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Genetic Diversity Analysis Based on Morphological Characters and Microsatellite Markers in Sugarcane (Saccharum officinarum L.)

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GENETIC DIVERSITY ANALYSIS BASED ON MORPHOLOGICAL CHARACTERS AND MICROSATELLITE MARKERS IN SUGARCANE (*SACCHARUM OFFICINARUM* L.)

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Dedicated

To My

Beloved Parents

DECLARATION

I hereby declare that the research work embodied in this thesis entitled "**Genetic Diversity Analysis Based on Morphological Characters and Microsatellite Markers in Sugarcane (*Saccharum officinarum* L.)**" has been carried out by me for the degree of **Doctor of Philosophy.** This research work was carried out under the supervision of Professor Dr. M. Firoz Alam, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh. This research work was carried out at DNA Lab. of Biotechnology Division and Experimental Field of Breeding Division, Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during 2011-2014. The research work was co-supervised by Dr. Md. Amzad Hossain, Chief Scientific Officer, Biotechnology Division, presently working as Director (Research), Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh. I also declare that the results presented in this dissertation are my own investigation and any part of this thesis work has not been submitted elsewhere for any degree/diploma or for similar purpose.

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CERTIFICATE

This is to certify that Md. Raihan Ali worked under our supervision as a PhD fellow, bearing student No. 10505, session: 2010-2011, Department of Botany, University of Rajshahi, Rajshahi-6205, Bangladesh. We are pleased to forward his thesis entitled "**Genetic Diversity Analysis Based on Morphological Characters and Microsatellite Markers in Sugarcane (*Saccharum officinarum* L.)"** which is the record of bonafide research carried out at DNA Lab., Biotechnology Division and experimental field of Breeding Division, Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh. He has fulfilled all the requirements of the regulations relating to the nature and prescribed period of research for submission of the thesis for the award of the degree of **Doctor of Philosophy** in the field of Plant Molecular Biology and Biotechnology. This work or part of it has not been submitted before as candidature for any other degree.

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ABSTRACT

Genetic diversity in sugarcane was investigated using 16 quantitative, 37 qualitative morphological traits and 23 microsatellite markers. Results showed that a moderate level of genetic diversity was present in 51 evaluated sugarcane genotypes. Agromorphological traits showed high Shannon-Weaver diversity indices (>0.80) for quantitative characters and very low (0.0) to high (>0.80) for most of the qualitative traits. Mean Euclidean distance for agromorphological quantitative traits was 87.33 between pairs of genotypes for all possible pair wise combinations, and was ranged from 6 to 251. Fourteen pairs of distantly related genotypes had Euclidean distances ≥ 200 while 37 pairs of closely related genotypes had Euclidean distance values ranging from 6 to 30.Diverse genotypes based on their mean Euclidean distance values can be utilized for parent selection in hybridization program. Integrating available information on their good combining ability with other genotypes to the phenotypic distance data, as a criterion in parental selection, ensures a higher chance of generating better performing hybrids. Thus, cross combinations between genetically closely related genotypes should be avoided. Crosses between genetically distant sugarcane genotypes might produce higher variances for quantitatively inherited traits in segregating population. Cane yield was found to be positively and significantly correlated with plant height, stalk length, number of tiller per clump, leaf width, internode diameter, number of millable cane and single cane weight. Cane yield of sugarcane could be improved by selecting sugarcane genotypes having high values of length of stalk, number of tiller per clump, internode diameter, single cane weight and number of millable cane. Principal component analysis conducted based on correlation matrix of 16 agromorphological traits resulted to five principal component axes accounted for 81.31 % of total variation. The first principal component that accounted for 28.82% of total variation was mainly attributed to variation in plant height and stalk length traits. This further indicates that plant height, stalk length, number of millable cane and leaf length were

among the most important traits which accounted for 81.31 % variation expressed in the evaluated sugarcane genotypes. It could be suggested that the use of these traits will save considerable amount of time, labour and cost for identification of superior sugarcane genotypes. The positive absolute values of two vectors revealed that plant height, internode length and diameter, number of internode per cane, single cane weight and pol percent had the greatest contribution to genetic divergence. Cluster analysis by UPGMA based on Euclidean distances classified 51 genotypes in to six clusters and cluster 3 was identified as the largest cluster. Fifty one genotypes were also grouped based on agromorphological traits into six clusters using Mahalanobis D^2 statistic. The highest inter-cluster distance (12.358) was found between clusters I and cluster V. The distance between cluster V and cluster VI was minimum (2.628).The crosses between genotypes in cluster I with genotypes in cluster V might produce a good hybrid which would exhibit highest heterosis.

The 23 microsatellite markers revealed high gene diversity (PIC) values in the 51 sugarcane genotypes. Average PIC value was 0.942. Primer pair SMC 226 CG showed the highest PIC value (0.979) that makes it the most discriminating among 23 markers used. The level of polymorphism indicates that distinction between any two genotypes is possible with appropriate SSR primer pair. This supports to the use of SSR markers, as an excellent tool, for diversity analysis and loci mapping in sugarcane. A total of 76 unique alleles were generated by 21 SSR markers. Most of the unique allele produce markers showed high PIC value. These 21 markers distinguished 88.24% sugarcane genotypes. Only two primer pairs viz. SMC 226 CG and SMC 278 CS produced unique allele in 19 genotypes i.e. 37.25% genotypes were distinguished. Cluster analysis showed that the genotype pair POJ 2878/ I 156-97 was the closet among all genotypes having the lowest Euclidean distance value (5.66).The most distant genotype pairs were I 6-04/ I 33-97 (8.77) and I 174-93/ I 33-97 (8.77). Among the 51 genotypes studied, genotype I 33-97 was found to be the most distant genotype with mean Euclidean distance 8.01 with other 50 genotypes. The difference between the lowest and the highest Euclidean distances indicated the presence of low to moderate level

of genetic diversity among the studied sugarcane genotypes at genotypic level. Dendrogram derived by UPGMA using Euclidean distance of SSR molecular genotyping data revealed two major clusters. Most of the genotypes (37) grouped in the cluster 1 and rest 14 genotypes concentrated in the cluster 2. On the other hand, five major clusters were formed when Jaccard's similarity coefficient was used for constructing dendrogram following UPGMA method. The clustering patterns using molecular marker data were different from that of clustering of genotypes constructed from agromorphological quantitative data. This might be due to nature of data and different clustering models used. The diversity analyzed by molecular (SSR) markers data was found to be more precise because these markers are not influenced by environmental factors. Both phenotypic and SSR profile data should be considered during the selection of clone/ genotype for conserving in the active collection of germplasm or parents for hybridization program. The knowledge obtained in this study might be useful for future breeding program for increasing genetic diversity of sugarcane germplasm to meet the demand of sustainable sugarcane production in Bangladesh. To widen the genetic base of BSRI sugarcane germplasm pool, incorporation of*S. spontaneum, S. barberi, S. robustum* and *Erianthus spp*. into the population should be initiated. *Saccharum* species other than *officinarum* can be utilized as female parents to broaden the cytoplasmic base. Phenotypic and molecular characterization of the entire collection should be done to determine the relationships among the genotypes available at "Field Gene Bank of BSRI, Ishurdi, Bangladesh.

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LIST OF ABBREVIATION

AFLP	: Amplified Fragment Length Polymorphism
ANOVA	: Analysis of variance
AYT	: Advanced Yield Trial
bp	: Base pair
BSRI	: Bangladesh Sugarcrop Research Institute
cm	: Centimeter
Conc.	: Concentration
CTAB	: Cetyl trimethylammonium bromide
CV	: Coefficient of variation
DF	: Degree of freedom
DNA	: Deoxyribo Nucleic Acid
dNTP	: Deoxynucleotide Triphosphate
e.g.	: Exampli gratia (for the sake of an example)
EDTA	: Ethylenediaminetetraacetic Acid
et al.	: Et alia (= and others)
Et Br	: Ethidium bromide
F	: Forward
g	: Gram
i.e	: That (is to say)
ISSR	: Inter Simple Sequence Repeats
LSD	: Least significant difference
M	: Molar
mg	: Milligram
MISRI	: Mauritius Sugarcane Industry Research Institute
ml	: Milliliter
mM	: Millimole
MS	: Mean square
MW	: Molecular weight
ng	: Nanogram
µg	: Microgram
nm	: Nanometer
^{o} C	: Degree Celsius

PCA	: Principal Component Analysis
PCoA	: Principal Coordinate Analysis
PCR	: Polymerase Chain Reaction
PHILSRIN	: Philippine Sugar Research Institute Foundation, Inc.
PIC	: Polymorphic Information Content
PVP	: Polyvinylpyrrolidone
PYT	: Preliminary Yield Trial
R	: Reverse
R & D	: Research & Development
RAPD	: Random Amplified Polymorphic DNA
RFLP	: Restriction Fragment Length Polymorphism
RNA	: Ribonucleic Acid
rpm	: Revolution Per Minute
Sdd H_2O	: Sterile double distilled water
SDS	: Sodium dodecyl sulfate
SMC	: Sugarcane Microsatellite Consortium
SP	: Sum of product
SS	: Sum of square
SSR	: Simple Sequence Repeats
STMS	: Signature Tagged Microsatellite Site
t/ha	: Ton/Hectare
Ta	: Annealing temperature
TBE	: Tris Borate EDTA
TE	: Tris-EDTA
TE	: Tris EDTA
TRAP	: Target Region Amplification Polymorphism
UGMS	: Unigene Derived Microsatellites
UPGMA	: Unweighted Pair Group Method using Arithmetic Averages
UV	: Ultra Violet
viz.	: Namely
w/v	: Weight/Volume
ZYT	: Zonal Yield Trial

Chapter I

INTRODUCTION

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an important food cum industrial crop grown in tropical and sub-tropical regions of the world. It is also an important export product of many developing countries (Heinz et al., 1977). Sugarcane is a member of the family “Poaceae” and belongs to the genus “*Saccharum*”. Sugarcane is being recognized as major source of sugar in the world and is produced in 120 countries in the world. Approximately 80% sugar is produced from sugarcane. Out of 120 countries, 70 countries produce sugar from sugarcane, 40 from sugar beet and 10 from both. About 75 % sugar is produced in 10 largest sugar producing countries of the world (Sucden, World Sugar Production, n.d.). The sugarcane producing countries lay between latitude 36.7 ^{o} N and 31.0 ^{o} S of the equator, spreading from tropical to sub-tropical zones (Introduction-Sugarcane, n.d.).

A large amounts (up to 23% w/v) of non-reducing disaccharide “sucrose” accumulates in the vacuoles of parenchyma cells of stem tissues of sugarcane plant (Hawker and Hatch,1965).Sugar is produced from sucrose containing juices of sugarcane extracted by crushing the cane followed by crystallization, refining and clarification. A number of products viz. white sugar, brown sugar (khandasari), jaggery (Gur) and ethanol are being produced from sugarcane juice. Bagasse and molasses are the main by-products of sugarcane.

Molasses is the chief raw materials for ethanol production and thus base for ethanol based industries. Spirits such as “Rum” and cacher are also produced from molasses. Bagasse is used for generating heat energy required for crystallization of extracted sugarcane juices in the sugar mills. Excess bagasse is also used for generation of electricity and making paper in the paper industry. In the refineries of Brazil, bagasse is burnt to provide heat for distillation of the fermented products required for purification and to co-generate electric power that is sold to the electric power grid (Scorteci et al., 2012).

Nowadays, sugarcane is considered as a first generation biofuel crop. The sucrose derived from sugarcane juice is used to convert ethanol biofuel through fermentation process (Scorteci et al., 2012) Sugarcane crop has the potential to lower green house gas (CO_2) emission, energy diversity and economic growth. Sugarcane ethanol cuts CO_2 emission by 90% on average compared to gasoline. Only 20 countries produce oil but more than 100 countries cultivate sugarcane. This ethanol biofuel would help to enhance energy security and reduce global dependence on fossil fuel. Moreover, sugarcane expansion could create rural jobs and increase access to electricity (Sugarcane Benefits, 2016).

Sugarcane is recognized as C_4 plant. It has much higher photosynthetic efficiency than C_3 plant. Because it has potential for efficiently converting solar energy in to chemical energy. Photosynthetic efficiency can be translated to biomass yield. Sugarcane is also one of the highest biomass producing crops (Chen et al., 2009). The highest efficiency (theoretical) of sugarcane biomass production is estimated to be 281 t/ha/year (Loomis and Williams, 1963). During photosynthesis, CO_2 is initially added to a 3-carbon acid to form a 4-carbon acid that is then transported to a region of the leaf where ribolosebiphosphate carboxylase is located. Reverse carboxylation enhances CO_2 concentration in the cell causing dramatically decrease in photorespiration (Wang et al., 2008).

In Bangladesh, sugarcane was cultivated in 0.116 million hectare of land and produced 4.67 million ton of sugarcane in 2010 (BBS, 2011). Sugarcane plays a vital role in the economy of Bangladesh. The economic importance of sugarcane in Bangladesh should not be signified by its share in total cropped area, which is about 0.72% (BBS, 2011), nonetheless, it plays pivotal role in partial fulfillment of the domestic requirement of sugar and jaggary, income and employment generation, rural poverty alleviation and also production of sugarcane by-product based allied products viz. ethanol from molasses, fuel and paper pulp from bagasse, etc. According to FAO, sugar requirement per capita/day are 29 g and Bangladesh requires 1.0 -1.2 million tons of sugar/year to meet the demand of domestic consumption (Hasan, 2003). Moreover, six distilleries utilize molasses

(a by-product of sugar mills) to produce ethanol. This ethanol is being mainly used in pharmaceutical industries, laboratories of universities and research organizations. Limited amount of ethanol is used to produce liquor like Ram in Carew & Company, Darshana, and Chuadanga, Bangladesh. The 15 sugar mills could not utilize their full sugar production potential (0.21 million ton/year) (BSFIC, 2008), due to unavailability of sugarcane supplied by the farmers. The national average yield of sugarcane is 40.21 t/ha, which is far below the world average yield (58.86 t/ha) (FAOSTAT, 2011). To meet the demand of sugar, horizontal expansion of sugarcane cultivation is not feasible in densely populated countries like ours. On an average, 1% cultivable land decreasing in each year in Bangladesh (Anonymous, 2010) due to urbanization, industrialization, housing of rural and urban people, construction of roads etc. In this perspective, vertical expansion of sugarcane production through increasing present yield potential by developing high yielding varieties tolerant to biotic and abiotic stress coupled with high management can be a viable solution to mitigate this problem.

Bi-parental cross or polycross technique is mainly used in sugarcane hybridization program in many sugar producing countries. Three steps are very important for variety development using hybridization viz. parent selection, crossing of genetically dissimilar (diverse) parents followed by selection of heterotic genotypes/clones having desirable traits. Parent selection is crucial /utmost important for getting useful heterotic progeny in crop breeding program and it requires knowledge and understanding of the genetic diversity of the available germplasm. Progeny selection will be inefficient if sufficient genetic variation is absent in the selected parents. Reservoir of diverse germplasm is the basic raw material of any breeding program of any crop. Long-term genetic gain depends on the availability and utilization of genetically diverse germplasm. Therefore, an accurate assessment of genetic diversity of available germplasm is indispensible in crop improvement program for identifying and selecting diverse parents to create segregants having maximum genetic variation useful for subsequent selection (Barrett and Kidwell, 1998) and insertion of desirable genes

from diverse germplasm into available genetic base (Thompson et al., 1998). In many crop breeding programs, significant priority has been given to comprehensive analysis of genetic diversity in numerous crops viz. rice (*Oryza sativa* L.) (Dilday, 1990; Cuevas-Perez et al., 1992), wheat (*Triticum aestivum* L.) (Cox et al., 1986), sorghum (*Sorghum bicolor* (L.)Moench) (Dje et al., 2000), corn (*Zea mays* L.) (Kantety et al., 1995); sugarcane (*Saccharum spp*.) (Muyco, 2002; Tai and Miller, 2002; Tahir et al., 2013), soybean (*Glycine max* (L.) Merr.)(Delannay et al., 1983), peanut (*Arachis hypogeal* L.) (Knauft and Gorbet, 1989), and the bean (*Phaseolus vulgaris* L.) (McClean et al., 1993). The amount of genetic diversity present in the genetic base depends on part in the amount and diversity of the original ancestors involved in the creation of a germplasm pool.

Germplasm collection, characterization, documentation, conservation and utilization are basic and continuous activities in plant breeding program. Characterization involves recording distinctly identifiable heritable characteristics of plants. Appropriate characterization of agro-morphological characters helps the breeder to utilize the germplasm. Morphological characterization provide information about extent of variation i.e., diversity, diverse parental lines for efficient hybridization program and also duplicate germplasm (Ribaut and Hoisington, 1998; Upadhaya et al., 2008). It is also crucial to describe distinctive characteristics of cultivated variety and landraces (UPOV, 1991). The present-day Plant Variety Protection (PVP) is dependent on morphological characters that are used for Distinctness, Uniformity, Stability (DUS) testing (UPOV, 1991). Sugarcane variety identification traditionally based on description of distinctive and heritable morphological characters. Calculation of genetic distances can also be performed by characterizing the parental lines of sugarcane (Babu et al., 2009). *Saccharum* species germplasm characterization would be helpful for better utilization of sugarcane varietal development program (Zhou et al., 2013).

Knowledge of germplasm diversity and relationships among elite breeding materials is basically important in crop improvement (Hallauer and Miranda,

1988). In breeding program, information regarding genetic associations between and among genotypes is very essential for categorizing germplasm resources. Ultimately, this process helps the breeder to choose parental clones for hybrid production (Nienhuis et al., 1995).

Genetic distance is quantitative measure of genetic divergence between two sequences, individuals, clones, genotypes, species, or populations within a species. Smaller genetic distances indicate close genetic relationship and vice versa. Greater amount of heterosis can be obtained from parents having greater genetic distance (Dje et al., 2000). Genetic diversity furnishes information about the amount of genetic divergence, genetic relationships among populations, and diverse parents useful for hybridization program (Thompson et al., 1998; Mostafa et al., 2011).

A number of data sets viz., pedigree record, morphological data, agronomic performance data, biochemical data (isozymes and seed proteins) and more recently, DNA based molecular markers data have been using to investigate genetic diversity and relationships among germplasm accessions, breeding lines and populations(Muyco, 2002; Mohammadi and Prasanna, 2003; Babu et al., 2009;). Molecular markers are advantageous over biochemical (isozymes and seed proteins) and morphological markers because they are numerous, not affected by environment, tissue non-specific and can be used at any stages of crop life cycle. These markers are also useful for making linkage map of the studied crop (Soiler and Beckman, 1983; Helentjaris et al., 1986; Esposito et al., 2007). Morphological and molecular analyses are among the most frequently used tools for the estimation of genetic distances within a group of genotypes. Combination of morphological and molecular markers increases the efficiency of diversity measured (Palaniappan and Murugaiah, 2012).

Information about relationships among genotypes in the breeding program is important in the analysis of diversity, selection of parental clones for hybrid production and prediction of variances for some traits in the F_2 and inbred

generations. Genetic distances between cultivars can be estimated from pedigree analysis or from multivariate analysis of agronomic and morphological traits and molecular markers. Genetic diversity can also be analyzed from morphological, biochemical and molecular marker data through using (most commonly used) multivariate analytical techniques viz. cluster analysis, principal component analysis (PCA), principal coordinate analysis (PCoA), and multidimensional scaling (MDS) (Melchinger, 1993; Jones et al., 1997; Thompson et al., 1998; Brown-Guedira et al., 2000; Muyco, 2002).

Phenotype of an organism is determined by its gene/s and environmental factors and also the interactions between the two. Phenotypic differences may also reveal genetic differences. Theoretically, phenotypic diversity should approximate genetic diversity. The number of genes involved in the control of phenotypic traits increases as the number of phenotypic traits being evaluated increases. Consequently, it improves the utility of phenotypic diversity in predicting genotypic diversity. Genetic relationships among cultivars and populations can be measured by similarity of any number of phenotypic characters. Differences between characters are assumed to reflect the genetic divergence of the genotypes. However, characterization of cultivars based on agronomic and morphological traits is subjective, labor intensive and can be influenced by genotype x environment interactions.(Muyco, 2002).

Although morphological traits (markers) have been using traditionally for characterization of germplasm, selection of parental lines, protection of crop variety, and diversity studies by the breeders but they have some inherent limitations such as low polymorphism, low heritability, delayed expression and influenced by environmental factors (Smith and Smith, 1992). In recent times, different types of DNA-based molecular markers have been using extensively in many crops for various purposes including finger printing varieties rice, Wheat, maize, sugarcane, soybean, potato etc. In contrast to morphological traits (markers), molecular markers have great potentiality to differentiate genotypes at DNA level, rendering more direct, reliable and efficient tool for germplasm characterization, conservation and

management (Tanksley et al., 1989). They are unlimited in number and not influenced by environment.

Molecular markers are important tools in crop improvement programs since they are useful for estimating genetic distance (GD), evolutionary and conservation studies. They are almost unlimited in number and are not influenced by the environment. In sugarcane breeding, genetic diversity has been estimated using various molecular markers, such as random fragment length polymorphism (RFLP) (D'Hont et al., 1994; Jannoo et al., 1999; Coto et al., 2002; Schenck et al., 2004), ribosomal DNA (Glaszmann et al., 1990), microsatellites (Piperidis et al., 2000; Pan et al., 2003; Corderio et al., 2003; Pinto et al., 2006), amplified fragment length polymorphism (AFLP) (Hoarao et al. , 2002; Lima et al., 2002 ; Aitken et al., 2006; Selvi et al., 2006), TRAP (Arro, 2005; Khan et al., 2011).

Simple sequence repeats (SSRs) also known as microsatellites are molecular markers based on tandem repeats of short (2-6 bp) DNA sequences (Litt and Lutty, 1989). These DNA sequences are highly polymorphic even among closely related cultivars due to mutation causing variation in the number of repeating units (Saghai-Maroof et al., 1994). SSRs can be analyzed by a rapid, technically simple and inexpensive polymerase chain reaction (PCR)-based assay that requires only small DNA quantities. Through PCR, different alleles at a locus can be detected by using conserved DNA sequences flanking the SSR as primers. SSR markers are co-dominant and can be transmitted in simple Mendelian segregation. Lastly, SSRs are abundant and uniformly distributed in plant genomes (Akkaya et al., 1992; Lagercrantz et al., 1993; Wang et al., 1994).

At BSRI, germplasm characterization based on agronomic and morphological traits has been initiated. But present day molecular markers could not substitute morphological descriptors in defining the genetic identity. Diversity analysis in the collection, however, has not been done to date.

This study was conducted with the following specific objectives:

1. To study the extent of genetic diversity among sugarcane clones (germplasm) using morphological characters.
2. To characterize sugarcane germplasm under study based on morphological characters.
3. To study genetic diversity among sugarcane clones (germplasm) using microsatellite markers
4. To elucidate information on the association of various agro-morphological traits that dictates the final performance of genotype under field condition pertaining to yield of cane sugar.
5. To determine the relationship among the 51 clones and to classify them into different clusters based on agro-morphological parameters;
6. To evaluate the utility and efficiency of microsatellite markers in evaluating diversity in the clones under study.

Chapter II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 Sugarcane

Knowledge of the origin, genetics and breeding of modern sugarcane is important in understanding how these affect and challenge the use o molecular markers specially microsatellites for sugarcane genome analysis.

2.1.1 Taxonomy and Origin of Sugarcane

Taxonomy of sugarcane is more complicated due to its frequent natural interspecific and / or intergeneric hybridization followed by various types of special mode of chromosome transmission, continuous evolution of new variability in genomic levels e.g., polyploids and aneuploids and finally natural as well as artificial selection pressure (Amalraj and Balasundaram, 2006).

