maps of Arabidopsis (Reiter et al., 1992) and loblolly pine (Neale and
Sederoff, 1991) which were constructed in two to four months as compared to conventional molecular markers like RFLP which might take several years.

Microsatellites belong to the new generation of molecular markers and are arrays of tandemly repeated DNA sequences occurring dispersed throughout the genomes of all eukaryotic organisms (Jeffreys et al., 1985). The chance to find a polymorphic microsatellite marker in a given region would be much higher than to find a RFLP marker in a cross involving within a species or between two species (Wu and Tanksley, 1993). Attempts are now on to map genomes of several crops using microsatellites.

Use of molecular markers in sugarcane was discussed as early as 1969 by Heinz, though molecular systematics in Saccharum was pioneered by Waldron and Glasziou (1971) through isozymes. Not much work was done in this line till Wood (1987) distinguished the two closely related species viz., S.sinense and S.barberi based on ribosomal DNA. Glaszmann et al. (1989) illustrated the use of isozyme variation and RFLP of ribosomal DNA in three Saccharum species and Erianthus for sugarcane taxonomy and breeding. Diversity among Saccharum species and between Saccharum and Erianthus for chloroplast and mitochondrial DNA of sugarcane were studied by Sobral et al. (1994) and Al Janabi et al. (1994) respectively. Latest report on genome mapping in Saccharum (da Silva et al., 1994) indicated construction of a partial linkage map of a wild species viz., S.spontaneum SES 208 (2n = 64).
Credits of all these achievements go to leading laboratories in advanced countries. Even in these cases, success in research in sugarcane lies far behind than that of other crops of comparable economic importance due to reasons cited earlier. From India, where biotechnology itself has not progressed much for want of infrastructural facilities and skill, published results on the molecular biology in relation to phylogeny of this crop were lacking. With the existing knowledge limitations and available facilities, this introductory study on assessing genetic diversity in *Saccharum* based on molecular markers like RFLP, RAPD, microsatellites and isozymes was made and has the following objectives:

1. To investigate the genetic diversity in *Saccharum* for RFLPs employing total genomic DNA of *S. spontaneum*, *Erianthus arundinaceus* and *Sorghum* species (sweet sorghum) as probes so as to understand the contribution of these genomes to *Saccharum* species and to explain the result in relation to species affinities and evolutionary trends

2. Rice sucrose synthase 1 (RSS 1) gene as a probe on sugarcane DNA for studying the homology between the rice and sugarcane with respect to this gene

3. to investigate genetic differences among *Saccharum* species and F$_1$ hybrids through RAPD techniques

4. to study the utility of two synthetic oligonucleotide probes viz., (GATA)$_4$ and (CA)$_8$ as markers of choice in genotype identification through DNA fingerprinting
5. to study the genetic diversity among sugarcane species and hybrids for various isozyme systems
6. to understand *Saccharum* species for their protein profile by SDS-PAGE
7. to construct dendrograms detecting genetic similarities among *Saccharum* species and hybrids based on molecular markers and to compare genetic similarities based on molecular markers with taxonomic or phylogenetic classification.