Sugarcane is a tall plant with sweet juicy stems cultivated in tropical and subtropical regions of the world, on both sides of the equator, up to approximately 35 ^{o} N and 35 ^{o} S (van Dillewijn, 1952; Gomes et al., 1964). It belongs to the genus *Saccharum* L., of the tribe Andropogoneae in the grass family-Poaceae (Jannoo et al., 1999). Another two important cereal crops viz. maize and sorghum are also belonging to the same tribe (Lu et al., 1994). The *Saccharum* is a complex genus, comprises of six species: *S*. *spontaneum* L., *S. officinarum* L., *S. robustum*, *S. edule*, *S.barberi* and *S. sinense* (D'Hont et al., 1998). "Saccharum complex" is an informal taxonomical group was first coined by Mukherjee (1957) and further extended by Daniels et al., (1975). This complex contains *Saccharum* and closely related interbreeding species from other genera such as *Erianthus* section *Ripidium, Miscanthus* section *Diandra*, *Sclerostachya* and *Narenga* (Daniels and Roach, 1987; Amalraj and Balasundaram, 2006).

The genus *Saccharum* was first described by Linneaus (1752) and had given the scientific name of sugarcane (*Saccharum officinarum* L.) in his book *Species Plantarum* (Daniels and Roach, 1987). The word Saccharum is thought to have been originated from ‘sankrit’ word ‘sharkara’ (Ritter 1841 as cited in Daniels and Roach 1987). This species is also known by the common name ‘noble cane’.

2.1.1.1 Cultivated and wild Species of Sugarcane

S. officinarum L. is an ancient cultivated species with 2n=80 (x=10, octaploid) (Bremer, 1961). Its stalks are thick, juicy, rich in sucrose and brightly colored but poor in disease resistance. This species is the primary source of genes for sucrose accumulation (Bremer, 1930; Li and Price, 1967; Lu et al., 1994). The first Dutch breeders in Java used the term ‘Noble’ to refer this species for vibrant coloured and large sized stalks (Brandes, 1956). The pure form of *S. officinarum* L. is found to be cultivated as field crop or garden species in Melanesia, which is not found in wild (Sreenivasan et al., 1987; Grivet et al., 2004). The maximum diversity of this species is found in New Guinea (Grivet et al., 2004). Most sugarcane geneticists and breeders have agreed that the centre of origin of *S. officinarum* L. to be in the New Guinea (Daniels and Roach, 1987) where it has been grown as a garden crop since 8000 B.C. (Fauconnier, 1993). It has been postulated that *S. officinarum* L. has been derived from the selection of sweet forms of *S. robustum*. This wild cane was previously used for house building, fencing and archery (Daniels and Roach, 1987) and may have been selected, possibly with the animals like pigs or rats that were attracted to sweeter plants (Daniels and Roach, 1987). It was disseminated to Southeast Asia, India, and the Pacific along the human migration route and was hybridized with wild canes. Later on, it arrived in Mediterranean around 500 B.C.(Fauconnier, 1993). From that place, it dispersed to Morocco, Egypt, Syria, Crete, Greece and Sicily followed by introduction to West Africa and lastly Central and South America and the West Indies (Fauconnier, 1993).Molecular marker such as amplified fragment length polymorphism (AFLP) marker analysis technique supports the view that

New Guinea area is considered to be the centre of diversity of *S. officinarum* L. (Daniels and Roach, 1987).

S. barberi Jeswiet (2n=80-124) is referred to as North Indian cane while *S. sinense* Roxb. (2n=60-80) is also referred to as Chinese cane (Bremer, 1966; Grivet et al., 2004). They have been cultivated in Indian and China respectively, since prehistoric times for sugar production. Both the species have some common characteristics. They possess thin to medium stalks and leaves, flatter colours and lower sucrose contents than *S. officinarum* L. These canes are more tolerant to stress conditions than nobles and adapted to sub-tropical climatic conditions (Grivet et al., 2004; Ming et al., 2010). *S. barberi* Jesw and *S. sinense* Roxb. are thought to be the ancient intergneric hybrids. *S. barberi* is believed to be the hybrid between *S. officinarum* and *Erianthus* (Sector *Rapidium*), while *S. sinense* is thought to be derived from *S. officinarum* x *Miscanthus* introgression (Daniels and Roach, 1987). Five morpho-cytological groups have been identified (Barber, 1922) and chromosome numbers of North Indian canes have been determined (Bremer, 1966). Four groups viz. Mungo (2n=124), Sunnabile (2n=82-116), Nargori (2n=107-124) and Saretha (2n= 91) belong to *S. barberi* Jesw. while the fifth group 'Pansahi' shares characteristics either *S. barberi* or *S. sinense* Roxb. (Barber 1922). Pansahi group is common in China. Currently, these two species are being maintained in germplasm bank (Grivet et al., 2004).

S. spontaneum L. is a wild and most ancient species. It is far more genetically diverse than *S. officinarum* L., and is highly polymorphic. Genotypes vary from short, grassy-appearing narrow-leafed types with no stalks, to large-stature types over 5m in height and 3 cm in stalk diameter. The stalk has no or very low sugar content with higher fiber content than *S. officinarum* L. (Jackson, 2005). It has contributed to the improvement in sugarcane vigour, hardiness, tillering, rationing ability and resistance to biotic stresses. This species resistant to most of the diseases including 'sareh' and mosaic except sugarcane smut disease (Segella, 1964). Moreover, *S. spontaneum* is highly tolerant to a wide range of abiotic stresses viz., droughts, floods, saline conditions, and freezing temperatures

(Mukherjee, 1957). It generally grows spontaneously near water source like river bank, lake, ponds etc. Its chromosome complement varies from 2n=40 to 2n=128. The chromosome complement is a multiple of eight in 80% individuals, indicating a polyploidy series with frequent aneoploidy (Panje and Babu, 1960). On the basis of chromosome number, Price (1965) reported three different forms of *S. spontaneum* L. available in Asia.These are: 1) The Java form of *S. spontaneum* L. (2n=112), 2) The Philippines form of *S. spontaneum* L. (2n=80), and 3) The Coimbatore form of *S. spontaneum* L. (2n= 64)). It has been thought that the centre of origin and diversity of *S. spontaneum* L. is in India (Ming et al., 2006). *S. spontaneum* L. grows in a wide range of habitats, extending from tropics to temperate regions, covering some Pacific islands, Melanesia, Tropical Asia, the Middle East and Part of Africa (Panje and Babu, 1960).

S. robustum Brandes & Jeswiet ex Grassl. Is considered another wild species of sugarcane. It is possibly the closest wild relatives of *S. officinarum* L. Its chromosome number vary generally from 2n=60 to 110 but most dominating cytotypes are 2n=60 and 2n=80 (Price, 1965). It possesses vigorous, long (up to 10m high), and thick stalks with little or no sugar, but like *S. officinarum*, it does not have rhizomes. Moreover, its stalk also contains high fiber, little juice and hard, which is useful for making hedges (Matsuoka et al., 2005; Mozambani et al., 2006). *S. robustum* is thought to be native to regions of southeast Sulawesi Island, where a large number of this species are found in natural habitats (Berding and Koike, 1980; Tew et al., 1991). It is spread in the islands of Kalimantan, Sulawesi, Maluku, and New Guinea and also in the Bismarck, Solomon, and Vanuatu archipelagos. In Kalimantan, it is grown as garden crop and used as medicinal plants (Grivet et al., 2004).

S. edule Hassk.is a non-sugar producing vegetable species cultivated in the gardens from New Guinea to Fiji for its edible aborted inflorescence. It has large thick canes. Its chromosome number ranges from 2n=60- 122(Roach, 1972). *S. edule* is believed to be an intergeneric hybrid between either *S. officinarum* or *S. robustum* and other *Miscanthus* sp. (Daniels and Roach, 1987).

2.1.1.2 Other Allied Genera

It has been hypothesized that sugarcane has been evolved from complex introgression between *Saccharum*, *Erianthus* (section *Ripidium,* 2n=20,30, 40,and 60), *Miscanthus* (Section *Diandra*, 2n=38, 40, and 76), Narenga (2n=30) and *Sclerostachya* (Hack) (Daniels and Roach, 1987; Lu et al., 1994). Although some data supports it originating from *S. robustum* (Amalraj and Balasundaram, 2006). *S. officinarum* has shared chromosomes with both the genera *Miscanthus* and *Erianthus* section *Ripidium* (Daniels and Roach, 1987; Basse et al., 1997).

2.2 Genome and Genetics of Sugarcane

Sugarcane is an efficient crop for harvesting sun light. Its genome is very large and the most complex among all field crops. The complexity and size of the sugarcane genome is a major limitation in genetic improvement of this crop. Knowledge about sugarcane genome size and organization is useful for planning and utilization of genetic resources (introgression) and biotechnological tools in breeding program (Butterfield et al., 2001; Zhang et al., 2012). Recently, genome size of *Saccharum spp*. has been estimated using flow cytometry. The genome size of different *S. officinarum* accessions ranges from 7.50 – 8.55 Gb with an average of 7.88 Gb while it ranges from 7.65- 11.78 Gb and 3.36- 12.64Gb in *S. robustum* and *S. spontanium*, respectively (Zhang et al., 2012). In comparison to other cereal crops of same tribe-Andropogoneae, the basic genome size of sugarcane (760-926 Mbp) is twice the size of rice genome (389 Mbp) and similar to sorghum's (760 Mbp) (D'Hont and Glaszman, 2001). The wide range of genome sizes indicating great variations in ploidy level and chromosome number among different species of *Saccharum* genus as well as various cultivars of the same species.

Saccharum species are autopolyploid with ploidy level ranges from 5x to 16x (Zhang et al., 2012). The domesticated species- *S. officinarum* L. is an octaploid

(2n=80) with a basic chromosome number of x=10, which is the basic chromosome number of its wild relative- *S. robustum* (2n=60-80) (D'Hont et al., 1998) and the members of Andropogoneae tribe (D'Hont et al., 1995; Cesnik and Miocque 2004; Nobrega and de Dornelas, 2006). Another wild relative *S. spontanium* (2n=40-128; 5-ploid to 16 ploid) has basic chromosome number of x=8 (D'Hont et al., 1996) but it shows great variation in chromosome numbers with five main cytotypes viz. 2n=62, 80, 96,112 or 128 (Daniels and Roach, 1987; Sreenivasan et al., 1987).

Modern sugarcane cultivars (*Saccharum spp*. hybrids) are generally advanced generations hybrid between *S. officinarum* L. (2n=80) and *S. spontaneum* L. (2n=40-128) (Panje and Babu, 1960). The hybrids are highly polyploid and aneuploid (D'Hont, 2005) with genomes composed of 2n=100-130 chromosomes (Purseglove, 1972; Grivet and Arruda, 2001). The *in situ* hybridization analyses have exhibited that genomes of modern hybrids are composed of 63-85% of *S. officinarum* chromosomes, 10-20 % of *S. spontaneum* chromosomes and 5-17% of recombinant chromosomes (Piperidis and D'Hont, 2001; D'Hont, 2005).

2.3 Traditional and Modern Cultivars

Traditional Cultivars

Traditional cultivars have been described as species and given Latin binomials by taxonomists. These are represented by domesticated *S. officinarum* L. (noble cane, 2n=8x=80), *S. barberi* (North Indian Cane, 2n=111-120) and *S. sinense* (Chinese cane; 2n=80-124) and were cultivated as sugar producing crop before the end of 19th century (Grivet et al., 2004). These are now being used as the potential sources of desirable traits for breeding program and maintained in the field gene bank. These clones were propagated by stem cuttings (Lu et al., 1994a) and have gradually replaced by modern cultivars during the 20th century (Grivet et al., 2004).

Modern Cultivars

The discovery of naturally germinating sugarcane seeds in Java (1858) and Barbados (1859) stimulated the sugarcane breeding program in 1888, for the first time (Stevenson 1965; Kennedy and Rao, 2000). The first man- made interspecific hybrids were produced in Java and India respectively (Lu et al., 1994b; Grivet et al., 1994). Modern sugarcane cultivars (*Saccharum spp*. hybrids) are virtually advanced generation hybrids between *Saccharum officinarum* L. (noble cane, 2n=80), and S*. spontaneum* L. (wild cane, 2n=40-128) (Sreenivasan et al., 1987; D'Hont et al., 1998). Both species are thought to have an autopolyploid origin (Sreenivasanet al., 1987; Grivet et al., 1996) while the hybrids (modern cultivar) are highly (~12x) polyploidy and often aneuploid (~130 chromosomes). These cultivars generally have between 100 and 130 chromosomes (Grivet and Arruda, 2001). The hybrids were then repeatedly backcrossed to *S. officinarum* to recover the thick sugar-containing stalks of this species. This also resulted in minimizing the negative effect of the wild parent and maximizing the sugar contents. This breeding procedure is known as 'nobilization' in sugarcane (Lu et al., 1994). Experimental results of molecular cytogenetic analyses (D'Hont et al., 1996; Piperidis and D'Hont, 2001; Cuadardo et al., 2004) and genetic mapping studies (Grivet et al., 1996; Hoarau et al., 2001) revealed that modern cultivars generally exhibit 70-80% of chromosomes entirely derived from *S. officinarum*, 10-20% from *S. spontaneum* and rest of the chromosomes derived from interspecific recombinations (D'Hont, 2005).

2.4 Nobilization of Sugarcane

S. spontaneum L. is the wild relative of commercially cultivated species *S. officinarum* L. The wild species possesses high vigour, heavy tillering potentiality, ratooning ability, biotic (diseases and insects) resistance and abiotic stresses (drought, floods, and salinity) tolerant capacity while these attributes are lacking in *S. officinarum* L except tall, thick juicy stem with high sucrose content. Bremer

(1961) was the first breeder who used nobilization technique to improve the characteristics viz. tillering, rationing, biotic and abiotic stresses of *S. officinarum* L. Nobilization is a special method of hybridization applied in sugarcane improvement. In this method, pollination of noble cane *S. officinarum* L. is done with pollen of its wild relative *S. spontaneum* followed by repeated backcrossing (up to 2nd backcrossing) to the noble canes (Figure 2.1). The wild cane is nobilized through this breeding process and selected hybrid progenies are the nobilized canes (Bremer, 1961). The nobilization process involved chromosome non-reduction plus introgression of additional genes through a system of crossing the noble with wild clones of *S. spontaneum* L. (x=8, 2n=32-128) (Ha et al., 1999; Irvine, 1999).

Table 2.1 Chromosome numbers at three stages of nobilization in crosses between sugarcane (*S. officinarum* L. (2n=80) and *S. spontaneum* L. (2n=64), assuming participation of 2n egg gametes in stage I and II. (source: Bhat and Gill, 1985)

Stages of nobiliza-tion	Gene-ration	Female x Male	Progeny Chromosome number	Proportion of (%) *S. officinarum* L. x *S. spontaneum* L.
I	F_1	*S. officinarum* L. (2n=80) x *S. spontaneum* L. (2n=64)	2n= 80+32=112	71.4:28.6
II	BC_1	*S. officinarum* L. (2n=80) x F_1	2n=80 +56=136	88.2:11.8
III	BC_2	*S. officinarum* L. (2n=80) x BC_1	2n= 40+68=108	92.6:7.4

Progeny of F1 and BC1 have the non-reduced somatic complement (2n) of the female parents plus the gametic number (n) of the male.

A number of hypotheses have been put forward to explain the mechanism of 2n + n transmission in sugarcane (Price, 1961). Among these are:

- Formation of unreduced egg cells
- Chromosome doubling through endo-duplication either at the dyad or tetrad stage
- Postmeiotic fusion of the two innermost megaspores
- Postmeiotic endomitosis in the egg cell

- Incompatability of n + n gametes due to either selective fertilization or a combination of selective fertilization and parthenogenesis, i.e. differential survival of 2n+n and n+n zygotes
- Failure of certain zygotic combinations due to faulty endosperm development (selective survival).

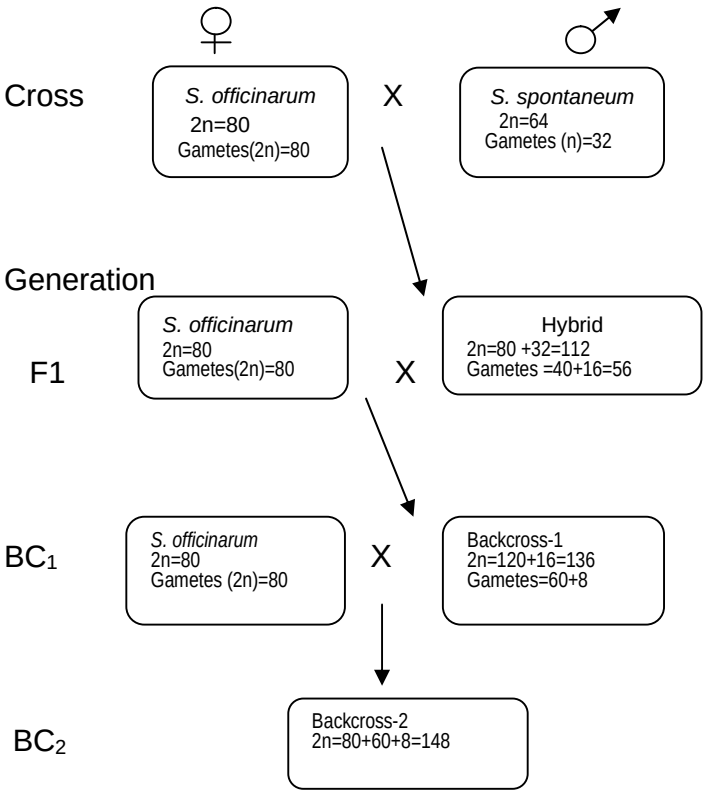

Figure 2.1 Diagram showing chromosome transmission pattern during nobilization of sugarcane.

The exact mechanism of the formation of egg cells with two haploid sets of chromosomes is not unambiguously established. The possibility of unreduced egg cell formation can be precluded. Since segregation of the maternal characters is observed among the hybrids from crosses between *S. officinarum x S. spontaneum*. The increase in chromosome number might occur by separation of chromatids of the *S. officinarum* chromosomes in the egg-nucleus either before or during fertilization with a sperm nucleus of *S. spontaneum* or chromosome doubling could occur in the chalazal megaspore by means of endoduplication, thus producing egg cells with n+n chromosomes, either at the dyad stage after the first

meiotic division or at the tetrad stage after second division but before fertilization. However, even this mechanism, while accounting for 2n+n zygotes, does not explain the transmission of the *n* chromosome number of *S. officinarum* L. on selfing or intraspecific crossing; 2n+n transmission occurs only after pollination and fertilization following interspecific crossing. From this it could be inferred that *S. officinarum* produce both reduced (haploid) and unreduced egg cells. Two explanations of such a phenomenon would be either that 2n and n egg cells are selectively fertilized or that the chromosome doubling in the egg cell occurs at the time the egg cell is fertilized by the sperm nucleus of *S. spontaneum*.

2.5 Modern Sugarcane Breeding

Improved varieties of sugarcane are being developed in many R & D (Government and corporate) institutions of sugarcane growing countries of the world following almost similar procedure. The selection of parents, crossing (biparental/polycross) followed by clonal selection and various field performance testing are the basic steps of sugarcane breeding methodology. Sometimes, mutation and somaclonal variation induction followed by clonal selection are being practiced. Recently, molecular breeding approach has been initiated to accelerate breeding program in several research organizations of the world. Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, is responsible for developing commercial sugarcane varieties in Bangladesh. The principal objectives of sugarcane breeding program are to develop sugarcane varieties possessing high tonnage, sugar and gur yield, and having good ratooning potential, resistance to red rot disease, suitable for cultivation in different climatic situations like drought, flood and salinity. (BSRI, 2014). In the breeding program, parents for hybridization are being chosen based on a number of different criteria. The traits of interest include high sucrose content, good agronomic characteristics viz. tall and thick cane, erect leaf, high tillering habit, early maturity, non-lodging, resistant to red rot disease and insect pests. Parents are chosen from 280 flowering germplasm conserved in the ‘Field Gene Bank’.

Germplasm comprise of local varieties, released varieties, hybrid clones developed at BSRI, exotic varieties/clones and wild species. Sugarcane variety development procedure followed at BSRI is described below:

Year	Activity	Selection Criteria
1	i) Crossing between selected parents ii) Harvesting , drying & storage of fuzz	-
	↓	
1	Pricking, Seedling production (60,000), & transplanting in the nursery bed	
	↓	
2	Stage-1 50,000 seedlings planting the in the field	Individual clone /clump selected based on vigour and other characters of cane
	↓	
3	Stage-2 3000-5000 clones planted in 1m x 3m plot	Brix and vigour of cane crop
	↓	
4	Stage-3 250-500 clones planted in 4m x3m plot	Brix, sucrose content, millable cane yield, natural insect pest and disease incidence.
	↓	
5	Stage-4 PYT (4mx3m plot) 4 Replications, at 2 locations 75-100 entry/trial/location	Brix, sucrose content, millable cane yield, natural insect pest and disease incidence.
	↓	
6	Stage-5 AYT (6mx5m plot) 4 Replications, at 2 locations 16-24 entry/trial/location	Brix, sucrose content, millable cane yield, natural insect pest and disease incidence and seed multiplication
	↓	
7	Stage-6 ZYT-I (6mx10m plot) 4 Replications, at 4 locations 6-10 entry/trial/location	Brix, sucrose content, millable cane yield, natural insect pest and disease incidence. Seed multiplication
	↓	
8	Stage-7 ZYT-II (6mx10m plot) 4 Replications, at 6-8 locations 6-10 entry/trial/location	Brix, sucrose content, millable cane yield, natural insect pest and disease incidence,Jaggery (Gur) recovery & quality checking.
	↓	
9	Stage- 8 ZYT-III (6mx10m plot) 4 Replications, at 6-8 locations 6-10 entry/trial/location	Brix, sucrose content, millable cane yield, natural insect pest and disease incidence, Jaggery (Gur) recovery & quality checking.

Figure 2.2 Flow diagram of sugarcane variety development procedure Followed at BSRI

2.6 Genetic Diversity

Genetic diversity is generally considered as the amount of genetic variability among individuals of a variety or population of a species (Brown, 1983). In other words, genetic diversity represents the heritable variation within and among populations of a species. Allelic variation present in the genome of individuals is the main cause of genetic diversity and this is reflected in morphological, physiological and biochemical differences of characteristics of a species (Frankham et al., 2002).Genetic diversity of a crop species plays a very important role in sustainable crop production and food security as well. A better understanding of genetic diversity and its distribution is very much essential for its conservation and utilization. Genetic diversity analysis provides information on the genetic base of the gene pool present in the germplasm collections. Genetic diversity study is a major breakthrough in the understanding intraspecies performance leading to crop improvement (Aremu, 2005).

2.6.1 Measures of Genetic Diversity

Success of crop breeding program depends on the availability of genetically diverse germplasm. Therefore, estimation of the extent of genetic diversity of existing germplasm collections is very essential for selecting diverse parental lines that would be used in hybridization program.

Generally genetic diversity is estimated by measuring genetic distance or similarity. Both the terms entail that either differences or similarities remain at the genetic level (Weir, 1990). Single or combinations of a number of statistical techniques are available for measuring genetic diversity of a group of individuals of crop genotypes (Weir, 1996; Warburton and Crossa, 2000; Aremu, 2005; Kubik et al., 2009). Various types of data from many sources have been used by several researchers including plant molecular biologists and breeders to measure genetic diversity of many crops. Both qualitative and quantitative data are used

for this purpose. These data source include morphological and agronomical, pedigree record, biochemical and molecular markers data (Liu et al., 2000 in cotton; Muyco, 2002 in sugarcane; Aremu et al., 2007 in cowpea; Kubik et al., 2009 in bent grass; Khodadadi et al., 2011 in wheat). The choice of statistical technique to be used depends on objectives of the experiment and availability of required data.

Knowledge of genetic diversity studies has been widely used in crop species to identify crop cultivars, breeding lines for the maintenance of genetic purity, to identify proprietary genetic resources and estimate genetic relationships (Prabhu et al., 1997). Nature and extent of genetic relationships within and between species is very much important to systematically organize germplasm collections, identify genetically diverse groups of breeding lines, selecting parents for hybridization program and elucidate genetic relationships (Muyco, 2002).

The commonly used measures of genetic diversity among various plant species are described below:

2.6.1.1 Coefficient of Parentage (COP)

Detail information of parentage is important for measuring genetic variation in the progeny. The coefficient of parentage (COP) estimates the probability that alleles of two individuals are identical by descent through their pedigrees and is commonly used to indirectly assess diversity within gene pools (Burkhamer et al., 1998). Coancestry is defined as the probability that a random gene chosen from an individual is identical by descent to a random gene at the same locus of another individual (Malecot, 1969). Coefficient of kinship (Falconer, 1989) and coefficient of parentage (COP) (Kempthrone, 1969) are synonymous to coancestry. The COP indicates a measure of the relatedness of two individuals. High COP value indicates that two individuals share greater number of identical alleles. Hybridization between them will practically produce a F_2 generation with lower number of allelic combinations and will be expected to show reduced

variation in segregating generations (Beer et al., 1995). The co-efficient of parentage (COP) is denoted by r and its value may vary from 0 (When two genotypes are unrelated) to 1 (When two genotypes are identical)(Martin et al., 1991a).

Besides estimation of genetic diversity among cultivars and parental germplasm, COP analysis can also be used to predict breeding behavior of the progeny of crosses, summarizing regional crop diversity, identifying parents that have contributed to higher yield, and monitoring trends in genetic divergence over time and space (Kim and Ward, 1997; Souza et al., 1998). Lack of accurate and complete pedigree information is one of the limitations for using COP to estimate genetic diversity (Carter et al., 1993). Furthermore, lack of suitable software to compute COP values create another problem. The COP is calculated according to the formula by Wright (1951):

$$F_I = (1/2)^n (1+F_A)$$

Where 'n' is the number of individuals including the common ancestor in the path leading to individual *I* that could be traced back to a common ancestor and F_A is the coefficient of inbreeding of the common ancestor (Carpena et al., 1993).

2.6.1.2 Shannon-Weaver Diversity Index

Characterization based on morphological characteristics of a large number of accessions in germplasm bank is a routine activity. Assessment of diversity is crucial to get information about the extent of variation of characterized crop germplasm for planning future breeding program. Moreover, it is also very much important for maximizing the amount of useful genetic variation within a collection (Bisht et al., 1999). Shannon-Weaver Diversity Index is commonly used to assess phenotypic diversity of crops. Phenotypic diversity of each trait is determined by calculating the Shannon-Weaver Diversity Index (H') as described by Hutcheson (1970) as:

$$H' = \sum_{i=1}^{n} \text{pi}(\log 2\text{pi})$$

Where n is the number of phenotypic classes for a character and pi is the proportion of the total number of entries belonging to the ith class or phenotype (i=1,2,….., n). Its value ranges from 0 to 1. The highest value of 1.0 indicates highest diversity while the lowest value of 0 indicates the least diversity. Each value of H' is divided by its maximum value, $\log_2$ n, and this normalizes the diversity index value in order to keep the values between zero and one. It can be assumed that the accessions can be classified into discrete classes with respect to a particular trait. This diversity measure is useful in hierarchical analyses of diversity in large data sets as in germplasm banks, due to its additive nature. In the recent times, it has also been used for molecular data, although it was used for morphological data in the past.

The Shannon-Weaver diversity Index has been widely used in studying diversity of germplasm collections of many crops (Bisht et al., 1995 in South Asian okra; Caldo et al., 1996 in rice; Bisht et al., 1999 in Indian sesame; Siopongco et al., 1999 in maize; Muyco, 2002 in sugarcane; Upadhyaya et al., 2002 in chickpea; Upadhyaya et al., 2003 in groundnut; Santesteban et al., 2009 in apple; Sourour et al., 2010 in durum wheat).

2.6.1.3 Mahalanobis Distance

Mahalanobis D^2 statistic was introduced by P.C. Mahalanobis in 1936. Later on, this statistic was extended by Rao (1952).The distance between clusters is measured using Mahalanobis D^2 statistic using the formula given by Souza and Sorrells (1991):

$D^2 = (zi-zj)' R^{-1} (zi-zj)$

where,

The distance D^2 is the squared difference between the *ith* and *jth* clusters' vectors (zi zj) of the quantitative characters. Each character distance is weighted by the inverse of the variance-co-variance matrix (R) of the characters among all entries in the *ith* and *jth* clusters.

Weighting the inverse of the variance-covariance matrix increases the emphasis of independent traits and reduces the emphasis to highly correlated traits. This is a unit less measure of distance and powerful and widely used technique to measure genetic divergence (Murty and Arunachalam, 1966).

2.6.1.4 Euclidian Distance

$E_{ij} = [\sum_k (X_{ki}-X_{kj})^2]^{1/2}$ where,

E_{ij} = 0 to ∞, the larger the value, the more distant the degree of relationship

Xi and Xj are the standardized values for the *ith* and *jth* characters in *kth* varieties.

2.6.1.5 Genetic Diversity Estimation from Molecular Data

Sun et al. (2001) compared the genetic diversity of common wild rice (*Oryza rupipogon* Griff.) and cultivated rice (*O. sativa* L.) based on RFLP markers using the six following diversity estimates.

Percentage of Polymorphic Loci (P)

P= (k/n) x 100%, where *k* is the number of polymorphic loci and *n* is the total number of loci investigated.

Average Number of Alleles per Locus (A):

A= $\sum$Ai/n, where Ai is the number of alleles at the *ith* locus and *n* is the total number of loci investigated.

Average Number of Alleles per Polymorphic Loci (*Ap*)

Ap= $\sum$Api/n_p, where Api is the number of alleles at a certain polymorphic locus and n_p is the total number of polymorphic loci investigated.

Average Number of Genotypes per Locus (G)

G= $\sum$g/n, where *g* is the number of genotypes at a certain locus and *n* is the number of loci investigated.

Average Heterozygosity per Locus (Ho)

Ho= $\sum$*Hoi*/n= (1- $\sum$qij)/n, where *Hoi* is the heterozygosity at a certain locus, *n* is the total number of loci investigated and *qij* is the frequency of homozygous genotypes of an allele at a certain locus.

Average Gene Diversity (Hs)

(Sano and Morishima, 1992)

Hs= 1-1/n$\sum q^2_{ij}$, where q_{ij} is the frequency of the jth allele at the ith locus and *n* is the number of loci investigated.

Gene Diversity at a Locus (Nei, 1987)

Nei's gene diversity at a locus=2pqn/(n-1), here *p* is the frequency of presence and *q* is the frequency of absence of a marker amplification at a locus and *n* is the number of individuals evaluated.

Gene Diversity (Heterozygosity)

According to Weir (1990), gene diversity=1-$\sum P^2ij$, where P^2ij is the frequency of the jth pattern for the marker *i* and is summed across *n* patterns.

Anderson et al. (1993) suggest that gene diversity is the same as the polymorphism information content. The relationship between two individuals based on single or multiple traits can be determined by the genetic divergence of loci throughout the genome. The more commonly used techniques to estimate genetic distance/similarity are the following:

Simple Matching Coefficient (Described by Gower, 1972*)*

A match is scored for a given locus when two accessions have the band or if both accessions lack the band. Genetic distance is calculated as the proportion of loci that do not match. Thus, the genetic distance between a pair of accessions is calculated as:

GDs =1-[$N_{(0,0)}$ +$N_{(1,1)}$]/N, Where

N is the number of loci or bands

$N_{(0,0)}$ is the number of loci where both accessions lack the band

$N_{(1,1)}$ is the number of loci where both accessions have the band

Sokal and Sneath (1963) suggested that the variance of a simple matching coefficient would approximate that of a binomial distribution. Goodall (1967) found that for 20 characters, the binomial variance consistently overestimated the calculated variance for the simple matching coefficient. Further, he recommended the setting of confidence limits to expected values. Since simple matching coefficient approximates a binomial distribution, high values of this coefficient have very low variances (Sneath and Sokal, 1973).

Jaccard's Coefficient (Gower, 1972)

Jaccard (1908), on the other hand, employed a method that gives more weight to the matches than to mismatches. A match is counted when both accessions show the presence of band in question. Jaccard's method does not consider pairs in which the band is absent in both accessions (negative matches).Thus, the coefficient between any pair of accessions can be expressed as:

GDj= 1-{$N_{(1,1)}$/[$N_{(1,1)}$ + $N_{(1,0)}$ + $N_{(0,1)}$]} where,

$N_{(1,1)}$) is the number of loci where both accessions have the band

$N_{(1,0)}$ is the number of loci where the band is present in the first accession and absent in the second

$N_{(0,1)}$ is the number of loci where the band is absent in the first accession and present in the second

Nei-Li Genetic Distance

The Nei-Li applies to systems it only two alleles and no co-dominance. This is equivalent to the coefficient of Dice (1945) and does not consider 0-0 matches (Nei and Li, 1979). This distance measure is expressed as:

$GD_N = -\ln\{2N_{(1,1)0})/[N_{(1,-)} + N_{(-,1)}]\}$ where

$N_{(1,-)}$ is the number of loci having a band present in the first accession.

$N_{(-,1)}$ is the number of loci having a band present in the second accession

$N_{(1,1)}$ is the total number of bands common between the two accessions

Modified Roger's Distance

The Modified Roger's distance considers each locus scored as an orthogonal dimension. It counts mismatches instead of matches.

$GS_R = [N_{(0,1)} + N_{(1,0)}/2N]^{½}$ where,

$N_{(0,1)}$ is the number of bands absent in the first accession and present in the second accession

$N_{(1,0)}$ is the number of bands present in the first accession and absent in the second accession and N is the number of loci or band

The General Similarity Coefficient of Gower (1971)

This is a general coefficient of similarity proposed by Gower (1971) that is applicable for two-state, multistate (ordered and qualitative) and quantitative characters. The genetic similarity between inbred I and j is estimated using the formula given by Hongtrakul et al. (1997):

$S_{ij} = \sum(W_{ijk} S_{ijk})/ \sum W_{ijk}$ where,

W_{ijk} is a weight for inbred *i* and *j* and fragment *k*

S_{ijk} is the marker phenotype or score (present or absent) for inbred *i* and *j* and fragment *k*, $i=j=1,2,\ldots, n$,

and *n* is the number of inbreds.

The similarity between two inbreds is estimated by ignoring null matches:

(1) if inbred *i* and *j* shared a band, then S_{ij}= 1;

(2) if inbred *i* and *j* did not share a band, then S_{ij}=0;

(3) if inbred *i* or *j* or *i* and *j* shared a band, then W_{ij}=1; and

(4) if inbred *i* and *j* lacked a band, then W_{ij}=0.

2.6.1.6 Multivariate Statistical Analysis Techniques

Univariate statistical analysis technique uses data on one variable while multivariate analysis deals with statistical analysis of the data recorded on two or more variables. Simultaneous analysis of multiple independent and dependent variables is carried out in multivariate statistical analysis (Tabachnick and Fidell, 2007). Multivariate analysis techniques are important for the study of diversity within germplasm collections (Edye et al., 1970; Lee and Kaltsikes, 1973; Narayan and Macefield, 1976; Chandra, 1977). Categorizing collected germplasm accessions in to homogenous groups on the basis of multivariate criteria instead of univariate criterion is essential for germplasm management and population improvement (Hintum, 1995). Multivariate data analysis technique is very much useful to know the relationships among the variables studied and allows in getting insight information about germplasm composition. It also helps to identify most relevant variables having significant contribution toward divergence in the collected germplasm (Leguizamon and Badenes, 2003). This technique is applicable for analyzing agro-morphological, physiological, biochemical or molecular markers data for the study of genetic diversity (Mohammadi and Prasanna, 2003), characterization, evaluation and classification of germplasm accessions (Peeters and Martinellis, 1989). Multivariate data analysis based various algorithms viz. Mahalanobis's D^2 statistics, (Mahalanobis,1936), principal component analysis (PCA) (Muyco, 2002; Sinha and Mishra, 2015), principal coordinate analysis (PCoA) [Sabaghnia et al., 2013), multidimensional scaling (MDS) (Johns et al., 1997; Thompson et al., 1998; Brown-Guedira et al., 2000) cluster analysis by Tocher's method(Rao, 1952; Thompson et al., 1998; Brown-Guedira et al., 2000), a non-hierarchical cluster

analysis (Beale, 1969), canonical analysis(Cliff and Krus, 1976; Anderson and Willis, 2003) and metroglyph analysis (Anderson, 1957) are most frequently used and valuable methods for diversity analysis and classifying germplasm into principal groups (Mohammadi and Prasanna, 2003; Singh et al., 2010). Classification (grouping of similar accessions) and ordination (description of spatial relationship among entities) are two notable multivariate statistical analysis techniques generally used in the field of numerical taxonomy, genetic analysis and molecular plant breeding (Crossa et al., 1995; Muyco, 2002).

Individuals or genotypes having similar values of all attributes are classified in to groups. Again simplification of data can be done by determining more homogenous sub-sets of accessions. There are two types of classification methods: cluster analysis and discriminate analysis. Multidimensional Scaling (MDS) can reflect the genetic relationship among the populations in a two-dimensional plot.

Cluster Analysis

The term cluster analysis was first introduced by Robert Tryon in 1939. Cluster analysis refers to a set of multivariate analytical methods where objects or individuals with similar characteristics are grouped together to form a cluster and remaining objects or individuals having different traits assembled in to different clusters through mathematical procedure. The members are more homogenous within a cluster but heterogeneous between clusters. In successful clustering analysis, objects/ individuals shall remain closer when plotted in scatter graph and member of different clusters shall be farther apart (Hair et al., 1995). Identification of distinct groups in a dataset is the main goal of cluster analysis (Tantrum et al., 2004). It has wide applications on various fields of research viz. statistics, mathematics, social sciences, Psychology, business, taxonomy, medicine, plant and animal sciences. Cluster analysis is frequently used in germplasm characterization, evaluation and conservation to study genetic divergence. By using this technique, germplasm accessions with similar

characteristics are grouped in to homogenous classes (clusters) in such a way that representative samples can be drawn from each cluster. It helps to establish core collections of germplasm bank.

There are chiefly two types of clustering methods, viz. (i) distance-based methods, in which a pair-wise distance matrix is used as an input for analysis of a particular clustering algorithm. Tree or dendrogram can be constructed from this analysis (Johnson and Wichern, 1992), and (ii) model-based methods, in which observations from each cluster are assume to be random draws from some parametric models. At present, distance-based methods are most frequently used (Mohammadi and Prasanna, 2003).

Distance-based clustering could be divided in to two types, such as hierarchical and non-hierarchical clustering. Hierarchical clustering is a widely used data analysis tool. Hierarchical clustering is connectivity based method. This technique is a stepwise algorithm, clustering process starts with each point connecting two closest objects (having least dissimilarity) to form a cluster. At each step, the two closest clusters combined in to a single cluster. This process continues until there is only one cluster containing all the points. The hierarchical clustering methods are frequently used in study of genetic diversity of various crop species. Hierarchical clustering may be used to assess genetic similarity and dissimilarity in germplasm collections, and the technique could also have applications for the selection of parental lines for which varying degrees of segregations are sought (Peeters and Martinelli, 1989).

Hierarchical clustering methods produce a tree or dendrogram. Distance matrix is used as clustering criteria in this method. Two separate systems are used to built tree or dendrogram. These are: i) agglomerative clustering (Bottom-up-starts from ‘n’ clusters to get 1 cluster), and ii) divisive clustering -(Top-down-starts from 1 cluster to get ‘n’ clusters)

Agglomerative hierarchical clustering method is the most popular method. In this method, data objects are grouped in a bottom-up fashion. This technique is a stepwise algorithm, clustering process starts with each point connecting two closest objects (having least dissimilarity) to form a cluster. At each step, closest pair of clusters combines into larger cluster. This process continues until all objects come under a biggest single cluster. In this method, seven clustering distance algorithms have been used.

(i) Single linkage (nearest neighbor i.e. smallest distance between points) (Sneath and Sokal, 1973),

(ii) Complete linkage (furthest neighbor i.e. largest distance between points) (King, 1967),

(iii) UPGMA (Unweighted pair group method using arithmetic averages) (Sneath and Sokal, 1973)

(iv) WPGMA(Weighted pair-group method using arithmetic averages) (Sneath and Sokal, 1973)

(v) UPGMC (Unweighted pair-group method using centroid/Centroid (Sneath and Sokal, 1973)

(vi) WPGMC (Weighted pair-group method using the centroid average)/Median (Sneath and Sokal, 1973)

(vii) Ward's method (Ward, 1963).

In UPGMA, the distance between two clusters is defined as the unweighted mean of the distances between all pairs of objects, one from each cluster. At each step, the two nearest clusters are joined.

Among various agglomerative hierarchical methods, UPGMA (Unweighted pair group method using arithmetic averages) (Sneath and Sokal, 1973) is the most popular followed by Ward's method (Ward, 1963) for the assessment of genetic diversity of germplasm collections (Panchen,1992; Odong et al., 2011).

In non-hierarchical methods, the objects or items are organized in to a set number of groups in the best possible manner. These methods frequently referred to as ‘K-means clustering’. In these clustering systems, dendrogram or trees are not constructed.

Strategies for hierarchical clustering generally fall into two types: agglomerative and divisive. Many workers successfully used these techniques to categorize variation patterns at both inter- and intra-specific levels (Sneath and Sokal, 1 973; Ariyo and Odulaja, 1991).

Ordination Analysis

‘Ordination’ means putting things in order. Ordination analysis is the collective term for multivariate techniques that arrange sites along axes on the basis of data. In other words, ordination is the term used to arrange multivariate data in a rational order usually in to a 2-D space (i.e., a diagram on paper). Ordination orders objects that are characterized by values on multiple variables (i.e. multivariate objects) so that similar objects are near each other and dissimilar objects are farther from each other. In this technique, multidimensional data converted to a low-dimensional space by using ordination analysis and similar accessions remain close to each other and dissimilar objects far apart. In germplasm characterization and conservation program, a large number of accessions are being characterized by recording multiple data of agro-morphological and physiological traits. For this reason, field data have high dimensions. Final results must be lower dimensions to take decision judiciously. In this technique, spatial representation of the objects in two or three dimensions will reflect their relationship in higher dimensions with minimum distortion. The output of ordination analysis is presented numerically and /or graphically. A number of ordination methods are available. Among them, following four methods are mainly used.

These are:

i) Principal components analysis (PCA)

ii) Correspondence Analysis (CA) and Detrended Correspondence Analysis (DCA)

iii) Principal Coordinates Analysis (PCoA), and

iv) Non-Metric Multidimensional Scaling (NMDS).

Principal Component Analysis

The concept of principal component analysis (PCA) was first introduced by Karl Pearson in 1901.PCA is very popular multivariate technique. It is the most common ordination technique. PCA can be defined as " a method of data reduction to clarify the relationships between two or more characters in to a limited number of uncorrelated new variables" (Wiley, 1981).The goal of PCA is to achieve parsimony by reducing the number of variables of interest (reduction of dimensionality) in to a smaller set of components. It is also used to identify hidden patterns in the data and classifying them according to the amount of information they represent. It summarizes the variation in correlated multi-attributes to a set of uncorrelated components known as principal components (PCs). Each component is estimated by linear combination of original variables. In PCA, uncorrelated PCs are extracted by linear transformation of the original variables so that the first few PCs contain most of the variation present in the original dataset. In the first step of PCA, eigenvalues are calculated, which explain the amount of total variation, which is displayed on the PC axes. The first PC captures most of the variance, present in the original dataset. The 2nd PC accounts for rest of the variability (2nd most) and uncorrelated with the first, and so on until all variance is accounted for (Jolliffe, 1986). PCs are orthogonal and independent to each other. Each PC exhibits distinct properties of the original dataset and may be interpreted independently (Mohammadi and Prasanna, 2003). The eigenvalues of PCs can be used as a criterion to determine how many PCs should be utilized. The PCs with eigenvalues >1.0 are considered as

inherently more informative than any single variable alone (Iezzoni and Pritts, 1991). PCA can be used to determine the optimum number of clusters in a study and also be used to drive 2- or 3-dimensional scatter plot of individuals, such that the geometrical distances among the individuals in the plot reflect the genetic distances among them with minimal distortion. Aggregations (Groups/ clusters) of individuals in such a plot will exhibits sets of genetically similar individuals (Melchinger, 1993; Karp et al., 1997; Warburton and Crossa, 2000).

2.6.2 Analysis of Genetic Diversity

Assessment of genetic diversity of a population can be done by various methods based on some criteria. These criteria include agro-ecological origin, pedigree records, morphological and agronomic characters, cytological characteristics, biochemical and DNA markers. Each criterion has its own merits and demerits with respect to the information content, methodology and aspects of diversity being presented. Therefore, the measures of diversity for each of these criteria vary. As a matter of fact, grouping based each of these different data can be expected to differ because all data types require their own measure of genetic diversity.

Genetic Markers

In most of the cases, genetic markers are being considered for genetic diversity analysis of many crop species. Genetic markers represent genetic differences between individual organisms or species. Generally they do not represent the target genes themselves but act as signs or flags (Collard et al., 2005). Genetic markers that are closely located to gene or genes (i.e. tightly linked) would be referred as gene tags. Such type of markers do not affect the phenotype itself but they are located at the near to genes controlled the traits. All genetic markers have the specific genomic position in particular chromosome like gene and that are called loci.

Three major types of genetic markers were stated by Jones et al. (1997) and Winter and Kahl (1995), these are: i) morphological markers which represent phenotypic traits or characters; ii) biochemical markers which include allelic variants of enzyme called isozymes; and iii) DNA or molecular markers, which reveal sites of variation in DNA. Morphological markers are visualized by phenotypic traits like flower color, seed color, seed shape, pigmentation on stems/branches/fruits etc. Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining. The major disadvantages of this two type of markers that they are limited in number and are influenced by environmental factors or developmental stage of the plants (Winter and Khal, 1995). However, despite these limitations, morphological and biochemical markers were often used by plant breeders (Eagles et al., 2001). Whereas, DNA markers are being frequently and widely used marker for diversity analysis. They arise from different types of mutation such as substitution, insertion, deletion or errors in replication of tandemly repeated DNA (Paterson, 1996a). These markers are selectively neutral because they are usually located in non-coding regions of DNA and also they are unlimited in number and are not influenced by environment or developmental stage of plants (Winter and Khal, 1995). Apart from the use of DNA markers in the construction of linkage maps, they have numerous applications in plant breeding such as assessing the level of genetic diversity within germplasm and cultivar identity (Baird et al., 1997; Henry, 1997; Jahufer et al., 2003).

2.6.2.1 Diversity Analysis Based on Agro-Morphological Markers

Recording of morphological and agronomic traits of various crops germplasm is a routine activity of many national and international Genetic Resources Centers of the world. Diversity analysis based on agronomic and morphological characteristics recorded at several stages of plant growth were carried out in several crops including sugarcane. Genetic diversity between parents is important to create transgressive segregants from the cross.

In chickpea (*Cicer arietinum* L.), a total of 1956 accessions of which 1465 desi, 433 Kabuli and 58 intermediate types were evaluated during 1999-2000 post-rainy season at ICRISAT, Patancheru, India. Seven morphological and 15 agronomic characteristics were studied for phenotypic diversity using Shannon-Weaver diversity index and principal component analysis. Data on flower color, plant color, growth habit, seed color, seed shape, dot on seed testa, seed testa texture and agronomic traits viz. days to flowering, flowering duration, plant height, number of branches , days to maturity, pod number per plant, number of seeds per pod, 100 –seed weight, plant yield and plot yield were analyzed . The average phenotypic diversity index was highest in the intermediate types (0.2653) and the lowest in the kabuli type (0.140). Principal component analysis indicated that days to 50% flowering , plant width, apical secondary branches, tertiary branches, dots on seed testa, 100-seed weight, flowering duration, basal secondary branches, seed color and seed testa texture were found important traits in explaining multivariate polymorphism (Upadhyaya et al., 2002).

A total of 306 landraces of cultivated common bean (*Phaseolus vulgaris* L.) were evaluated in three locations in Columbia during the 1987-1988 cropping season. Data on pigmentation, growth habit, and leaflet, pod, seed and phenology traits, as well as reaction to four important diseases and an insect pest were analyzed by multivariate statistical analysis. Characterization of these landraces based on electrophoresis for phaseolin seed protein and nine allozymes was done. Multivariate analysis on phaseolin or allozymes data validated by analysis of morpho-agronomic traits indicated distinct separation of these landraces into Mesoamerican and Andean groups with the presence of subgroups distinct in morphology, adaptation and disease resistance (Singh et al., 1991).

Kanwal et al. (1983) studied the genetic architecture of rice yield in diverse population. Multivariate analysis by Mahalanobis distance and canonical analysis raveled that panicle weight, days to maturity, plant height and seed size contributed significantly to variation. The grouping patterns among the varieties

were at random which indicated that the geographical and genetic diversity were not related.

A total of 41 traits showed highly significant differences among the ancestral rice lines and 33 traits among the modern varieties based on morphological traits. Euclidian distance estimates ranged from 30 to 17.4 in parental lines and from 2.2 to 16.7 in modern varieties. The most important sources of variation among the genotypes were plant height, culm length, apiculus color, internode color, blade color, stigma color, collar color and basal leaf sheath color. Among ancestral lines, plant height, culm length, leaf length, heading, maturity and productive tillers were the most important sources of variation. However, clustering based on morphological traits was not able to provide the real relationship between genotypes (Caldo, 1996).

Tai et al. (1996) evaluated a group of *Saccharum spontaneum*, which represent a wide geographical distribution of the World Collection of Sugarcane and Related Grasses in Miami, Florida based on morphological and chemical characters. Data on four juice-quality characters sucrose, glucose, fructose and brix and five morphological characters (fiber content, stalk diameter, leaf length, leaf width and leaf module) were assessed by cluster analysis and principal component analysis. A considerable variation existed in the *S. spontaneum* collection for the characters studied. It was pointed out that conservation and utilization of *S. spontaneum* germplasm can be enhanced by characterization.

Twenty seven morphological descriptors were evaluated to determine the phenotypic diversity of the world collection of sugarcane germplasm at the Sugarcane Breeding Institute-Research Center, Cannanore, India using the Shannon-Weaver diversity index. Another ten quantitative characters were also considered to compute for the cumulative contribution of individual accessions to the overall variability and the classification of 690 sugarcane accessions into clusters based on Euclidian distances (Balakrishnan et al., 2000). Appropriate size for the germplasm core collection was attained by a logistic regression

model between the cumulative contribution of accessions to the total variability and the number of accessions. This method utilized agronomic and morphological data to evaluate the diversity in the base collection based on principal component scores and Shannon-Weaver diversity index.

Muyco (2002) assessed genetic diversity for agromorphological traits of 81 sugarcane (*Saccharum spp*.) germplasm of PHILSURIN, Philippines using Shannon-Weaver diversity index, Euclidian distance and multivariate statistical analysis. Agromorphological traits showed high Shannon-Weaver diversity indices (>0.80) for quantitative traits and moderate (0.5 -0.75) to high (>0.75) for most of the qualitative traits. Mean Euclidian distance was 52.17 between pairs of cultivars for all possible pair wise combinations and ranged from 11.06 to 131.27.Principal component analysis conducted based on correlation matrix of 16 agromorphological traits resulted to four principal component axes that accounted for 76.22% of total variation. The first principal component that accounted for 29.31% of total variation was mainly attributed to variation in juice quality, yield and stalk diameter traits. Cluster analysis by UPGMA based on Euclidian distances classified the 81 cultivars in to one major and four minor clusters.

Fourteen agronomic characters were evaluated to assess genetic divergence of 25 sugarcane genotypes at Sugar Crops Research Institute (SCRI), Mardan, Khyber Pakhtunkhwa, Pakistan during 2008-2009. Data on germination, tillering, plant height (growth), cane yield, millable canes, pol%, recovery, sugar yield and crop growth rate were analyzed by multivariate statistical analysis. Cluster analysis using Ward's method on the newly created variables using principal components revealed that there were three clusters at a linkage distance of 4.5. Cluster I and II had 11, and Cluster III had 3 genotypes. The genotypes in cluster I and II could be used as source for future selection of hybridization program of sugarcane (Tahir et al., 2013).

2.6.2.2 Diversity Analysis Based on Biochemical Markers

Biochemical marker is a kind of protein that can be extracted and observed; for example isozymes and storage proteins. Isozymes are the earliest molecular markers developed. Isozymes can be defined as structurally different molecular forms of an enzyme with qualitatively, the same catalytic function. They occur as a result of variations in nucleotide sequence that results in the substitution of one amino acid for another. Such a substitution may result in the alteration of the net electrical charge on a protein or the spatial structural (conformation) of the enzyme molecules. These enzymes usually display different kinetic parameters or different regulatory properties. The charge difference is subsequently detected as an alteration in the migration rate of a protein through an electrical field. Electrophoretic separation is then used to measure protein mobility variation within a population .After specific staining the isozyme profile of individual samples can be visualized (Hadacova and Ondrej, 1972; Vallejos 1983; Soltis and Soltis, 1989; Klug and Cummings, 2000). Enzyme specific stains are available to visualize the resulting electromorph bands, which can be from one to several bands depending on the number of loci, their state of homo- or heterozygosity, and the enzyme molecule configuration (Weising et al., 1995). Thus, electrophoretically distinct forms of a protein (isozymes) could imply that they are encoded by different alleles, i.e., genetic variation.

Isozymes analysis has received more attention in recent years as the data reflect more truly the genetic variability because they are the direct product of genes (Khalil, 2013). Isozymes reflect the products of different alleles rather than different genes because the difference in electrophoretic mobility is caused by point mutation as a result of amino acid substitution (Xu, 2010).Therefore, isozyme markers can be genetically mapped onto chromosomes and then used as genetic markers to map other genes. Isozymes analysis has been used for various purposes in biological sciences, viz. to define phylogenetic relationships, to estimate genetic diversity and taxonomy, to study population genetics and

developmental biology, to characterize plant genetic resources management and plant breeding (Bretting and Widrlechner, 1995; Staub and Serquen, 1996).

Isozyme analysis has several advantages as compared not only with morphological and physiological characters, but also with other genetic markers. Isozymes are mostly co-dominant with a simple Mendelian inheritance pattern in most loci, so that the frequency of individual allele is directly counted. Moreover, isozymes can be resolved for most plant species regardless of habitat, size or longevity. The use of SDS-PAGE and isozymes are the cheapest and simplest methods that offer sufficient information and served as a starting point for DNA band studies (Khalil, 2013). Zymograms (the banding pattern of isozymes) can be readily interpreted in terms of loci and alleles, or they may require segregation analysis of progeny of known parental crosses for interpretation (Kumar et al., 2009).

Although isozymes have some advantages but these are inferior to DNA markers due to the low number of markers they generate. Additionally, because isozymes are the products of gene expression they are often affected by environmental conditions, tissue type and the developmental stage of a plant. Proteins are also subject to posttranslational modifications that may alter their electrophoretic mobility (Kumar, 1999). In addition, since not all substitutions change the net electrical charge on the molecule, approximately 30% of the actual variation due to amino acid substitutions is electrophoretically detected (Klug and Cummings, 2000).

Several investigators (Thom and Maretzki, 1970; Nagai et al., 1991; Almeida and Crocomo, 1994; D'Hont et al., 1995; Oropeza and de Garcia, 1997; Roughan et al., 1997; Barrett et al., 1999), had used isozyme electrophoresis techniques for sugarcane genetic diversity studies instead of morphological markers studies.

In sugarcane, isozymes have been used to discriminate between wild and noble canes and to show progeny-parent relationships. Nine isozymes were used to distinguish among 39 wild and noble sugarcane clones. It was possible to

separate *Erianthus* clone from *Saccharum spontaneum* and also clones from *S. robustum* and *S. officinarum* clones but later two were not differentiated from one another (Glaszmann et al., 1989).

Isozyme research with sugarcane has also revealed that most of the diversity within sugarcane varieties is related to the presence or absence of *S. spontaneum* genes (Eksomtramage et al., 1992). However, Glaszmann et al. (1989) found that the use of isozymes in sugarcane is often encompass with practical difficulties due to the high number of bands that may migrate at similar distances and the occurrence of multiple bands of unequal intensities, both of which arising due to the high ploidy of sugarcane. This is further compounded by the fact that isozyme detection is often weak and unreliable, and consequently may produce different results in different laboratories (Gallacher et al., 1995).

Twenty isozyme systems were used to evaluate 100 *Saccharum spp*. hybrid clones randomly selected from the Bureau of Sugar Experiment Station (BSES's) parental germplasm collection of Meringa. Three enzyme systems viz. alcohol dehydrogenase, peroxidase and posphoglucomutase yielded 11 reliable, interclonally variable markers. The remaining enzyme systems were invariant or could be scored reliably. Of all possible pair wise combinations, 97% were separate with an average of 3.5 band difference. Isozyme markers were not able to discriminate completely all the clones but they are reliable for checking the identity of suspected mislabeled clones (Gallacher et al., 1995).

2.6.2.3 Diversity Analysis Based on DNA /Molecular Markers

Although morphological traits can be used to identify and classify clones, most of the traits are influenced by the environment under which the clones are grown or selected. Variability caused by genotype x environment interactions and inadvertent mislabeling of clones can adversely influence data derived from phenotypic evaluation and clonal records.

With the advent of molecular markers, it is now possible to make direct inferences about genetic diversity and inter-relationships among organisms at the DNA level without the confounding effects of the environment and/or faulty pedigree records. Indeed, a vast number of molecular marker techniques such as isoenzymes (Glaszmann et al., 1989), RFLP (D'Hont et al., 1994; Jannoo et al., 1999; Coto et al., 2002), ribosomal DNA (Glaszmann et al., 1990 ; Pan et al., 2000), microsatellites (Piperidis et al., 2001; Cordeiro et al., 2003), AFLP (Besse et al., 1998; Lima et al., 2002) and molecular cytogenetics (D'Hont et al., 1996) have been instrumental in explaining genetic diversity and inter relationships among accessions in sugarcane germplasm collections. A reproducible and informative molecular marker system has application in the following areas (Cordeiro, 2001; Lee and Henry, 2001):

i) Ensuring field grown cane is true to type;

ii) Determination of genetic diversity in commercial sugarcane cultivars;

iii) Management of breeding programs through marker assisted selection;

IV) Determination of genetic diversity of parents in breeding programs (heterosis);

v) Confirmation that parents selected in breeding programs are true to type;

vi) Protection of plant breeders' right.

2.7 DNA Markers

There are three major types of DNA markers based on the methods of their detection. These are: viz.

i) Hybridization based. e.g., RFLP

ii) Polymerase Chain Reaction (PCR) based, e.g., RAPD, AFLP, SSR, ISSR,

iii) DNA sequence based, e.g. SNP

Hybridization Based Marker

In hybridization based marker system, DNA profiles are visualized by hybridizing the restriction enzyme-digested DNA, to a labeled probe, which is a DNA fragment of known origin or sequence. Restriction fragment length polymorphism (RFLP) is the most widely used hybridization-based molecular marker.

Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphisms (RFLPs) were initially used in man (Botstein et al., 1980), but were rapidly accepted as a plant DNA marker (Beckmann and Soller, 1986). RFLP's are caused by DNA rearrangements such as insertions and deletions or point mutations. They are codominant, simply inherited and naturally occurring Mendelian characters, which exhibit environmental stability and nearly unlimited availability, making them a useful tool for genome analysis (Graner et al., 1990,1991).

RFLP's are detected as differences in the lengths of homologous restriction fragments following hybridization of genomic DNA to a labeled probe. In RFLP, the genetic material itself is screened and the same RFLPs will be detected in DNA isolated from organs and tissues in the plant irrespective of the age of the particular tissue. This is a significant advantage over biochemical tests, such as isozymes, that assay gene products (Beckmann and Soller, 1986; Ainsworth and Sharp, 1989). Comprehensive studies of taxonomic variation and genetic relationships have been reported in sorghum, Solanaceae, *Oryza*, soybean, *Brassica*, maize, *Saccharum* among others.

RFLP technique involves extraction of genomic DNA followed by its digestion with specific restriction endonucleases. The restriction enzyme cuts the DNA in to fragments (Morell et al., 1995). An RFLP results when variation in restriction enzyme cleavage sites, arising due to base substitutions, insertions, deletions or translocations in the genomic DNA (Gupta et al., 2002), is detected by Southern hybridization using either a pre-existing probe for a specific gene from a closely

related species or a probe generated for a specific sequence that occurs in the region of interest (Ainsworth and Sharp, 1989; Parker et al., 1998). It is a common practice to screen a number of probes for RFLP analysis, as well as to utilize an array of restriction enzymes to determine the most suitable combination. If two individuals differ at a restriction site this will affect the length of a particular DNA fragment, homologous to the probe, bringing about a screenable polymorphism. In this way, a restriction site polymorphism at the DNA level is detected as a restriction fragment length polymorphism (Beckman and Soller, 1986; Weising et al., 1995).

In sugarcane, RFLPs have been used to show a strong molecular differentiation between *S. officinarum* and *S. spontaneum* (Lu et al., 1994b; Jannoo et al., 1999), and that the major part of the diversity among sugarcane cultivars arises solely from the *S. spontaneum* chromosomes (Lu et al., 1994a). Moreover, RFLP maps have been or are being constructed for many crop plants (Graner et al., 1991), including sugarcane (D'Hont et al., 1994; Grivet et al., 1996), to assess genetic variability, determine correlations between RFLP markers and qualitative or quantitative traits (Tang et al., 2000; Xu et al., 2002) and in some cases to maximize the benefits of marker assisted selection or elucidate phylogenetic relationships (Debener et al., 1990; Da Silva et al., 1993; Besse et al., 1997).

Despite numerous applications of RFLPs, it has few inherent weaknesses. RFLP research is most often hampered by the requirement of large quantities of DNA by the fact that Southern hybridization is time consuming and expensive and lastly by the lack of suitable probes (Ainsworth and Sharp, 1989). Consequently, it has not been widely adopted for fingerprinting purposes (D'Hont et al., 1994; Parker et al., 1998).

Intergeneric sugarcane hybrids were characterized by using RFLP, *in situ* hybridization and two enzyme systems viz. GOT and MDHB. Many *Erianthus*-specific RFLP bands were observed. The basic chromosome numbers in the two parental lines were identified trough *in situ* hybridization technique with the

18s-5.8s-25s rDNA probes. In the intergeneric hybrids, distinguishing of *S. officinarum* chromosomes from chromosomes contributed by *E. arundinaceus* was possible through *in situ* hybridization technique. These techniques showed potential in the monitoring of the Eriantus genome during introgression process (D'Hont et al., 1995).

Coto et al. (2002) investigated genetic diversity of 35 wild sugarcane germplasm of Laos using 10 RFLP probes. RFLP analysis revealed that 35 wild *Saccharum* complex comprised of three distinct groups with an independent gene pool. It was also found that a group of bands was exclusively exhibited by Laos wild sugarcane complex clones.

Burnquist et al. (1992) analyzed genetic variability of 59 sugarcane germplasm representing six species viz. *Saccharum officinarum, S. barberi, S. robustum, S. sinense, S. spontaneum* and *Erianthus arundinaceus* using RFLP marker. RFLP analysis revealed clear-cut and accurate biological clustering of the genotypes and permitted the identification of misclassified clones. Genetic distance as measured by RFLP placed *S. sinense* and *S. barberi* close to other and intermediate to *S. officinarum* while *E. arundinaceus* formed a distinct and distant group when compared to the others.

Jannoo et al. (1999) used RFLP marker to analyze molecular genetic diversity of 162 clones of sugarcane. One hundred and nine of them were modern cultivars of interspecific origin. Twelve low-copy nuclear DNA probes were used in combination with one or two restriction enzymes. A total of 386 out of 399 fragments were found polymorphic. A high number of fragments per probe/enzyme combination were exhibited for each sugarcane clone which indicated its high ploidy level. The largest variability was found among the clones from New Guinea. The clones from New Caledonia formed a separate group that could correspond to *S. officinarum* clones that were modified through introgression with other members of the *Saccharum* complex. The cultivars from Mauritius and Barbados formed two separate groups essentially due to the presence of *S. spontaneum* alleles in Mauritian cultivars and absent in Barbadian ones.

PCR Based Molecular Markers

PCR is a versatile technique invented during the mid-1980's (Saiki et al., 1985). PCR based markers involve *in vitro* amplification of particular DNA sequence or loci, (that lies between two regions of known DNA sequence) with the help of specially or arbitrarily chosen oligonucleotide sequences (primers) and a thermostable DNA polymerase enzyme. The amplified fragments are separated electrophoretically and banding patterns are detected by different methods such as staining followed by autoradiography. This technique allows for the amplification of any DNA sequence of interest to high copy number, thereby by passing the need for molecular cloning (Erlich, 1989; Weising et al., 1995). Some of the advantages of PCR based marker systems are that: (1) PCR requires only small amount of DNA, and often crude miniprep procedures yield DNA of sufficient quantity and quality; (2) PCR is relatively quick to perform and technically straight forward, once PCR conditions have been established and (3) the range of primer sequences possible gives PCR-based techniques great diagnostic power (Morell et al., 1995). Various molecular techniques have developed using PCR, namely: Random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and microsatellites or simple sequence repeats (SSRs).

Random Amplified Polymorphic DNA (RAPD)

RAPD is a PCR-based technology developed by Welsh and McClelland in 1990. This method is based on enzymatic amplification of target or random DNA segments with the help of single arbitrary primers that are nine or 10 nucleotides to generate a set of DNA fragment. The primer anneals to the genomic DNA at two different sites on complementary stands of DNA template. Amplification occurs when the same sequence complementary to the primer is present in inverse orientation within an amplifiable distance (Gupta et al., 2002). If these priming sites are within an amplifiable range of each other, a discrete DNA product is formed through thermo cyclic amplification. On an average, each

primer detects amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals (Willium et al., 1990). Amplified products (usually within the 0.5-5 kb size range) are being separated using agarose gel electrophoresis technique. The amplified products (i.e. bands) are stained with ethidium bromide and viewed under ultraviolet light (Jones et al., 1997). Agarose gels stained with ethidium bromide are easy and fast but are limited to detection of major amplification products only (Prabhu, 1997). Polymorphisms at DNA level evolved from mutations or rearrangements either at or between the primer-binding sites are visible in the electrophoresis as the presence or absence of a particular RAPD band (Jiang, 2013). RAPD is dominant marker, which is inherited in a Mendelian fashion (Welsh and McClelland, 1990; Dawson et al., 1993).

RAPD analysis technique has many advantages. This technique is very simple and easy to assay. During PCR, very low quantities of genomic/template DNA is required, about 10ng per reaction, the procedure can be automated, and higher levels of polymorphism can also be detected compared with RFLP. Neither DNA probe nor DNA sequence information is required for the design of specific primers. Moreover, no marker development is required and primers are non-species specific and can be universal. Additionally, the RAPD products can be cloned, sequenced and then converted into or used to develop other types of PCR based markers viz. simple sequence repeats(SSR), sequenced characterized amplified region (SCAR), single nucleotide polymorphism (SNP) etc., (Jiang, 2013, Senan et al., 2014). However, it has also some limitations. The major drawback of RAPD is low reproducibility (Schierwater and Ender, 1993) and incapability to detect allelic differences in heterozygotes (Jiang, 2013). RAPD analyses generally require purified, high molecular weight DNA and precautions are necessary to avoid contamination of DNA samples because short random primers are used that are able to amplify DNA fragments of other contaminating organisms. RAPD markers are not locus specific, band profile cannot be

interpreted in terms of loci and alleles (dominance of markers) and similar sized fragments may not be homologous (Jones et al., 1997; Kumar et al., 2009).

RAPD analysis has many promising applications and may be used to assess kinship relationships (Tinker et al., 1993) or genetic diversity (Dawson et al., 1993; Muyco, 2002), constructing genetic maps (Saliba-Colombani et al., 2000) or create specific probes (Xu et al., 1995). The RAPD analysis of NILs (Non-isogenic lines) has been successful in identifying markers link to disease resistance genes in tomato (Martin et al., 1991), lettuce (Paran et al., 1991 and common bean (Adam-Blondon et al., 1994).

Thirteen random amplified polymorphic DNA (RAPD) markers were used to study the genetic identities of nine nearly phenotypically identical germplasm of butterhead lettuce (*Lactuca sativa* L.). These oligonucleotide markers generated 93 polymorphic bands. Within line variation ranged from 0.0 to 12.0%. Between lines, similarity ranged from 0.919 to 0.985. The relationship between the crisphead accession and a composite of all butterhead accession was 0.84. A positive correlation was indicated between variation based morphological data and the variation detected at the DNA level. Results showed that RAPD analyses may serve as a major source of information for separation of closely related accessions, especially when integrated with phenotypic measures (Waycott and Fort, 1994).

In another study, using RAPD analysis with 28 arbitrary 10-mer primers, 21 celery (*Apium graveolens* L.var. dulce) cultivars, one celeriac (var. rapaceum) and one annual smallage (var. secalinum) cultivar were screened. A total of 309 bands were obtained, 29 (9.3%) showed polymorphism in the 23 cultivars screened. Only 19 (6.1%) markers were polymorphic within the 21 type dulce cultivars and were sufficient were 6.4 between 2 celery cultivars used. The average marker difference was 6.4 between celery cultivars, 16.7 between celery and annual smallage and celeriac. The celery cultivars surveyed were classified into 3 groups or clusters based on marker differences. Relationship among the

dulce type cultivars was basically consistent with the known lineage of the cultivars and previous study using stem protein and isozyme markers. Yang and Quiros (1993) concluded that RAPD technology provides a new alternative for cultivar identification and classification in celery.

In *Brassica oleracea* genetic similarity among 45 genotypes was compared based on two molecular markers, random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLPs). Fifty-six polymorphic RFLP bands and 181 polymorphic RAPD bands were generated using 15 random cDNA probes and 62 10-mer primers, respectively. Based on RFLP and RAPD data, rank correlation between the Nei-Li genetic similarity values for all pairs of genotypes was 0.745. RFLP and RAPD dendograms of the relationships among genotypes based on genetic similarity values were consistent with known pedigrees. However, inconsistencies between clustering based on RFLP and RAPD dendograms were observed. Results indicated that RAPD's are equivalent to RFLP's in the estimation of genetic similarity among the 45 *B. oleracea* genotypes. Moreover, because of their relative simplicity and lower cost, RAPD's are considered more practical than RFLP's for studies on germplasm organization and characterization (dos Santos et al., 1994).

Tinker et al. (1993) used RAPDs to analyze 27 inbred lines with varying amounts of common ancestry and 20 double-haploid (DH) barley lines from a biparental cross. Out of 33 arbitrary, 10-base primers tested, 19 showed a total of 31 polymorphisms which were scored as dominant genetic markers. There was 1 that indicated the presence of 2 codominant amplification products through Southern analysis. Genetic distance (d) from RAPD data indicated a linear relationship when compared to kinship coefficients (r) between the same pairs of lines. Cluster analysis showed that groups of inbred lines based on r were similar to those based on d with some notable exceptions. RAPD markers, therefore, can be used to gain information about genetic similarities or differences that are not evident from pedigree information.

Shahid et al. (2012) evaluated sugarcane parent BF-162 (susceptible to red rot) and its 15 somaclones generated through tissue culture, for studying genetic variability using 20 RAPD markers. A total of 83 DNA fragments (loci) were generated by 20 markers. Out of 83, 28 DNA fragments were found polymorphic while 55 were monomorphic indicating 33.7% polymorphism. This study has identified the usefulness of RAPD markers to find out the diversity among somaclones along with parents. Detection of somaclonal variation through RAPD marker has also been applied in sugarcane by many workers (Taylor et al., 1995; Saini et al., 2004; Devarumath et al., 2007).

Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a multiplex PCR based dominant marker developed by Zabeau and Vos in 1993. AFLP analysis combines the reliability of restriction enzyme digestion with the utility of PCR. This method is robust and relatively insensitive to reaction conditions. As a result, reproducibility is high (Jones et al., 1997; McGregor et al., 2000) and the genetic background is likely to result in artifactual polymorphisms (Williams et al., 1990; Maughan et al., 1996; Mueller and Wolfenbarger, 1999). AFLP analysis allows the reliable identification of over 50 loci in a single reaction (Zabeau and Vos, 1993; Vos et al., 1995). AFLP analysis is able to assay a large number of DNA loci, to reveal more polymorphic bands in one gel lane, than RAPDs, RFLPs or microsatellites (Cho et al., 1998; Saliba-Colombian et al., 2000). No sequence data is required for AFLP primer construction.The AFLP technique involves three steps: (1) digestion of total genomic DNA with two restriction enzymes and ligation of restriction half-site specific oligonucleotide adaptors to all restriction fragments; (2) selective amplification of only a sub-set of the restriction fragments with two PCR primers that have corresponding adaptor-and restriction-site-sequences as their target sites and (3) electrophoretic separation of the PCR products on a denaturazing polyacrylamide gel (Janssen et al., 1996; Vos et al., 1995). The selective amplification is achieved by the use of primers that extend

into the restriction fragments, amplifying only those fragments in which the primer extensions match the nucleotides flanking the restriction sites. The PCR primers consists of a core sequence (part of the adaptor), and a restrict enzyme specific sequence and 1-5 selective nucleotides. The primer pairs used for AFLP analysis usually produce 50-100 bands per assay. When the AFLP method is applied to complex genomes, like plants, two cycles of selective amplification are performed. The first step is known as pre-amplification step, where the genomic DNA is amplified with AFLP primers both having a single selective nucleotide. This pool of PCR products is then amplified with primers both having three selective nucleotides. This two step amplification process reduces the amplicons to a manageable number, with only 1 out of every 4096 possible amplicons being amplified (Vos et al., 1995). The AFLP banding profiles are the result of variations in the restriction sites or in the intervening region. The number of amplicons per AFLP assay is a function of the number of selective nucleotides in the AFLP primer combination, the selective nucleotide motif, GC content and physical genome size complexity (Agarwal et al., 2008).

Segregation analysis and linkage studies indicate that AFLP markers are inherited in a Mendelian fashion (Maughan et al., 1996) but it cannot distinguish heterozygotes and homozygotes, and as such are grouped as dominant markers (Bradshaw et al., 1998).

The basic difference between RFLP and AFLP polymorphisms is that for RFLPs, an area is scanned that is defined by the number of nucleotides in the restriction sites, whereas for the AFLP technique an additional number of nucleotides defined by the selective nucleotides is scanned. Therefore, it is expected that AFLP markers will detect more point mutations per 100 nucleotides than RFLPs, but should detect more or less the same frequency of insertions or deletions (Becker et al., 1995).

It has wide range of applications in genetic studies of many organisms at molecular level. AFLP can be applied in studies involving genetic identity,

parentage analysis and identification of clones and cultivars, and phylogenetic studies of closely related species at sub-species level (Althoff et al., 2007; Kumar et al., 2009). High genomic abundance and generally random distribution throughout the genome make AFLPs a widely valued technology for gene mapping studies (Vos et al. 1995). AFLP markers have successfully been used for analyzing genetic diversity in some plant species such as peanut (Herselman, 2003), soybean (Ude et al., 2003), maize (Lübberstedt et al., 2000) and sugarcane (Besse et al, 1998; Xu et al, 1999). AFLP markers have also been used for DNA fingerprinting (Powell et al., 1996a), the construction of linkage-maps (Becker et al., 1995; Cho et al., 1998; Hoarau et al., 2001) and to locate traits of interest or track their transmission (Gupta et al., 1999; Hartl et al., 1999). This technique is useful for breeders to accelerate plant breeding program through marker assisted selection (MAS) and positional cloning for special character.

However, although AFLP is a powerful molecular marker, some reproducibility issues have been raised in sugarcane, and it is believed that these originated from: (1) partial digestion of the template DNA as a result of insufficient enzymatic conditions or due to unexpected or inconsistent methylation of template DNA; (2) poor amplification of PCR fragments or (3) DNA contamination (Cordeiro, 2001).

2.8 Microsatellite Markers

Litt and Luty first used the term “microsatellites” in 1989 when analyzing the abundance and disruption of (TG)n in the human cardiac actin gene. Microsatellites are tandemly repeated nucleotide motifs of variable lengths that distributed throughout the eukaryotic nuclear genome in both coding and non-coding regions (Jarne and Lagoda, 1996). These nucleotide motifs are arranged in head-to-tail (Hancock, 1999) and are 1-6 bp long (Gupta et al., 1996; Thiel et al., 2003). They also found in prokaryotic and eukaryotic organellar genomes e.g., chloroplast (Powell et al., 1995) and mitochondria (Soranzo et al., 1999).

They are also designated as Simple Sequences (Tautz, 1989), Short Tandem Repeats (STRs) (Edwards et al., 1991) and Simple Sequence Repeats (SSRs) (Jacob et al., 1991). Microsatellites are otherwise called as Sequence Tagged Microsatellites (STMs).

2.8.1 Characteristics of Microsatellites

A number of important characteristics of microsatellite markers which they possess are as follows:

(1) Locus-specific and multi-allelic in nature; in contrast to multi-locus markers such as minisatellites or RAPDs, (2) Co-dominant transmission and therefore, the heterozygote can be distinguished from homozygotes, in contrast to RAPD and AFLP, which are dominant in nature, (3) Highly polymorphic and hypervariable, (4) High information content and produce considerable pattern, (5) Wide genomic distribution, (6) Higher mutation rate than standard sequences (up to 0.002 gametes/generation), and (7) High probability of back reverse mutation (Powell et al., 1996a; Mital and Dubey, 2009; Parida et al., 2009).

2.8.2 Classification of Microsatellites

Microsatellites can be classified based on size, occurrence and source of development, the nature of the repeated unit or their position within the genome. With respect to the number of nucleotides per repeat unit, microsatellites can be classified as mono-, di-, tri-, tetra-, penta- or hexa-nucleotide repeats viz., (A)n, (CA)n, (CGT)n, (CAGA)n, (AAATT)n, or (CTTTAA)n, where n= no. of variable, respectively. Based on occurrence and source for development, microsatellites can be grouped in to three categories. These are:(1) genomic or nuclear microsatellites (gSSRs)-microsatellites isolated from the nuclear genome (genomic DNA of an organism with or without the construction of genomic DNA library), (2) EST or genic microsatellites (EST-SSRs) - microsatellites developed by data-mining or exploiting EST sequences deposited in public databases, and (3) organellar microsatellites [chloroplast SSRs (cpSSRs) and mitochondrial

SSRs (mtSSRs)- microsatellites developed from the chloroplast or mitochondrial genome of an organism. On the basis of repeat sequence, Olivera et al.(2006) classified microsatellites into four groups, viz. (i) Perfect microsatellite- the repeat sequence is continuous and is not interrupted by any base not belonging to the motif, e.g. GAGAGAGAGAG or (AG)6, (ii) Imperfect microsatellite- a pair of bases is present between the repeat motif that does not match the motif sequence, e.g. AGAGAGAGAGCTAGAGAG or (AG)5CT(AG)3, (iii) Interrupted microsatellite- a small sequence within the repeated sequence that does not match the motif sequence, e.g. AGAGAGAGCGTGAGAGAGAG or (AG)4CGTG(AG)4, (iv) Compound/ composite microsatellite- two adjacent distinctive repeats present within the sequence e.g. AGAGAGAGAGTCTCTCTC or (AG)5(TC)4. Microsatellites have also been classified according to the length (bp) of repeat motif present. Two types of microsatellites viz., (1) Class I microsatellites (SSRs) containing ≥20 nucleotides in length, and (2) Class II microsatellites (SSRs) containing ≤20 nucleotides in length (Temnykh et al*.,* 2001).

2.8.3 Distribution and Frequency

Microsatellites are found in both eukaryotic and prokaryotic genomes (Field and Wills, 1998;Toth et al., 2000) but occur in a lesser extent in prokaryotic and eubacterial genomes (Tautz, 1989), at higher frequencies than would be expected purely on the basis of base composition (Hancock, 1999). Microsatellites are distributed throughout the eukaryotic nuclear genome in both coding and non-coding regions (Jarne and Lagoda, 1996). Recently, many reports have showed that a large number of SSRs located in transcribed regions, including protein-coding genes and expressed sequence tags (ESTs) (Morgante et al., 2002), in general, repeat numbers and total lengths of SSRs in these regions are relatively small. In cereals (maize, wheat, barley, sorghum, and rice) 1.5%–7.5% of ESTs consist of SSRs (Kantety et al. 2002; Thiel et al., 2003).

The microsatellite frequency was higher in transcribed regions, especially in the untranslated portions, than in genomic DNA. After completion of initial draft

sequence of human genome analysis, it was estimated that microsatellites account for 3% of the genome. The human genome is estimated to contain on average 10 fold more microsatellites than plant genomes (Powell et al., 1996) or at least one simple sequence stretch every 10kb of DNA sequence (Tautz, 1989). There are more than one million microsatellite loci in the human genome, although the exact number greatly depends on the parameters of the search algorithm (for example, gap and mismatch penalties). This number also includes an appreciable proportion of interrupted microsatellites and many that are probably monomorphic. Dinucleotide repeats dominate, followed by mono- and tetranucleotide repeats, and trinucleotide repeats are least dominant. Again, however, it is a matter of how microsatellites are defined. Among dinucleotides, (CA)n repeats are most frequent, followed by (AT)n, (GA)n and (GC)n, the last type of repeat being rare. Note that there are only four possible types of dinucleotide repeat, because CA = AC = GT = TG, GA = AG = CT = TC, AT = TA, and GC = CG (Ellegren, 2004).

Frequency of microsatellites is correlated with the genome size. Microsatellite density tends to positively correlate with genome size (Hancock, 1996; Toth et al., 2000; Katti et al., 2001). Among fully sequenced eukaryotic genomes, microsatellite density is highest in mammals while microsatellite frequency is negatively correlated with genome size in plants (Morgante et al., 2002). This has been attributed to the fact that microsatellites are underrepresented in the repetitive parts of the plant genome that are involved in genome expansion, such as the long terminal repeats of retrotransposons (Morgante et al, 2002). Another peculiar feature of most plant genomes is that (AT)n is the most common motif among dinucleotides (Lagercrantz et al., 1993).

In the last few years, surveys of DNA sequence databases have revealed an abundance of SSR loci in plants, and subsequent studies have demonstrated the informativeness of these markers in several genera (Liu et al., 1995). Database searches indicate that (AT)n, (A)n, (GA)n, (TAT)n and (CA)n repeats are the most frequently occurring SSRs among the plant species examined (Lagercrantz

et aI., 1993; Morgante and Olivieri, 1993; Wang et al., 1994),and that tetranucleotide repeats are rarer than trinucleotide, which are in turn rarer than dinucleotide repeats (Hokanson et al., 1998).The frequencies of the (GA)n and (CA)n repeats based on DNA library screening have been reported for several plant genomes, and there is one (GA)n repeat every 125-250kb and one (CA)n repeat every 250-480kb in *Arabidopsis thaliana* (Bell and Ecker, 1994); *Brassica napus* (Lagercrantz et al., 1993); rice, *Oryza sativa* (Wu and Tanksley, 1993) and seashore paspalum, *Paspalum vaginaturn* Swartz (Liu et al., 1995). In the barley *(Hordeum vulgare)* genome it is estimated that a (GA)n will be present every 330kb and one (CA)n repeat every 620kb, which means that there are a total of 1.5 x 104 (GA)n and 7.9 x 10^3 (CA)n repeats in the genome (Liu et al., 1996). While in wheat *(Triticum aestivum)* these repeats are observed every 440 kb and 704 kb, respectively (Roder et al., 1995). The most frequent trinucleotide and tetranucleotide repeats found in plant genomes are (AAT)n, (AAC)n, (AGC)n, (AAG)n, (AATT)n and (AAAT)n (Wang et al., 1994; Gupta et aI., 1996). Initial studies utilizing fluorescent *in situ* hybridization (FISH) (Schmidt and Heslop-Harrison, 1996) and Southern hybridization (Broun and Tanksley, 1996) showed a clustering of microsatellites around the centromere of chromosomes. More recently, in contrast to these earlier reports, genetic and physical mapping have shown that microsatellites are not clustered in specific regions but rather are uniformly distributed in different regions (Panaud et al., 1996; Senior et al., 1996; McCouch et al., 1997; Gianfranceschi et al., 1998; Roder et al., 1998a, 1998b, Cregan et al., 1999). However, although mapping suggests a more or less even (i.e. random) distribution of microsatellites at the gross level, even the highest resolution maps contain some long gaps and low-density regions, many near telomeres (Wu and Tanksley, 1993; Dib et al., 1996; Dietrich et al., 1996).

Microsatellites may be found within expressed regions of the genome, although this is a rare event particularly for microsatellites not based on repeat units of three or more nucleotides, such as (CA)n, as these can give rise to frameshifts if they mutate, a situation seen in some genetic diseases (Weber, 1990; Bruland et

al., 1999). Furthermore the detection of a size ceiling on allele size among microsatellites in exons, suggests that these loci are under selective pressure. As a result microsatellites might be excluded from the immediate vicinity of coding regions, as well as from the coding regions themselves (Broun and Tanksley, 1996). Whether size limitation also applies to loci within introns is unclear; however, they may be more prone than loci located outside genes to selective influence acting on nearby exons through background selection (Charlesworth et al., 1993).

2.8.4 Mutational Mechanism of SSR Variation

Microsatellites are subject to mutations during evolution but molecular mutational processes of simple sequence repeats (SSRs) in complex genomes are poorly understood. However, few mechanisms have been described by several investigators. In general, microsatellites have high mutation rate (10^{-2} to 10^{-6} events per locus per generation) as compared to point mutations in coding gene loci (Li et al., 2002). Microsatellite mutation rates in *in vitro* systems are estimated around 10^{-2} events per locus per replication in *E.coli* (Levinson and Gutman, 1987a) and 10^{-4} to 10^{-5} in yeast (Henderdon and Petes, 1992; Strand et al., 1993), which is high compared to rates of point mutation that are of the order of 10^{-9}- 10^{-10} (Hancock, 1999). Estimates from pedigree analysis in humans suggest a microsatellite mutation rate of around 10^{-3} events per locus per generation (Weber and Wong, 1993). Many factors could be important for the mutational processes in microsatellites such as allele size, motif size, gender, and G/C content (Chakraborty et al., 1997; Anderson et al., 2000; Brohede et al., 2004; Whittaker et al., 2003). Mutation pattern may also depends on the genomic context such as the particular location on the chromosome and functional potential of the transcribed products (Chakraborty et al., 1997; Schlotterer et al., 1998; Tomiuk et al., 2006; Hawk et al., 2005) as well as the effectiveness of mismatch repair enzymes (Modrich and Lahue, 1996; Harr et al., 2002). Li et al.

(2002) indicated that the microsatellites instability is predominantly displayed as changes in the number of SSR repeats.

There are two proposed mechanisms to explain these high rates of mutation. The first involves only a single DNA double helix and slipped strand mispairing (slippage) during DNA replication (Levinson and Gutman, 1987b; Tachida and Iizuka, 1992), the second involves recombination between DNA strands (Jeffreys et al., 1994; Harding et al., 1992). Slippage during replication can take place when the nascent DNA strand dissociates from the template strand. When non-repetitive sequences are being replicated this does not pose a problem because there is only one way in which the nascent strand can re-anneal precisely to the template strand before replication is recommenced. If the replicated sequence, however, is repetitive in nature the nascent strand may re-anneal out-of-phase with the template strand. When replication is continued after such a mis-annealing, the eventual nascent strand will be longer or shorter than the template, depending on whether the mis-annealing gives rise to looped-out bases in the template strand, in which case the product will be shorter; or the nascent strand, in which case it will be longer (Levinson and Gutman, 1987a; Hancock, 1999).

Recombination could potentially alter the lengths of microsatellites in two ways, by unequal crossing-over or by gene conversion. Unequal crossing-over involves crossing over between chromosome strands (DNA molecules) that are misaligned, giving rise to a deletion in one DNA molecule and insertion in another and can occur both between chromatids in the same chromosome or between chromosomes (Smith, 1976). This occurs most easily for long, tandemly repeated sequences where the recombination machinery cannot easily determine the correct register between the two strands. Gene conversion involves unidirectional transfer of information by recombination, probably as a response to DNA damage, and can transfer sequences in an out-of-phase manner from one allele to another. This has been suggested to generate diversity at minisatellite loci (Jeffreys et al., 1994), which are tandemly repeated arrays of basic motifs longer

than those found in microsatellites (Hancock, 1999). Nonetheless, slippage is the commonly accepted mutation model for microsatellites and evidence for the primary role of replication slippage in the generation of length mutation in microsatellites comes from genetic analyses of the process in yeast and *E.coli.* In both systems, length instability of tandem repeats is unaffected by mutants with greatly decreased recombination frequencies (Henderson and Petes, 1992). Furthermore length mutations in microsatellites represent gains or losses of single repeat units, while recombination based mutation would be expected to give rise to a wider range of novel mutants (Hancock, 1999). Microsatellites exhibit high mutation rates even in species otherwise characterized by low levels of genetic diversity, and consequently they are useful for many applications from plant varietal identification to population studies because a single locus with numerous alleles can be examined (Saghai-Maroof et al., 1994). Informative microsatellite variability has been found in insect species with little or no allozyme variability (Gupta et al., 1994), and the utility of SSR loci is also apparent in self-breeding plants. In highly inbred soybean cultivars *(Glycine max.),* Rongwen et al., (1995) reported 11 to 26 alleles per locus and an average heterozygosity of 0.87 at seven SSR loci, substantially exceeding that obtained with allozyme and RFLP markers.

2.8.5 Significance of Microsatellites

In earlier times, microsatellites were considered as “junk DNA” which is generally found on non-coding region and the variation is mostly neutral. In humans, 90% of known microsatellites are found in non-coding regions of the genome (Pokhriyal et al., 2012). Recent investigations indicated that microsatellites can be present in both non-coding and coding regions of the genome (Field and Wills, 1998; Toth et al., 2000). The significance of microsatellites is described below:

(1) Genotype of an organism/individual is decided by microsatellites. They do not have measurable effect on phenotype, and after mutation, may cause a change in the genotype of an individual.

(2) Microsatellites provide a necessary source of genetic variation. The variation in microsatellite alleles in coding regions is thought to be the cause of adaptation in different environments. For example, a short allele may be adaptive in one environment, and a long allele with many repeats may be adaptive in different environments.

(3) Variation in the coding regions may also cause disease in humans. Microsatellites may act as a marker for some genetic diseases of human i.e., they serve a role in biomedical diagnosis as markers for certain disease conditions viz. cancer, Huntington's diseases, myotonic dystrophy, Fragile X syndrome etc. (Pokhriyal et al., 2012).

(4) Microsatellites may help regulate gene expression and protein function. Kashi and Soller (1999) indicated that microsatellites may have regulatory roles in gene expression. Variation in microsatellite alleles have been shown to be associated with quantitative variation in protein function and gene activity. The presence of SSRs in the coding regions lead to the appearance of repetitive patterns in the amino acid sequences (Katti et al*.,* 2001) and thus involve in regulating gene expression or molecular functions.

(5) Presence of SSRs in the promoter region influences transcriptional activity (Kashi et al*.,* 1997), whereas their presence in non-coding regions influences gene regulation, transcription (Martin et al*.,* 2004; Lawson and Zhang, 2006) and recombination events (Bagshaw et al*.,* 2008).

(6) Microsatellites present on flanking and/ or promoter regions can regulate gene expression. For example, over-representation of CT/GA and CTT/GAA repeats in the 5'-flanks of *Arabidopsis thaliana* suggest their potential involvement in regulating gene expression (Zhang et al*.,* 2004). The (GA)n repeats in promoters govern the regulation of certain plant genes (Meister et al*.,* 2004) and exhibit protein-binding affinity (Kooiker et al*.,* 2005).

(7) The CT/GA repeat variation in the 5′ UTR of the *waxy* gene is correlated with amylase content in rice (Bao et al*.,* 2002). In maize, presence of (CCG)n in the 5' UTR of ribosomal protein genes regulate fertilization (Dresselhaus et al*.,* 1999). Polystretches of glutamine (Gerber et al*.,* 1994) and proline (Perutz et al*.,* 1994) encoded by rapidly evolving repeats are known to modulate the activity of transcription factors. Similarly, the presence of the trinucleotide repeats like (GAA)n within 5'UTR of *ntp303* regulate transcription and translation (Hulzink et al*.,* 2002).

2.8.6 Signature Tagged Microsatellite Site (STMS) Amplification

A number of strategies (both hybridization based and PCR based) have been designed to exploit microsatellite sequences for the study of plant genomes (Joshi, 1999; Gupta and Varshney, 2000). Signature Tagged Microsatellite Site (STMS) amplification is the most widely used method utilizing microsatellite DNA or simple sequence repeats. It involves the amplification of a SSR by designing primers that flank and hence define the microsatellite site, revealing variation in the length of the repeat motifs between individuals, following electrophoresis through an acrylamide or agarose gel (Parker et al., 1998). This method is referred to commonly, though incorrectly, as microsatellites or simple sequence repeats. Due to their ubiquity, PCR typability, Mendelian co-dominant inheritance, and extreme polymorphism, microsatellites or STMS markers have assumed an increasingly important role as markers in genome analysis (Koreth et al., 1996).

2.8.7 Microsatellite Marker Development

The sequences flanking microsatellite loci in a genome are believed to be conserved within a particular species, across species within a genus and rarely even across related genera (Gupta et al., 2002). In order to generate a new set of polymorphic SSR marker for species, microsatellite repeats must be isolated or identified along with sufficient flanking nucleotide sequence information to expedite primer designing. The resultant SSRs usually identifies a single locus

that, because of the high mutation rate of SSRs, is often multi-allelic (Jones et al., 1997; McCouch et al., 1997; Tautz, 1989). However, the main limitation of microsatellites is that they need to be isolated *de novo* from species being examined for the first time (Zane et al., 2002). The PCR conditions need to be optimized and the primers need to be screened in a set of related and unrelated individuals for estimation of their polymorphic potential.

The various methods used for the effective isolation of SSR loci were reviewed earlier by Zane et al. (2002) and Kalia et al. (2011). However, with the advancement in genomics, molecular tools, bioinformatics and sequencing platforms for exploring genomic information, several new protocols have been developed after first discovery of microsatellite isolation. Recently, Senan et al. (2014) have also reviewed several strategies of microsatellite isolation/development, sum up those methods and outlined a comprehensive methodology of microsatellite development.

Exploring public sequence databases such as Gene bank or the European Molecular Biology Laboratory (EMBL) is the easiest and simplest way of finding SSRs. This is the least costly in terms of time and resources (Brown et al., 1996). In species where databases of expressed sequence tag (EST)/ cpSSRs data have been compiled, identification of microsatellites is also possible. EST SSRs markers are being developed using this method. The advantage of using SSRs present in EST sequences is that genes of known function can be mapped (Holton, 2001). However SSRs derived from ESTs are generally less polymorphic (Da Silva, 2001) than those from other approaches and of the 8678 sugarcane sequences scanned by Cordeiro et al. (2001) only approximately 250 (2.9%) revealed microsatellites.

Alternatively, SSR primers designed for closely related species to the particular species in question can be used to obtain polymorphic bands. This is called cross-genetic amplification or cross-species amplification/transferability. The taxonomic distance of the species of interest and the conservation of the

flanking sequences, determines whether the microsatellite sequence is amplified and the level of variation observed. Often the reactions need to be optimized and the products sequenced to verify the presence of microsatellite regions (Maguire, 2001).

The percentage of cross genetic amplification is zero for *Paspalum* SSR primers used on *Sorghum,* 18% for *Zea* SSR primers used on *Sorghum* and 22% for *Picea* SSR primers used to amplify regions on the *Pinus* genome (Brown et al., 1996; Peakall, 1997). The screening of public libraries and the use of SSR primers of related species are the least costly methods in terms of time and resources and are, therefore, useful starting points in the search for SSR primers, considering that the SSRs of many species have already been characterized, including maize *(Zea mays)* (Senior and Heun, 1993) and soybean *(Glycine max)* (Akkaya et al., 1992).

A third approach involves constructing and screening SSR enriched/non-enriched genomic libraries or by utilizing the products generated by other molecular markers(e.g. RAPD, ISSR, SSR/AFLP) or by application of next generation sequencing systems. Genomic SSR markers can be developed using this approach (Senan et al., 2014).

Genomic SSR Markers Development

Genomic SSRs can be isolated in two ways, viz. (i) development of microsatellite markers from SSR-enriched genomic libraries, and (ii) development of microsatellite markers from non-enriched genomic libraries.

Microsatellite Development from SSR- enriched Genomic DNA Libraries

Enrichment by hybridization is the most popular approach for the isolation of microsatellites. There are several advantages to this method: (1) it is applicable to many plant species, (2) it is quick and relatively inexpensive, and (3) it results in the production of a large number of clones containing many different

microsatellite repeats, thus eliminating the need for further library construction with different microsatellite oligonucleotides (Maguire, 2001). The isolation of SSRs by constructing genomic libraries can be categorized in to two types, e.g.

(i) Selective hybridization methods

These methods assist in selective isolation of microsatellite containing DNA portions of the genome by hybridization with repeat-specific probes. Generally, microsatellite loci have partial genomic library of the target species. High quality genomic DNA is fragmented either using restriction enzymes (Brown et al., 1995; Glenn and Schable, 2005)) or less commonly by sonication (Geng et al., 2010) or by nebulisation (Kumpatla et al., 2004). In the first case, the choice of restriction enzyme depends on the desired average length of DNA fragments, the microsatellite repeat to be found, and the type of ends (cohesive or blunt) of the restriction fragments. Fragmented DNA is then size-selected to preferentially obtain small fragments (300-700bp). DNA fragments obtained from above methods are the ligated in to a common plasmid vector either directly or after ligation to specific adaptors/linkers. This step is most critical, due to the risk of obtaining low numbers of recombinants and the formation of concatamers between genomic fragments. Transformation of bacterial cells with ligation product generally yields thousands of recombinant clones that can be subsequently screened for the presence of microsatellite sequences. Screening for positive clones is generally carried out by means of Southern hybridization using repeat-containing probes, after blotting bacterial colonies on to nylon membranes. Colony transfer can be carried out either by classical replica plating or by picking single colonies and ordering them in new arrayed plates. While the later method is more time consuming and limits the total number of screened clones, it avoids the requirement of reprobing positive clones for confirmation. Repeat-containing probes can be synthesized de novo, alternatively a genomic clone, which contains a microsatellite locus that has already been isolated, can be used.

DNA is then denatured and subjected to enrichment by hybridization. Hybridization probe(s) can be labeled by both radioactive (^{32}P,^{33}P) or non-radioactive (digoxigenin) methods. Enrichment by hybridization can be done by any one of the following methods:

(a) hybridization with biotinylated oligos followed by capture of biotinylated hybrids (oligo bound DNA fragments) in vectrex-avidin matrix (Kandpal et al., 1994) or

(b) oligonucleotides bound to nylon membrane (Karagyozov et al., 1993; Edwards et al*.,* 1996) or

(c) 5′ biotinylated repeat oligos and subsequent capture of biotinylated hybrids by streptavidincoated magnetic beads (Brown et al., 1995; Refseth et al*.,* 1997; Connell et al*.,* 1998; Kumpatla et al*.,* 2004; Dixit et al*.,* 2005; Glenn and Schable, 2005; Geng et al*.,* 2010) or

(d) 'biotinylated SSR probe-streptavidin coated magnetic bead complex' ('Triplex Affinity capture' protocol (White and Powell, 1997).

The enriched DNA fragments were then amplified, either cloned and sequenced or sequenced directly and searched for the presence of SSR motifs. The efficiency of this approach entirely depends on the specific binding of streptavidin coated beads to the biotin labeled DNA fragments harboring SSRs.

ii) Primer extension methods

This enrichment procedure usually involves PCR amplification of inserts before ligation in to vector. In this method, selective amplification of microsatellite containing genomic DNA is carried out using SSR specific primers (Ostrander et al., 1992; Paetkau, 1999). Initial library construction is done as in traditional (non-enrichment) protocols and the enrichment is involves selection of clones rather than selection of genomic DNA fragments before ligation. In the first step, 5'-biotinylated microsatellite primer is annealed sequentially to microsatellite containing clones and extended by Klenow polymerase. After completion of first

strand extension, the 5'-biotinylated clones are selected using streptavidin coated magnetic beads. The single stranded (ss) DNA is then eluted from the bound molecules, and the extension reaction is repeated to convert double stranded (ds) DNA. Linear molecules attached to the magnetic beads are discarded. A second round of strand extension enhances transformation efficiency for microsatellite containing clones (Paetkau, 1999). The primer extension steps that selectively generate double stranded products only from vectors containing the desired repeats are transformed in to E. coli cells (Ostrander et al., 1992.

This protocol takes less time than is normally required for a single round of filter hybridization (Paetkau, 1999). Moreover, it is relatively simple, reproducible and cost effective approach for isolating microsatellites from diverse plant species with higher efficiency (Kalia et al., 2011).

Development of microsatellite markers from non-enriched genomic DNA libraries

The generation of non-enriched genomic library protocol involves genomic DNA digestion by restriction enzymes or sonication, ligation of DNA fragments into a suitable plasmid vectors and transformation in to *E. coli*. Clones are then spotted onto gridded nylon filters and screened with radiolabelled SSR probes or subjected to enrichment with 'biotin labeled probes-streptavidin capture system', positive clones are identified and sequenced. Cloning of DNA fragments prior to enrichment steps makes it ideal to screen for a wide range of SSR motifs and reduce/avoid redundancy when compared to enrichment protocols. This method was successfully employed for the isolation of SSR markers from common bean (*Phaseolus vulgaris* L.) (Blair et al*.,* 2009).

STMS Methodology

In STMS amplification variation in the number of tandem repeats at a microsatellite site, which are primarily due to slippage-based mutations, gives rise to simple sequence length polymorphisms (SSLPs) (Brown et al., 1996). Such

variations in tandem repeat number accumulate in populations more rapidly than point mutations, insertions or deletion events, which are events responsible for RFLPs (McCouch et al., 1997). These differences in length of PCR products or SSLPs are resolved using either agarose, polyacrylamide gel electrophoresis (PAGE), denaturing PAGE or capillary electrophoresis (Jones et al., 1997). The allele size differences are difficult to resolve on agarose gels with ethidium bromide staining (Becker and Heun, 1995; Holton, 2001), but high resolutions can be achieved through the use of polyacrylamide gels in combination with either ethidium bromide staining, silver staining (Scrimshaw, 1992), radiolabelling or fluorescence labeling (Holton, 2001). Although both denaturing and non-denaturing PAGE have been used to resolve small size differences between alleles (Lagoda et al., 1998), single nucleotide resolution of DNA fragments requires the use of denaturing PAGE or capillary electrophoresis (Holton, 2001).

The use of fluorescent primers in combination with a semi-automated DNA sequence has been shown to be a very promising alternative (Ziegle et al., 1992; Schwengel et al., 1994), and has greatly increased the throughput of microsatellite based systems used to assay variation in humans (Levitt et al., 1994); soybean, *Glycine max* (Diwan and Cregan, 1997); *Brassica* (Mitchell et al., 1997) and tomato, *Lycopersicon esculentum* (Bredemeijer et al., 1998).

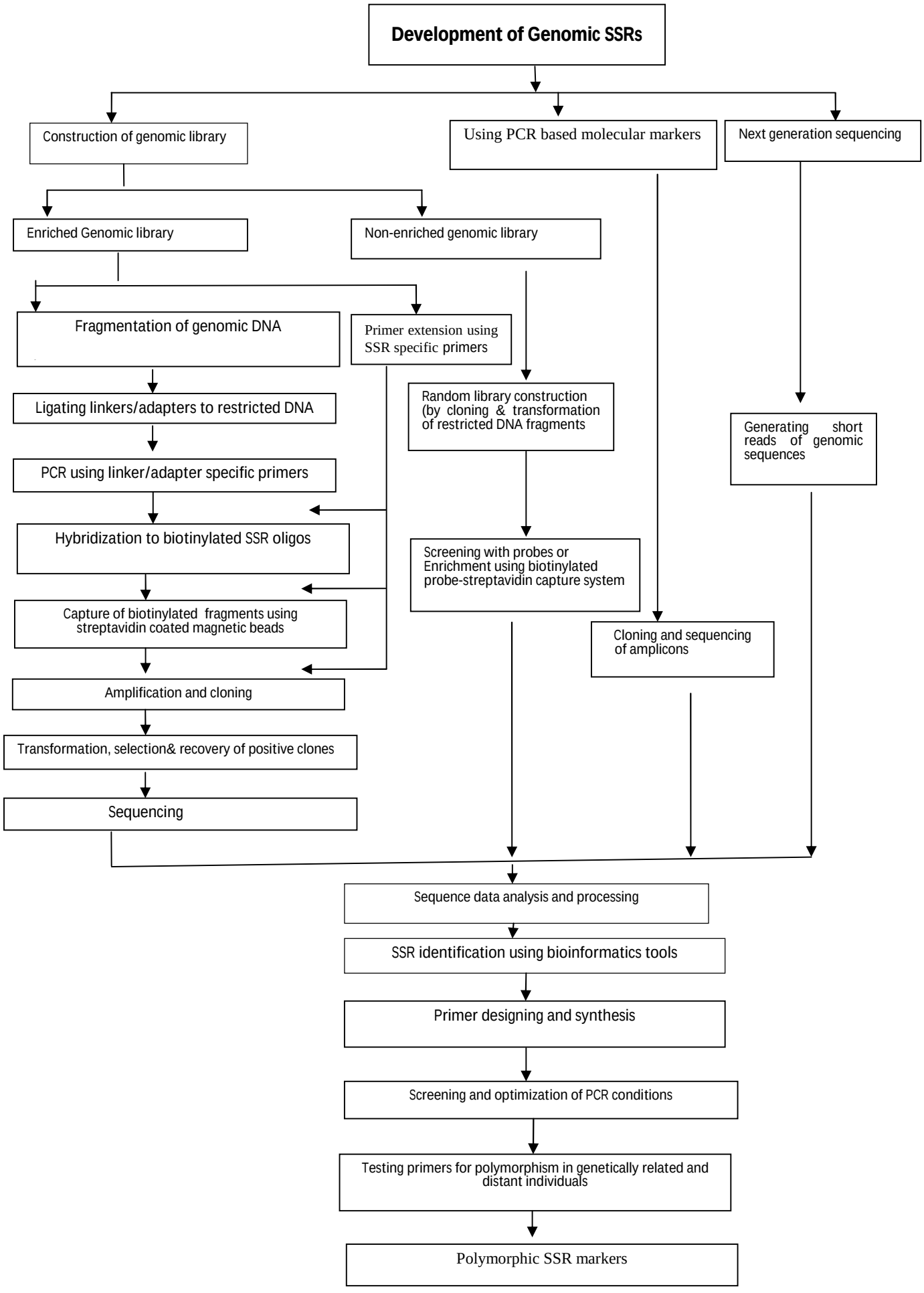

Figure 2.3 Schematic representation of genomic SSRs development (After Senan et al., 2014)

However, certain groups have raised doubts as to the accuracy of the internal size standard based sizing in automated electrophoresis systems (Schwengel et al., 1994; Delmotte et al., 2001). Yang et al., (1994) referred to the sequence amplified by each microsatellite primer pair as a particular locus, and any variants thereof (these will be detected by a difference in length) as an allele of the particular locus under consideration. In polyploids, such as sugarcane, a specific banding pattern is generated per microsatellite primer pair/template combination with the origin of each band being unknown. The bands visualized may be PCR amplicons from the same microsatellite locus, multiple microsatellite loci or perhaps even nonspecific PCR products, and as a result these bands are not referred to as alleles (Kaye et al., 1999).

2.8.8 Applications of Microsatellite Marker Systems

In plants STMS amplification is particularly attractive as a molecular marker system and its development is accelerating. In fact, it provides a higher incidence of detectable polymorphisms in relatively unpolymorphic species, such as wheat *(Triticum aestivum)* (Roder et al., 1995) and barley *(Hordeum vulgare)* (Liu et al., 1996), than RFLPs and is more reproducible than RAPDs (Powell et al., 1996b). There are numerous applications available to utilize the polymorphism detected by microsatellites, but only the most significant applications are explored below.

Fingerprinting and Genotyping

In plant species morphological or phenotypic characteristics have long been used to classify or distinguish plant genotypes; however their screening is subjective and often influenced by the environment (Russell et al., 1997; McGregor et al., 2000). Furthermore, examination of morphological characters is labour intensive; for example, over 80 separate morphological markers are examined for a barley *(Hordeum vulgare)* genotype (Cooke, 1984), while at present 52 phenotypic characters have been suggested by the Union for the Protection of New Varieties (UPOV) (http://www.upov.int) for the establishment of Plant Breeders' Rights in

sugarcane. Moreover, with an ever-increasing number of cultivars and the finite number of morphological characters, it has become apparent that such traits will not suffice to establish uniqueness in the future (Rongwen et al., 1995).

DNA markers offer a superior approach for varietal identification revealing genotypic rather than phenotypic polymorphisms, with STMS amplification approach, detecting a large number of alleles accurately and repeatedly. This means that microsatellite data from a number of loci has the potential to provide unique allelic profiles that can be used in fingerprinting and varietal identification (Cordeiro et al., 2000).

In the STMS approach, microsatellites, have been used in many different plant species for varietal identification, such as barley (*Hordeum vulgare*) (Russell et al., 1997b), wheat (*Triticum aestivum*) (Donini et al., 1998; Gupta et al., 1999), rice (*Oryza sativa*) (Garland et al., 1999), grapevine (*Vitis vinifera*)(Thomas and Scott, 1993). Bredemeijer et al. (1998) found that four microsatellites were sufficient to differentiate between all 16 cultivars of tomatoes (*Lycopersicon esculentum*) investigated; while Rongwen et al. (1995) used seven microsatellites to discriminate between 94 diverse soybean (*Glycine max*) genotypes and McGregor et al, (2000) was able to use two microsatellites to yield unique profiles for 20 potato (*Solanum tuberosum)* cultivars. In sugarcane, Piperidis et al. (2001) have demonstrated that by using only five microsatellite primer pairs in the STMS approach, 40 Australian varieties could be resolved.

Verification of Pedigree

In crop plants, the utilization of germplasm in the process of developing new breeding lines or cultivars is complicated, requiring generally a number of cycles (one cycle in sugarcane) of crossing and selection. This provides the opportunity for human error and incorrect record keeping, which could potentially result in a recorded pedigree being incorrect (Warburton and Hoisington, 2001). Molecular markers, such as microsatellites, provide a means of verifying pedigrees of

valuable germplasm. The term ‘parentage’ analysis refers to the process whereby the identity of both parents and the seed parent is revealed using the genotype of the progeny, the genotype of the seed parent (if known), and the genotypes of all potential parents at a defined set of gene loci (Gillet, 1999). This approach has been used in humans (Jeffreys and Pena, 1993), chimpanzees (Morin et al., 1994) and even in plant species such as sweet potato, which is a polyploid species (Buteler et al., 2002).

Microsatellites can also be used to screen the potential progeny of a cross to ensure that all are legitimate. Jannoo et al. (2001) used one microsatellite to screen 186 sugarcane progeny and successfully detected the presence of 16 illegitimate clones.

Gene Tagging and Marker-Assisted Selection

Plant improvement either by natural selection or through the efforts of breeders, has always relied upon creating, evaluating and selecting the right combination of alleles. However, various obstacles hinder conventional plant breeding during selection of desirable plants from a segregating population. Such as having to screen a large segregating population for a desirable trait e.g., disease resistance and the associated difficulty in screening the population for a desired trait, when the environment influences the trait in view of these difficulties the concept of indirect marker aided selection at the seedling stage in early generations is very appealing. The availability of tightly linked molecular marker for a trait will facilitate plant breeding by saving time and expense, although, in many cases the occurrence of linkage disequilibrium will make gene tagging difficult (Gupta and Varshney, 2000).

A large number of monogenic and polygenic loci for various traits have been identified in a number of plants, which are currently being exploited in marker-assisted selection (McCough et al., 1997). A number of genes for disease resistance have already been tagged in wheat (*Triticum aestivum*) (Fahima et al.,

1998; Korzun et al., 1998), and rice (*Oryza sativa*) using microsatellite markers. In soybean (*Glycine max*), an $(AT)_{15}$ repeat was located within a soybean heat shock protein gene, which is about 0.5cM from (RsV), a gene conferring resistance to soybean mosaic virus. Furthermore, several other resistance genes including peanut mottle virus (Rpv), phytophthora (Rps3) and Javanese root knot nematodes are clustered in this region of the soybean (*Glycine max*) genome (Joshi et al., 1999).

Study of Genetic Diversity

Microsatellites have been used for investigating genetic diversity of sugarcane in Mauritius Sugar Research Institute in early 2000s. Eighty six microsatellite primers were screened for polymorphic patterns. Eighty primers were highly polymorphic with *S. spontaneum* clones compared to commercial cultivars. Twenty-eight primers exhibited discrete and easily scorable bands. Further, five primers were evaluated to determine the diversity of 96 cultivars. A total of 57 polymorphic bands out of 61 were identified with an average of 5 bands per primer individual. A single primer was able to generate up to 20 different bands. Results also indicated that one primer discriminated 88 cultivars and a minimum of two primers was sufficient to give the same results as the five primers (Jannoo et al., 2000).

At SASEX, South Africa, 35 microsatellite primer pairs were tested on two closely related varieties N18 and NCo 376, *S. officinarum* variety Black Cheribon and *S. spontaneum* variety Kloet. N18 and NCo 376 showed similar profiles while the two ancestral varieties were polymorphic for all the primer pairs tested. The total number of alleles per marker across the four varieties ranged from 1 to 18. Results from evaluation of these primer pairs on additional 18 varieties suggested that microsatellite markers would be useful in sugarcane varietal identification, mapping and pedigree control (Bester, 2000).

The world first SSR marker-based sugarcane (*Saccharum* spp.) database for molecular identity was reported in 2010. A total of 1025 sugarcane clones consisting of 811 Louisiana, 45 Florida, 39 Texas, 130 foreign, and eight consultant/seed company clones were genotyped using 21 highly polymorphic SSR markers. These markers generated 144 distinct DNA fragments. The molecular data base developed by these SSR markers useful for registration of cultivars, identification of mis-labeled sugarcane clones in the crossing program, determination of paternity of cross progeny and cultivar identification/verification grown in the farmers field (Pan, 2010).

A total of 26 microsatellite primers pairs were used to determine the genetic diversity of 40 sugarcane genotypes including their parents. Out of 26 SSR markers, only 10 (38.4%) displayed polymorphism with polymorphism information content (PIC) values ranged from 0.15 to 0.67. The observed homozygosity (Ho) and Nei's gene diversity for individual loci ranged from 0.000 to 0.277 and 0.129 to 0.473, respectively. The UPGMA clustering method based on Nei's (1978), unbiased genetic distance classified all sugarcane genotypes into two major groups (I and II) comprising six clusters. The results from this investigation indicated that microsatellite markers would be useful to select the parents in sugarcane breeding program (Sharma et al., 2014).

Genetic diversity of 115 sugarcane parents of Chinese Breeding Program was evaluated using five genomic simple sequence repeats (gSSR) markers. A total of 88 alleles of loci were detected by capillary electrophoresis. These SSR markers were found to be highly robust and showed high PIC value (0.84 on average). The values of genetic diversity parameters across the population indicated much higher intra-population variation (90.5%) than that of inter-population (9.5%). Cluster analysis revealed that 115 parents were grouped in to three distinct clusters. Principal component analysis (PCA) indicated that the first and second principal components accounted for a cumulative 76% o the total variation in which 43% were for common parents and 33% were for new parents, respectively (You et al., 2013).

Chapter III

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Genetic Diversity Analysis Based on Morphological Characters of Sugarcane

The experiment on the study of genetic diversity of sugarcane genotypes based on morphological characters was carried out in the experiment field of Breeding Division, Bangladesh Sugarcrop Research Institute (BSRI) Ishurdi, Pabna, Bangladesh during 2010-2011.

3.1.1 Experimental Material

The experimental material for the present study consisted of 51 genotypes of sugarcane involving 36 BSRI developed clones, 12 exotic clones and 3 released varieties. The list of all genotypes and their parentage are given in Table 3.1.

3.1.2 Experimental Design

An augmented block design (Federer, 1956; Petersen, 1985) was used to conduct the experiment. A total of 51 genotypes of sugarcane were planted in an augmented block design II with six blocks in November 28, 2010. Three check varieties viz. Isd 38, Isd 39 and Isd 40 were randomly repeated in each block. Each block is comprised of tested genotypes and three checks. This design is most appropriate for preliminary evaluation of large number of germplasm accessions in the same experiment where amount of planting materials (seeds, setts, bulbs, tubers etc.) are not sufficient for replicated trial. Augmented design incorporates the provision of accommodating single replication of all treatments by spreading it over all blocks (b), while a set of checks (c), numbering three or more are replicated in each block. Error degree of freedom {(b-1)(c-1)} must be at least 10 for a valid augmented design (Petersen, 1985) i.e. if three checks are included, then the number of block would be at least six to fulfill the requirement

of 10 d.f. Randomization was done in such a way that all the checks and a part of test genotypes fall only once in each block. Equal number of test genotypes was planted in each block to facilitate statistical analysis.

Each plot consisted of 2 rows 5m long. Distance between rows was 1.0 m. Three budded setts were planted end to end method with a plant population of 3 setts per m^2 or 15 setts per row.

3.1.3 Cultural Management

The experimental field was ploughed and harrowed thrice to make the soil friable. Chemical fertilizer viz. NPKS and Zn was applied @ of 150-50-90-34-3.5 kg/ha. Full amount of TSP, gypsum, and zinc sulphate, one third of urea and MP fertilizers were applied as basal dose in the trenches and mixed thoroughly with the soil before planting. Rest amount of urea and MP were top dressed in two equal splits at 120 days after planting (DAP) and at 180 DAP i.e. at tiller completion stage. All other fertilizers were applied as basal dose during planting in the trench. Three times irrigation was applied, 1st irrigation just after planting, 2nd irrigation after first top dressing of urea and MP and 3rd irrigation was applied after 2nd top dressing of urea and MP. Mulching was done after each top dressing of urea followed by irrigation. Weed control, insect control and fungal disease control were done as and when necessary. Earthing up was done 7 months after planting while tying of trash was done 8 months after planting.

Table 3.1 Parentage of 51 sugarcane genotypes used in the experiment

Sl. No.	Accession of genotype	Parentage (Female x Male)
1.	I 112-01	I 273-91 x Isd 20
2.	I 39-04	I 273-91 x I 64-98
3.	I 152-04	CPI 85-80 x I 216-92
4.	I 111-03	Phil 48-15 x I 61-90
5.	I 6-04	?
6.	I 189-04	COL 33 x I 326-86
7.	I 174-93	I 91-79 x ?
8.	SC 5d	Self cross
9.	I 255-06	I 92-00 x I 101-66
10.	CPI 96-80	Exotic
11.	B 34-231	Exotic
12.	I 562-85	ZH 238 x F
13.	SC 2d	Self cross
14.	I 326-86	Isd 16 x ?
15.	Bo 43	Exotic
16.	I 156-97	I 281-85 x CP 50-50
17.	POJ 2878	POJ 2364 x EK 28 (Exotic)
18.	I 134-70	?
19.	CP 69-1052	Exotic
20.	IC 7a	?
21.	Co 635	Exotic
22.	CL 41-229	Exotic
23.	I 40-00	I 327-86 x I 523-85
24.	CP 36-105	Exotic
25.	CP 75-361	Exotic
26.	I 14-96	I 95-78 x I 144-86
27.	SC 10d	Self cross
28.	I 98-98	I 281-85 x Co 635
29.	I 64-98	I 281-85 x Isd 25
30.	I 17-01	CPI 38-80 x I 325-86
31.	I 137-96	I 457-85 x CP 55-30
32.	I 127-96	I 457-85 x B 34-231
33.	I 91-79	CP 44-154 x Bo 32
34.	I 33-97	?
35.	Saipan 17	Exotic
36.	I 26-04	I 273-91 x I 46-63
37.	Co 630	Exotic
38.	I 21-00	CPI 85-80 x B 34-231
39.	SC 5b	Self Cross
40.	I 82-98	I 281-85 x Co 635
41.	I 181-03	POJ 2878 (Self.)
42.	I 1-05	I 176-97 x I 216-92
43.	I 143-01	I 322-86 x Co 530
44.	I 23-05	Isd 33 xCP 55-30
45.	I 48-05	H 37 x 1933 x I 137-96
46.	Co 642	Exotic
47.	SC 6d	Self cross
48.	I 108-01	I 273-91 x Isd 20
49.	Isd 38	Isd 28
50.	Isd 40	Isd 27 x Isd 24
51.	Isd 39	BC5 x Isd 25

3.1.4 Data Collection

3.1.4.1 Data Collection of Agro-morphological Traits of Sugarcane

Data on following 16 agro-morphological traits were collected in the month of December 2011 i.e. 12 months after planting following the PHILSURIN descriptors.

1. Number of tiller per clump: Randomly ten stools were selected in each entry and the tillers were counted in each stool. Average of ten stools was recorded as tillers per clump at physiological maturity stage.

2. Plant height (cm): Ten randomly selected stalks from each entry were tagged. Shoot length was measured from the base (soil surface) of the stalk to the tip of the largest leaf at harvest and the average height is expressed in centimeters.

3. Stalk length (cm): Ten randomly selected stalks from each entry were tagged. Stalk length was measured from the base (soil surface) of the stalk to the top visible dewlap at harvest and the average height is expressed in centimeters.

4. Leaf length (cm): Leaf length was measured on the fourth leaf from the top most open leaf. The fourth leaf from the top most open leaf of ten selected stalks was measured from ligule to the leaf tip. The average length is expressed in centimeters.

5. Leaf width (cm): Leaf width is measured at the widest point of fourth leaf from topmost open leaf of ten selected stalks. The average length is expressed in centimeters.

6. Bud length (mm): Bud length was measured at the longest point from ten buds from ten randomly selected stalks using digital slide calipers. The average bud length is expressed in millimeters.

7. Bud width (mm): Bud width was measured at the widest point from ten buds from ten randomly selected stalks using slide calipers. The average width is expressed in millimeters.

8. Number of internode/cane: The number of internode was counted from the base internode up to the top visible dewlap from ten randomly selected stalks and expressed as average number of internodes per stalk at the time of harvest.

9. Internode length (cm): The internode length of basal (3rd visible internode from base), middle (largest internode) and top (1st internode below the top visible dewlap) of ten randomly selected stalks were measured at harvest. The average of three internodes of ten stalks is expressed in centimeters.

10. Internode diameter (cm): The internode diameter of basal (3rd visible internode from base), middle (largest internode) and top (1st internode below the top visible dewlap) of ten randomly selected stalks were measured using slide caliper at harvest. The average of three (basal, middle and top) internodes of ten stalks is expressed in centimeters.

11. Single cane weight (kg): The ten randomly selected stalks were cut at harvest, de-trashed, cleaned and tops were removed. The weight of ten stalks was recorded using top load balance and average weight was worked out and expressed as single cane weight in kilograms.

12. Number of millable cane (x10^3 ha^{-1}): All the canes in each plot were cut, dressed, counted and recorded as the number of millable cane per plot (10 m^2) at harvest. Finally number of millable cane/ha was calculated by multiplying the total number of millable cane counted per plot multiplied by 1000.

13. Cane yield (t/ha): All the canes in each plot were cut close to the ground level. The tops and trash were removed and cane weight per plot was recorded and expressed as cane yield per plot in kilograms. Finally plot yield was converted to cane yield in t/ha.

14. Brix per cent: Brix (soluble solids) was determined after 12 months after planting of sugarcane by Brix Hydrometer standardized at 20^{o} C. Ten randomly selected canes were harvested from each entry followed by detrashing, cleaning and removing the tops. Then these canes were crushed in a three roller mill (power crusher). The collected juice was first thoroughly mixed and strained with fine mesh or cloth to remove the debris and particles of bagasse, wax and other suspended impurities. A 500-ml measuring cylinder was filled up with cleaned juice and it was kept for few minutes for escaping of air bubbles and floating impurities was again removed. Then a Hydrometer was put in the juice filled cylinder and was kept a while for the adjustment of hydrometer and juice temperatures. Hydrometer reading was recorded by reading just near lower meniscus. The temperature of juice was recorded simultaneously and the corrected brix was calculated from the temperature correction table.

15. Pol per cent: At physiological maturity (360 DAP) of sugarcane, ten randomly selected canes were harvested, stripped, cleaned and crushed in power crusher machine to extract juice. Horne's Dry Basic Lead Sub-acetate method is widely used for the clarification of cane juice. About 100-200 ml juices were taken in a stopper reagent bottle and 2-3 g of Horne's lead sub-acetate was added. The content of the reagent bottle was then shaken vigorously for about a minute and then filtered through Whatman No. 40 filter paper and the clarified juice was filled in to 200mm polarimeter tube and Pol reading was recorded from the polarimeter. The corrected pol readings were obtained by comparing the Pol reading measured with the corresponding corrected brix reading referring to Schmitz table.

16. Juice purity per cent: The ratio of sucrose percent to the corrected brix was expressed as purity of the juice, which indicates the proportion of sucrose in the total solids present in the juice. It was calculated at 360 DAP. After recording brix per cent and Pol per cent of each entry, the purity per cent was calculated by the following formula:

$$\text{Juice purity percent} = \frac{\text{Pol \%}}{\text{Brix \%}} \text{x } 100$$

3.1.4.2 Data Collection of Qualitative Morphological Traits of Sugarcane

Data on 36 qualitative morphological traits were recorded according to the procedures developed jointly by Institute of plant Breeding (IPB), College of Agriculture, UP Los Baňos (UPLB) and Philippines Sugar Research Institute Foundation Inc. (PHILSURIN) and followed by Breeding Division of BSRI, Ishurdi, Pabna, Bangladesh. Descriptor for sugarcane germplasm characterization developed by PHILSURIN is shown in the AppendixTable 4.3.

3.1.5 Data Analysis

3.1.5.1 Analysis of Variance and Descriptive Statistics

The collected agro-morphological data were analyzed following the procedure outlined by Federer (1956) and Petersen (1985). Analysis of variance (ANOVA) and adjusted means of 16 agro-morphological data were calculated by using online software developed by Indian Agricultural Statistics Research Institute, New Delhi, India, following the procedure developed by Federer (1956, 1961) available at its website (www.iasri.res.in/Spad/web). Other descriptive statistics viz. mean, coefficient of variation, standard error, LSD values and critical difference (CD) were calculated following the procedure given by Steel and Torrie (1980) and Petersen (1985). The block effect was also estimated from the replicated check means and adjusted means following the procedure outlined by Petersen (1985).

3.1.5.2 Pearson's Correlation Coefficients

All possible pair-wise combinations of 16 agro-morphological traits were subjected to Pearson's correlation analysis. Pearson's Correlation coefficients (r) were calculated among pairs of all agro-morphological traits using the following formula:

$$r = \frac{\text{SP (xy)}}{\sqrt{\text{SS(x) SS (y)}}}$$

Where r denotes Pearson's correlation coefficient

SP (xy)= $\sum (x - \overline{x}) \sum (y - \overline{y})$; denotes sum of products of x and y

SS (x)= $\sum (x - \overline{x})^2$; denotes sum of square of x;

SS (y)= $\sum (y - \overline{y})^2$; denotes sum of square of y;

Therefore, correlation coefficients becomes

$$r = \frac{\sum (x - \overline{x})}{\sqrt{(x - \overline{x})^2}} \frac{\sum (y - \overline{y})}{\sqrt{\sum (y - \overline{y})^2}}$$

Test of significance of correlation coefficient (r) was carried out by referring to t-table given by Snedecor and Cochran (1967) using n-2 degree of freedom.

3.1.5.3 Shannon-Weaver Diversity Index

All the 16 agro-morphological characters were analyzed for their Shannon-Weaver diversity index. Shannon-Weaver diversity index was calculated using the following formula defined as:

$$H' = \frac{\sum pi \; log2 \; (pi)}{log2 \; n}$$

Where, n is the number of phenotypic classes of a character and pi is the proportion of the total number of entries belonging to the ith class (Jain et al., 1975).The Shannon-Weaver diversity index was standardized by dividing H ′ by the log $_2$ of the total number of phenotypic classes (Yu Li et al., 1996).

All the 16 agro-morphological traits were analyzed for their Shannon-Weaver diversity index. Class intervals of 0.5 above and below the mean were designated on the range of particular trait. Class marks were then formulated based on the mean+ (class interval x standard deviation). A total of 36 qualitative characters of sugarcane were subjected to Shannon-Weaver diversity index analysis. Classes

were based on the number of descriptor states (phenotypic classes) used for a particular trait.

3.1.5.4 Genetic Divergence Analysis

Euclidean Distance

To measure genetic divergence, Euclidean distance (Sneath and Sokal, 1973) between genotypes was calculated using the following formula:

Euclidean $d_{ij}=[\sum(X_{ih}-X_{jh})^2]^{½}$

Where i and j are the two accessions and p is the number of quantitative traits observed.

Mahalanobis Distance

Genetic diversity among the 51 genotypes was also studied on multivariate scale following Mahalanobis (1936) generalized distance (D^2) statistic extended by Rao (1952). All the 16 agro-morphological data were subjected to analysis of Mahalanobis' D^2 statistic using Genstat 5.1 software. Based on the D^2 values, the studied genotypes were grouped in to clusters according to the Tocher's method (Rao, 1952). Intra -cluster and inter-cluster distances, cluster means and contribution of each trait to divergence were estimated as suggested by Singh and Chaudhury (1985).

3.1.5.5 Multivariate Statistical Analyses

Characterization and evaluation data of quantitative characters were subjected to Multivariate statistical analyses using Genstat ver. 5.1. Cluster Analysis and Principal Component Analysis (PCA) were done to classify the different accessions in to groups and to identify the principal components that explain variability.

Principal Component Analysis

Raw data were first standardized to zero mean and unit variance followed by computation of numerical measures of likeness/similarity and construction of distance matrix using variance-covariance coefficients. Eigenvalues and Eigenvectors of the variance-covariance matrix were then computed. The first two principal components were plotted in two dimensional scales to view the graphical representation of the association among 51 genotypes of sugarcane.

Cluster Analysis

Using standardized data, numerical measures of likeness/similarity were computed and distance matrix constructed using Euclidian Distance coefficients. Cluster Analysis (Sequential, agglomerative, Hierarchical) using UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method was executed.

3.2 Genetic Diversity Analysis of Sugarcane Based on Microsatellite Markers

The experiment on the study of genetic diversity of sugarcane using microsatellite markers was carried out at DNA Laboratory of Biotechnology Division, Bangladesh Sugarcrop Research Institute (BSRI), Ishurdi, Pabna, Bangladesh during 2012-2014.

3.2.1 Plant Materials Collection and Preparation

Top of 8-month old sugarcane plant of each entry (Table 3.1) was tagged and was cut from experimental field. The collected samples were kept in plastic bucket containing tap water to keep the materials alive and fresh. After bringing the materials in the laboratory, the outer leaf sheaths and leaf blades were removed leaving only very young inner spindle to get meristem cylinder. Then the meristem cylinder (spindle base) was cut in to small pieces (about 5 mm long

with 3mm diameter) with sterile scissors and about 0.2 g sample was taken in each cleaned, autoclaved and labeled ceramic mortar. This sample was used to extract genomic DNA and the extracted, purified DNA sample was used for DNA fingerprinting and genetic diversity studies using SSR markers.

3.2.2 Equipments and Chemicals

A number of sophisticated equipments and, molecular biology grade chemicals were used for DNA isolation, purification, quantification, PCR amplification, gel electrophoresis and gel documentation. List of all the materials has been presented in Appendix Tables 3.1 and 3.2

3.2.3 Stock Solutions Preparation for DNA Isolation

A number of stock solutions were used to isolate genomic DNA from sugarcane leaf sample. The procedures of stock solution preparation are given below:

1M Tris-HCl Stock Solution Preparation

About 75 ml ddH_2O was taken in 150 ml- conical flask and 12.11 g Trisma base was added to it. About 5 ml 0.1N HCl was added in the flask slowly and was stirred slowly until the chemical dissolved completely. The pH of the solution was adjusted to 8.0. The final volume of the stock solution was adjusted to 100 ml by adding required amount of ddH_2O. The Tris-HCl stock solution was autoclaved and stored at 4 ^{o} C in the refrigerator.

0.5M EDTA Stock Solution Preparation

About 75 ml ddH_2O was taken in 150 ml- conical flask and 18.61 g EDTA was added to it. About 2 g NaOH pellet was added in the flask slowly and was stirred slowly until the chemical dissolved completely. The pH of the solution was adjusted to 8.0. The final volume of the stock solution was adjusted to 100 ml by adding required amount of ddH_2O. The EDTA stock solution was autoclaved and stored at 4 ^{o} C in the refrigerator.

5M NaCl Stock Solution Preparation

About 75 ml ddH_2O was taken in 150 ml- conical flask and 29.22 g molecular biology grade NaCl was added to it. The content was vigorously stirred with magnetic stirrer for an hour. The flask was then heated in water bath at 65 ^{o} C for 2 minutes for dissolving the salt completely. The final volume of the stock solution was adjusted to 100 ml by adding required amount of ddH_2O. The NaCl stock solution was autoclaved and stored at 4 ^{o} C in the refrigerator.

5% SDS Stock Solution Preparation

About 75 ml ddH_2O was taken in 150 ml- conical flask and 5.0 g SDS was added to it. The flask was heated in the water bath at 65 ^{o} C and gentle shaking was done until the chemical dissolved completely. The final volume of the stock solution was adjusted to 100 ml by adding required amount of ddH_2O. The SDS stock solution was stored at 4 ^{o} C in the refrigerator.

10% PVP Stock Solution Preparation

About 75 ml ddH_2O was taken in 150 ml- conical flask and 10.0 g PVP was added to it. The flask was shaking gently until the chemical dissolved completely. The final volume of the stock solution was adjusted to 100 ml by adding required amount of ddH_2O. The PVP stock solution was stored at 4 ^{o} C in the refrigerator.

20 % CTAB Stock Solution Preparation

About 75 ml ddH_2O was taken in 150 ml- conical flask and 20.0 g CTAB was added to it. The flask was heated in the water bath at 65 ^{o} C and gentle shaking was done until the chemical dissolved completely. The final volume of the stock solution was adjusted to 100 ml by adding required amount of ddH_2O. The CTAB stock solution was stored at room temperature.

TE (10:1) Buffer Preparation

About 494 ml deionized distilled water was taken in 1L beaker. In this beaker, 5.0 ml of 1M Tris-HCl and 1.0 ml of 0.5M EDTA were added and mixed well by swirling the beaker. This TE solution was filter sterilized by using 0.22 µm Millipore filter in the laminar hood and transferred to an autoclaved 1L conical flask. The mouth of the flask was sealed with aluminum foil and stored at room temperature.

Preparation of TE Saturated Phenol

The bottle containing phenol crystal was melted by heating at 65 ^{o} C in the water bath for 30 minutes. About 50 ml melted phenol and 50 ml TE buffer were taken in 200 ml beaker. This mixture was stirred by magnetic stirrer for 10 minutes and kept rest for 5 minutes. The upper phase was discarded by dropper carefully and again 50 ml TE buffer was added. The above procedure was repeated six times until pH of phenol raised up to7.75. The saturated phenol was kept in dark colored (amber) bottle in the fridge at 4^{o} C.

Preparation of Phenol: Chloroform: Isoamylalcohol (25:24:1)

In a dark colored bottle, 25ml saturated phenol; 24ml chloroform and 1ml isoamylalcohol were added and mixed by vortexing under fume hood for 1 minute. The mouth of the bottle was sealed and kept in amber bottle at 4 ^{o} C in the fridge.

Preparation of 10ml Extraction Buffer

In a autoclaved 100 ml conical flask, 2 ml of 1M Tris-HCl (pH 8.0), 1 ml of 0.5M EDTA (pH 8.0), 3.6 ml of 5M NaCl, 0.2 g of CTAB and 0.006 g of sodium sulphite were added. At last, 3.124 ml of ddH_2O was added and mixed well by swirling. The mixture was heated at 65^{o} C in the water bath until all the components dissolved completely. Freshly prepared extraction buffer was used in DNA isolation.

3.3 DNA Isolation Protocol

A modified method of DNA isolation developed by Al-Janabi et al. (1999) was used for DNA isolation from sugarcane leaf sample. The following steps were followed for isolation of genomic DNA from sugarcane meristem tissues.

Step 1 About 0.2 g meristem tissue was taken in a cleaned and autoclaved mortar.

Step 2 About 800 µl extraction buffer was added to the sample and again finely grounded so that cells wall ruptured and genomic DNA comes out from cells

Step 3 The extraction buffer mixed grounded material was transferred to 2-ml Eppendorf tube.

Step 4 About 150 µl of each 5% SDS, 10% PVP and 20% CTAB were added in the above 2-ml Eppendorf tube and mixed well by inversion several times and incubated at 65 ^{o} C in water bath for 40 minutes. During incubation period, 3-4 times inversions of Eppendorf tube were done.

Step 5 The Eppendorf tube was removed from water bath and cooled at room temperature. Equal volume (800 µl) of Phenol: chloroform: isoamyl alcohol (25:24:1) was added and mixed well.

Step 6 The Eppendorf tube was centrifuged at 10,000 rpm for 30 minutes.

Step 7 About 650 µl of aqueous upper phase was transferred to 1.5 ml Eppendorf tube and equal volume (650 µl) of ice-cold isopropanol and 150 µl of 5M NaCl were added. The Eppendorf tube was kept at -20^{o} C in the freezer for an hour for precipitation of crude DNA.

Step 8 The Eppendorf tube was centrifuged at 10,000 rpm for 20 minutes at room temperature for DNA pellet formation.

Step 9 The supernatant was discarded by micropipette carefully.

Step 10 About 500 µl (2.5 times) 70% ice-cold ethanol was added to it.

Step 11 The Eppendorf tube was centrifuged at 10,000 rpm for 10 minutes and supernatant was discarded carefully (DNA pellet was formed in this step.

Step 12 DNA pellet was washed with 200-300 µl 70% ethanol. This washing procedure was repeated 2-3 times to removes phenolic compounds and excess salt.

Step 13 After discarding ethanol, DNA pellet containing Eppendorf tube was kept in inverted position (Upside down) on the filter paper for about 30 minutes to dry the pellet.

Step 14 About 50 µl TE (10:1) was added in each Eppendorf tube to dissolve the DNA pellet.

Step 15 DNA sample was kept at -20 ^{o}C in the freezer for future use.

3.4 Quantification and Quality Checking of Isolated DNA

Sometimes, isolated genomic DNA may contain large amount polysaccharides, phenolic compounds pigments and RNA which usually cause over estimation of DNA concentration in a spectrophotometer. For that reason, the DNA sample was evaluated both qualitatively and quantitatively by Nanodrop 2000 (spectrophotometer) machine. Measurement of isolated DNA concentration can be done by estimating the absorbance of DNA by spectrophotometer at 260 nm wave length of UV light and quality of isolated DNA was measured by checking absorbance ratio (A 260nm/ A 280nm).

Procedure of Measuring Template DNA Concentration and Quality Checking By Nanodrop 2000 (Spctrophotometer)

Following steps were followed during measurement of genomic DNA concentration and quality checking.

1. Pedestal surfaces were cleaned before opening the software.
2. After thawing the stored genomic DNA sample (Stored at -20 ^{o} C), the DNA samples were heated to 55 ^{o} C in the water bath before measurement to make more homogenous DNA sample.

3. After opening the pedestal arm of Nanodrop, blank sample TE of about 1-2 µl was loaded on to the lower measurement pedestal and then the sampling arm was lowered in to the down position.
4. Cursor of mouse was kept on the blank icon and was clicked on
5. After completion of measurement, blanking buffer was wiped from both pedestals using a laboratory wipe.
6. About 1 µl of extracted genomic DNA sample was taken by micropipette and was loaded on the lower Pedestal and then arm was closed.
7. Cursor of mouse was kept on the “measure” icon and was clicked on
8. The DNA concentration (ng/µl) and A260/A280 appeared on the screen of the computer. These results were saved in a folder and print out of results was taken later on.
9. After completion of measurement, the pedestal surfaces were wiped with laboratory wipe.

The quality of the extracted DNA was assessed by measuring the absorbance of the sample at 260nm (A260) and 280nm (A280) on a Nanodrop 2000 spectrophotometer. The purity is indicated by (A260)/(A280), and this ratio is 1.8 for pure DNA, which is free from protein.

Different DNA extraction methods provide DNA of widely different concentrations and purity. Thus, it is necessary to optimize the amount of DNA used in SSR analysis to achieve reproducibility. Below a certain concentration of genomic DNA, rapid amplification is no longer reproducible. Thus, it is essential to keep on above this critical concentration. It is best to do a series of SSR reaction using a couple of primer pairs and a set of serial dilutions of each genomic DNA to identify empirically the useful range of DNA concentration, for which reproducible SSR patterns are obtained.

Good quality DNA should give the A260/A280 in the range of 1.8 - 2.0. The A260/A280 ratio higher than 2.0 generally indicates RNA contamination. For A260/A280 ratio lower than 1.8 normally indicates protein contamination during extraction process.

3.5 Preparation of working solution (25 ng/µl) of DNA sample for SSR- PCR

Original stock solution concentration of each DNA sample was adjusted to a unique concentration (25 ng/µl) using the following formula:

$S_1V_1=S_2V_2$; Where,

S_1= Initial DNA concentration (ng/µl) of stock

V_1= Initial volume of DNA solution (µl)= 2µl

V_2= Final volume of DNA solution (µl) = TE buffer (µl) to be adjusted

S_2=Final DNA concentration (ng/µl) = 25ng/µl to be adjusted

Original stock DNA (2 µl) was taken in an Eppendorf tube and required amount of TE buffer calculated using the above formula was added to it. Required amount (volume) of TE buffer for each sample was calculated and used for DNA working solution preparation. These working solutions were used for preparation of SSR-PCR master mix solution.

3.6 Preparation of dNTPs (400 µl)

Equal amounts (10 µl) of each dATP, dTTP, dCTP, dGTP (each with 100 mM conc.) were mixed in a 1.5 ml autoclaved tube and 360 µl SddH_2O was added. After adding all components, the mixture was vortexed for 30 seconds for better mixing. So, 400 µl dNTPs was prepared.

3.7 Microsatellite (SSR) Primers Used

Twenty three SSR primers were used for PCR amplification of genomic DNA isolated from 51 sugarcane genotypes. List of primers used along with their forward and reverse sequences, and their annealing temperatures are presented in the Appendix Table 3.3

3.8 Preparation of PCR Master Mix

PCR reaction mixture i.e., master mix/cocktail for each DNA sample with each primer was made for 10 µl volume in a 0.2ml thin walled PCR tube. Each PCR reaction mixture contained 5.0 µl of master mix and 2.5 µl of each forward and reverse primer, respectively. The master mix contained the following reagents. The amount and final concentration of each component are mentioned in the following table:

Table 3.2 Composition of PCR Mastermix

Sl. No.	Components	Final Conc.	Vol.(µl)/ Reaction
1.	Sterile de-ionized distilled water	-	0.8
2.	10X PCR Buffer with 15mM $MgCl_2$	1X	1.0
3.	dNTP (2.5mM)	0.25mM	1.0
4.	Template DNA (25ng/µl)	5ng/µl	2.0
5.	Taq DNA polymerase(5U/µl)	1U	0.2
6.	Forward primer		2.5
7.	Reverse primer		2.5
	Total	=	10.0 µl

During the experiment, PCR buffer, dNTPs, primers and DNA sample solution were thawed from frozen stocks, mixed by vortexing and kept on ice flacks. Template DNA (25ng/µl) were pipette (2.0 µl) first into PCR tubes (0.2ml) compatible with the thermal cycler.

3.9 Performing PCR Reaction in Thermal Cycler Machine

PCR tubes containing all the components of PCR reactions were placed in the Thermal cycler (PCR machine). After closing the upper lid of previously programmed PCR machine, electric switch was put on. The DNA amplification was performed in oil-free Thermal Cycler (Genius, Techne, Cambridge Ltd. UK) following the SSR-PCR profile mentioned below:

Table 3.3 PCR Profile of Thermal Cycler Machine "Genius"

Program	Cycle	Link To	Segment	Temperature (°C)	Function	Rate	Hold Time
1	1	2	1	94	Initial denaturation	Maximum	4 Minutes
2	35	3	1	94	Denaturation	Maximum	30 Sec.
			2	55	Annealing	Maximum	30 Sec.
			3	72	Extension	Maximum	1 Minute
3	1	4	1	72	Final Extension	Maximum	7 Minutes
4	1	E	1	4	-	Maximum	Hold Time

3.10 Electrophoresis of PCR Products

After completion of PCR amplifications, PCR products (amplicons) were resolved on 2% agarose gel using a horizontal electrophoresis unit (CBS Scientific USA). After electrophoresis, the gel was taken out carefully from electrophoresis chamber and placed in gel Documentation system (Alpha View 3.2, Cell BioSciences Inc.USA) for visualizing the DNA bands.

Preparation of 2% Agarose Gel

About 130 ml agarose gel is needed for 15 cm x 15cm tray making 0.5cm thickness of gel. The following steps were followed for casting the gel.

Step1. Initially 2.6 g agarose powder (Sigma Co.) was weighing out and placed into a 250 ml conical flask.

Step 2. Then 130 ml of 1X TBE buffer was added into the 250 ml flask.

Step 3. The flask was then placed in a microwave oven. Using a low to medium temperature setting, the timer was set for two minutes. The oven was stopped and swirled the container gently to suspend the agarose which was not dissolved. The solution was swirled until all of the small translucent agarose particles were dissolved. Visually it seems clear.

Step 4. When the Agarose solution was cooled to about 50^{o} C (the flask was cool enough to hold comfortably with bare hand), about 13 µl of ethidium bromide solution (conc. 10mg/ml) was added in the gel solution, so that in the gel the concentration of ethidium bromide is maintained as 0.8 µg/ml. The final concentration of ethidium bromide in the melted agarose solution may be in the range of 0.5-1.0 µg/ml) and mixed well by gentle shaking to make DNA visible under UV light.

Agarose Gel Casting

1. The ends of the gel casting tray was sealed or fitted with casting dams. The dams were fitted so that there was no gap between the sides of the tray and the groove in the dams.
2. The gel tray was leveled on a leveling table or working bench using the leveling mercury bubble.
3. Then the comb was placed into the appropriate groove or slot of the tray.
4. The molten agarose (Temp. about 50 ^{o} C) was poured on to the tray. Hot agarose (Temp. above 60 ^{o} C) might cause the comb to warp or craze and would decrease the lifetime of the comb. Warping might also result in sample wells of uneven depth. Air bubbles were removed by pushing away to the side of the casting tray by using a disposable micropipette tip.
5. The gel was allowed to solidify at room temperature for 20-30 minutes.
6. After solidification of gel, the comb was removed carefully from the solidified gel.

7. Then the casting dams or gates were removed from the edges of the gel tray. Casting dams were removed carefully so that the gel does not slide off the tray.

Preparation of PCR Products (Amplicon) for Electrophoresis

1. In each PCR tube, 1-2 µl 1X loading buffer was added in the PCR products by micropipette and mixed properly.

Preparation of working Solution of DNA Ladder

The supplied 100 bp DNA ladder (Conc. 500 µg/ml) was diluted to five times with TE buffer and 6X loading dye to make working solution. Therefore, 20µl DNA ladder + 20 µl loading dye + 60 µl TE buffer were added together and mixed properly by vortexing.

Loading of the DNA Sample in the wells

The total volume of the loading sample was 10-12 µl. The loading volume is dependent upon the type of comb used (i.e., well thickness and length of comb's teeth) and the thickness of the gel. The prepared sample was then loaded slowly in each well to allow them to sink to the bottom of the wells.

Loading 100bp DNA Ladder

First and last wells of the solidified agarose gel were loaded with 5 µl working solution of 100 bp DNA weight marker for the measuring unknown band size (bp) of amplified DNA sample (Amplicon).

Running the Gel (Electrophoresis)

1. The agarose gel casted tray was placed on to the base/floor of the electrophoresis chamber by keeping the gel horizontal, so that the sample wells remain near the cathode (negative end generally marked as black). The DNA sample would migrate toward anode (positive end generally marked as red) during electrophoresis.

2. Sufficient amount of 1X TBE running buffer (about 600 ml) was added to cover the gel to a depth of about 2mm (1-5 mm may be used). The volume of electrophoresis buffer should not be above the maximum buffer mark on electrophoresis chamber.

3. Electrophoresis was carried out at 120 volt volts for 1.5 hours. The separation process was monitored by the migration of the dye in the loading buffer. When the bromophenol blue dye had reached about three-fourths ($3/4^{th}$) of the gel length, the electrophoresis was completed and electricity connection was switched off.

3.11 Visualization and Documentation of PCR Amplification Products

1. After completion of electrophoresis, the gel was taken out carefully from electrophoresis chamber and placed in Gel documentation system (FluorChem FC2, Cell BioSciences, USA) for visualizing the DNA bands.

2. The amplification products (Amplicon) were observed as DNA bands and the image was photographed using above Gel documentation system. The image was saved in separate folder in the PC attached with the gel documentation system.

3. The size of the band found in each lane was estimated with the help of Alpha View 3.2, (Cell BioSciences Inc.USA) software available in the Gel documentation system (FluorChem FC2, Cell BioSciences, USA) using 100 bp DNA ladder (Genei, India) as size standard.

3.12 Molecular Data Analysis

The SSR-PCR amplification products visualized as bands in the image of the gel were scored of all genotypes for their presence (1) or absence (0) in all primer combinations employed. Only polymorphic bands were used in the construction of binary matrix as discrete variables. The number of alleles per locus was determined. The following genetic diversity parameters were calculated.

(1) Percentage of Polymorphic Loci (P) was calculated using the formula described by Sun et al. (2001) was as follows:

P= (k/n) x 100, where 'K' is the number of polymorphic loci and 'n' is the total number of loci investigated.

(2) Average Number Allele per Locus (A) was calculated using the formula described by Sun et al. (2001) was as follows:

A= ∑Ai/n, where 'Ai' is the number of alleles at ith locus and 'n' is the total number of loci investigated.

(3) Polymorphic Information Content (PIC): The frequency of microsatellite polymorphism was calculated based on presence or absence of common band (Ghosh et al., 1997) .To measure the informativeness of the markers, polymorphism information content (PIC) value described by Anderson et al. (1993) of each of the SSR marker was computed as follows:

$$PICi = \sum_{i=0}^{n} \text{Pij } 2$$

Where, P_{ij} is the frequency of the jth allele for ith marker and summation extends over 'n' alleles.

(4) **Effective Allele per Locus (Aep):** The effective number of allele per locus was calculated according to Weir (1990) with the formula 1/(1 - Hep), where Hep, the genetic diversity per locus, is equal to 1 – Pi^2 and Pi is equal to the frequency of the ith allele at the locus.

(5) **Euclidean Distance:** Euclidean distance values between the genotype pairs were computed by using Statistica 6.0 software. Based on Euclidean distance, dendrogram was constructed using UPGMA method with the help of same software.

(6) **Jaccard's Similarity Coefficient**: Genetic similarity (GS) was calculated by making a pair wise comparison among the accessions using Jaccard similarity

Coefficient (GS_{ij}) (Jaccard, 1908).The formula is given as follows:

$GS_{ij} = a/a + b + c,$

where GSi j is the measure of genetic similarity between individuals i and j, a is the number of polymorphic fragments that are shared by i and j, b is the number of fragments present in i and absent in j, and c is the number of fragments present in j and absent in i. This definition of similarity excludes bands, which are absent in both individuals. The binary data matrix was used further analysis. The Excel file containing the binary data was imported into NT Edit of NTSYS-pc 2.02J. The 0/1 matrix was used to calculate Similarity matrix as Jaccard's coefficient using SIMQUAL subroutine program in SIMILARITY routine. The resultant similarity matrix was employed to construct dendrogram using Sequential Agglomerative Hierarchical Nesting (SHAN) based Unweighted Pair Group Method with Arithmetic Means (UPGMA) as suggested by Sneath and Sokal (1973). Finally, a dendrogram was created using derived genetic distances to infer genetic relationships.

Chapter IV

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1 Genetic Diversity Analysis Based on Agromorphological Traits

Diversity analysis based on agronomic and morphological characters recorded at several stages of plant growth were carried out in sugarcane. Genetic diversity between parents is important to generate transgressive segregants from the cross.

4.1.1 Analysis of Variance of Agromorphological Traits

The experiment was laid out in augmented complete block design with six replications using three check varieties viz. Isd-38, Isd-39 and Isd-40. Analysis of variance revealed the presence of highly significant differences among the genotypes for all the characters studied (Appendix Table 4.1), thereby indicating the presence of sufficient amount of genetic variability in the studied population. It was also indicated that all the genotypes differed each other with regard to the traits that broaden the way to proceed for further improvement through simple selection (Punia, 1982).

In sugarcane, many studies on estimating variability had been conducted on different population in different sugarcane regions in the past (Hooda et al., 1989; Nair et al., 1980; Chaudhary et al., 1982; Patil, 2005). Table 4.1 summarizes the means, ranges, coefficient of variations (CV %) and standard deviations, (mean ± SE) of 16 agromorphological traits. Appendix Table 4.2 shows the means (adjusted) of agromorphological traits of 51 genotypes of sugarcane.

4.1.2 Mean Performance of Different Quantitative Traits

Basic statistics for the quantitative traits presented in Table 4.1 revealed high coefficient of variations (23.98 % to 30.44%) for number of tiller per clump, single cane weight, number of millable cane and cane yield indicating selection for these characters will be expected to achieve profitable gain. Whereas, low

variability was found for plant height (9.45%), brix percent (7.87 %) and juice purity percent (3.08 %). The coefficient of variation for some of the morphological characters such as internode length, mid-rib width, leaf width, plant height, and stalk height was studied by Govindaraj et al. (2014) and they reported coefficient of variation between 15 and 30 %, which indicates a very high variability within the collection of sugarcane.

Plant height

Analysis of variance revealed significant variation among the genotypes studied (Appendix Table 4.1). The mean plant height was 430.34 cm ranging from 323.20 cm to 515.77 cm (Table 4.1). The genotype SC 10d was the tallest (515.77 cm) and the genotype I 111-03 was the shortest among 51 genotypes evaluated (Appendix Table 4.2). Plant height of sugarcane increases with advance in age of the crop up to maturity. Sabitha and Prasad Rao (2008) reported in their study on sugarcane that clones with higher plant height recorded higher yield.

Number of Tiller per Clump

Number of tiller plays a pivotal role in enhancing the final yield of sugarcane. Highly significant differences ($P<0.01$) were recorded for number of tillers per clump among the genotypes in this study. High variability (28.08 %) in tiller per clump was found among the studied germplasm. Mean number of tiller per clump was 4.53 and ranged from 2.69 to 9.29. The genotype POJ 2878 exhibited the highest number of tiller per clump (9.29) followed by B 34-231 (7.13) while the genotype I 40-00 produced the lowest number of tiller (2.69) per clump. The best performing genotype POJ 2878 produced more number of tillers than the best standard check variety Isd 38 (5.30) (Appendix Table 4.2). Similar result was reported by Tiwari and Chatterjee (1998). Greater tillering potentiality is one of the most important quantitative morphological characters to be kept in mind while selecting a variety of sugarcane for commercial cultivation. Different clones of sugarcane differ in their trend in tillering and ultimate tillers at harvest (van

Dillewijn, 1952). It has been observed that tillering is influenced by internal and external environmental factors. Some genotypes have early rapid tillering capacity while others are slow in tillering at the initial stage of crop growth and tillering in these genotypes increases gradually (Akhtar et al., 2001).

Stalk Characters

The number of millable cane and single cane weight showed high variability while moderate variability (>10.0 to 20.0 %) was found in stalk length, internode length, internode diameter and number internode per stalk (Table 4.1). The height of a cane contributes significantly towards final cane yield. Average stalk length was 311.00 cm and ranged from 230.56 cm to 371.90 cm. The stalk length was found maximum in the check variety Isd 38 (371.90 cm) followed by genotype I 91-79 (363.39 cm) while minimum stalk length was recorded in the genotype I 111-03 (230.56 cm). None of the tested genotype superseded the standard check variety Isd 38. According to Jackson and MC Rae (2001) under good growing conditions, individual seedling clone may produce up to about 2.0 m of cane can be planted to the next selection stage. Muyco (2002) observed highest stalk length in the cultivar-H 65-2209 (285.10 cm) during investigating morphological diversity of 81 sugarcane cultivars of the Philippines during 1999-2001. The research work carried out by Panhwar et al. (2006) is in accordance with the present finding.

Single cane weight is the product of its length and girth of cane, and contributes substantially to final cane yield. The analysis of variance showed highly significant differences among the genotypes for single cane weight (Appendix Table 4.1). Single cane weight showed high variability (26.62%). The average single cane weight was 0.90 kg. The single cane weight ranged from 0.42 to 1.47 kg. The genotype I 174-93 produced the heaviest stalk (1.47 kg). This genotype is heavier than the check variety Isd 38 (1.24 kg). The genotype Bo 43 produced the lightest cane (0.42 kg). Shanmuganathan et al. (2015) recorded that single cane weight ranged from 1.10 kg (CoN 09071) to 1.62 kg (Co 09006) with a

mean of 1.38 kg. The deviance of these results from the present investigation might be due to dissimilar genetic background of the genotypes used in different experiments.

Table 4.1 Means ±SE, ranges, coefficient of variations (CV) % and least significant difference (LSD) values of 16 agro-morphological traits in sugarcane.

Sl. No.	Characters	Mean ± SE	Range	CV (%)	$LSD_{0.05}$
1.	No. of tiller/clump	4.53 ± 0.179	2.69 -9.29	28.08	0.18
2.	Plant height (cm)	430.34 ± 5.69	323.20-515.77	9.45	11.43
3.	Stalk length (cm)	311.00 ± 4.98	230.56-371.90	11.43	10.00
4.	Leaf length (cm)	142.16 ± 2.07	104.25 -171.32	10.37	4.16
5.	Leaf width (cm)	4.04 ± 0.11	2.46 -6.95	19.47	0.22
6.	Bud length(mm)	6.78 ± 0.16	4.64- 9.19	16.91	0.32
7.	Bud width (mm)	5.80 ± 0.13	3.88- 8.02	15.74	0.26
8.	Internode length (cm)	10.91 ± 0.16	8.27 -13.35	10.46	0.32
9.	Internode diameter (cm)	1.83 ± 0.03	1.10-2.39	13.17	0.07
10.	No. of internode/stalk	25.95 ± 0.44	20.38 -32.38	12.16	0.89
11.	Single cane weight (kg)	0.90 ± 0.03	0.42-1.47	26.62	0.07
12.	No. of millable cane/ 10 m^2*	91.71 ± 0.31	61.44-166.44	23.98	6.18
13.	Brix (%)	19.44 ± 0.21	13.60 - 22.38	7.87	0.43
14.	Pol (%)	13.55 ± 0.19	8.41- 15.94	10.19	0.39
15.	Juice purity (%)	88.10 ± 0.38	76.54-91.86	3.08	0.77
16.	Cane yield (t/ha)	81.82 ± 3.49	33.60-136.61	30.44	7.01

*: ($x10^3$ ha^{-1})

The data regarding number of internode per stalk (Table 4.1) reveals that the average number of internode per stalk was 25.95 and ranged from 20.38 to 32.38. The genotype Saipan 17 exhibited the highest number of internode per stalk (32.38) followed by SC 10d (31.88) while the genotype I 112-01 displayed the lowest number of internode per stalk (20.38). The best performing check variety Isd 38 showed 29.73 internode per stalk. Out of 51 genotypes studied, only five genotypes namely CP 69-1052 (30.48), CP 75-361(29.98), SC 10 d (31.88), Saipan 17 (32.38) and Co 642 (31.18) exhibited higher number of internode than the check variety Isd 38. The results are in agreement with the findings of Soomro et al. (2007) who reported highest 33.32 internodes per stalk with a mean of 26.61 internode per stalk.

Highly significant differences were observed among the genotypes studied for internode length but the variability among the genotypes was moderate (10.46%). Internode length displayed a mean of 10.91 cm and varied from 8.27 cm to 13.35 cm. The genotype I 189-04 exhibited the longest internodes (13.35 cm) while the genotype I 134-70 had the shortest internode (8.27 cm). The genotype I 189-04 produced longer internode than all the three check varieties (Appendix Table 4.2). Similar results were reported by Muyco (2002) who recorded the highest value for internode length (13.93 cm) and average length of internode (11.55 cm) from the diversity studies of 81 cultivars of sugarcane of the Philippines.

Stalk diameter is one of the important yield contributing traits of sugarcane and large stalk diameter would enhance the acceptability of varieties from economic point of view. Canes that grow tall and thin may be more susceptible to lodging; the tall clones with thick stalked canes that resist lodging. Regarding the internode diameter character, the mean internode diameter was 1.83 cm and ranged from 1.10 cm to 2.39 cm. The thickest internode diameter (2.39 cm) was found in the genotype I 174-93 where as the thinnest (1.10 cm) was recorded in the genotype I 134-70. The genotype I 174-93 produced thicker cane than the check varieties. The results of the present investigation are in agreement with the findings of Singh and Singh (2000), who concluded that sugarcane genotypes of different groups respond differently even under similar climatic and edaphic conditions for cane girth. However, Kadam et al. (2007) reported that higher cane diameter showed positive influence on cane yield.

The number of millable cane/ha is one of the important yield contributing traits of sugarcane and it directly influences cane yield (Munir et al., 2009; Khalid et al., 2014). More number of millable canes having greater stalk height and girth contribute towards higher cane tonnage and higher per unit area production (Singh and Sharma, 1983). The number of millable cane ranged from 61.44 to 166.44 ($x10^3$ ha^{-1}) with a mean of 91.71 ($x10^3$ ha^{-1}). The genotype I 127-96 produced the highest number of millable cane (166.44) ($x10^3$ ha^{-1}) while the genotype Saipan 17 displayed the lowest number of millable cane (61.44) ($x10^3$

ha^{-1}). The best check variety Isd 38 produced 94.00 ($x10^3$ ha^{-1}) millable canes. Shanmuganathan et al. (2015) recorded 95.75 millable canes in the clone CoN 09072 to 116.91 in the clone Co 09004 with a mean of 107.71 ($x10^3$ ha^{-1}). Reddy et al. (2014) reported the highest number of millable cane (95,433 ha^{-1}) produced by the clone 2006T36 and it was at par with the standards Co 94008 (94,300 ha^{-1}) and CoC 671 (92,767 ha^{-1}). Whereas, maximum (135) ($x10^3$ ha^{-1}) millable cane were exhibited in variety Thatta-34 against check variety Thatta-10 (128.8) (x 10^3 ha^{-1}) (Arian et al., 2011). The differences in millable cane count observed by different authors might be due to the inherent potential of the genotypes and interaction between genotype and environment.

Leaf Characters

Leaf length was measured on leaf number 4. Leaf length varied from 104.25 cm to 171.32 cm with a mean of 142.16 cm. The genotype I 17-01 produced the longest (171.32 cm) leaves while the genotype I 156-97 displayed the shortest (104.25 cm) leaves. Mean leaf width was 4.04 cm and ranged from 2.46 cm to 6.95 cm. The widest leaf was observed in the genotype I 14-96 (6.95 cm). The narrowest leaf was found in the genotype Bo 43 (2.46 cm).

Bud Characters

Bud length and width were recorded from 7-8 month old sugarcane plants. Sugarcane bud is one of the key identifying characters of sugarcane variety. Bud length was varied from 4.64 mm to 9.19 mm with a mean of 6.78 mm. The longest bud was found in the genotype I 33-97 (9.19 mm) whereas, the shortest bud was recorded in the genotype SC 2d (4.64 mm).

Juice Quality Characters

Juice quality characters viz. brix percent, pol percent and juice purity percent were recorded during harvesting period i.e. at the age of 11-12 months old sugarcane plant. These three biochemical properties of cane juice are important

for high sugar yield. A major objective of sugarcane variety improvement program is getting higher sugar yield. Brix percent of cane juice plays an important role in determining the sugar recovery per cent of the sugarcane. Improvement of sugar content in the harvested sugarcane is highly attractive from a commercial point of view since it increases revenue from increased sugar production without increased harvesting, cane transport and cane crushing costs that are associated with higher cane yield.

The brix content in the cane juice ranged from 13.60 % to 22.38% with a mean of 19.44%. The check variety Isd 38 exhibited the highest brix value (22.38%) while the genotype I 255-06 showed the lowest (13.69%) brix value. The results revealed that none of the genotype exceeded the check variety Isd 38 in respect of brix percentage (Appendix Table 4.2). Burio et al. (2003) recorded the highest brix content (27.27 %) in the variety ML-7 followed by Thatta-10 and Gulabi-95 (25.45 % and 24.48%) respectively, while the lowest brix content (19.07%) was recorded in FAC-81/745. During the evaluation of more than 200 exotic sugarcane clones, Habib et al. (1992) had observed large variations in brix percentage in different genotypes. These results of this investigation are in agreement with those of Das et al. (1996) and Singh and Singh (2000) who studied a number of sugarcane varieties and found different levels of brix content.

In regard to pol percent, moderate variability was found among the genotypes studied (Table 4.1).The pol percent in cane juice ranged from 8.41 % to 15.94 % with a mean of 13.55%. The check variety Isd 38 showed the highest (15.94%) polarity reading followed by another check variety Isd 39 (15.39%). The genotype I 255-06 had the lowest (8.41%) polarity reading. Out of 51 genotypes, 46 genotypes had lower (<15.0 %) juice polarity reading. Juice purity is the major factor that is used in maturity and quality judgement.The analysis of variance displayed highly significant variation among the genotypes for juice purity percentage (Appendix Table 4.1). Mean pol reading was 16.57 % of 81 sugarcane varieties of Philippines (Muyco, 2002). Juice purity values ranged from 76.54 % to 91.86 % with a mean of 88.10%. More than 98 % of the genotypes

displayed mean juice purity values greater than 80%. The genotypes Isd 39, Isd 40, I 112-01, CPI 96-80, I 562-85, Poj 2878, I 40-00, CP 75-361, Saipan 17 and I 82-98 showed greater than 80% purity of juice (Appendix Table 4.2). Akhtar et al. (2001) reported maximum juice purity of 86.57% in variety CP 89-846 during evaluation of six exotic sugarcane germplasm. Quality of the cane juice also depends on many factors like genotype (Hatam and Pazir, 1989), age of the crop (Yadav and Sharma, 1982) and other factors like management, balanced fertilization and the environment (Akhtar, 1999).

Cane Yield

Analysis of variance revealed highly significant variation observed in cane yield (t/ha) (Appendix Table 4.1). The highest variability (30.44%) was found in cane yield among the genotypes studied. Cane yield ranged from 33.60 t/ha to 136.61 t/ha with a mean of 81.82 t/ha. The highest cane yield was obtained from the genotype SC 10d (136.61 t/ha). Out of 51 genotypes evaluated, 29.41% were considered high yielding with more than 94.28 t/ha, 43.14% were medium yielders with 69.36 to 94.28 t/ha, and 27.45 % were low yielding produced less than 69.36 t/ha. The check variety Isd 38 was found to be the best among three checks used in respect of cane yield (116.63 t/ha). Among high yielding groups of sugarcane genotypes, I 174-93 (122.24 t/ha), I 255-06 (127.49 t/ha), Poj 2878 (117.37 t/ha), SC 10d (136.61 t/ha) and SC 5b (134.89 t/ha) out yielded the best check variety Isd 38 (116.63 t/ha) (Appendix Table 4.2). Begum et al. (2013) recorded highest cane yield (114.6 t/ha) in the genotype I 189-04 under saline condition in the coastal areas of Bangladesh. Many factors namely good germination, good tillering capability and taller and thicker stalks are the most important traits responsible for improving cane yield (Singh and Sharma, 1983). Cane and sugar yield also differ considerably in different locations and varieties. Favourable climate and proper management factors ensure better cane and sugar yield in cane crop (van Dillewijn, 1952).

Plant characteristics such as number of tiller per clump, single cane weight and number of millable cane showed high variability, which could be considered during selection breeding program but few other important traits like plant height, brix percent and juice purity percent showed low variability (<10.00%) that restricts the scope of selection for considering these characters in the present germplasm collection. Therefore, large scale testing of broad base germplasm need to be build up by making extensive local collections and obtaining germplasm from abroad to develop a sound breeding program.

4.1.3 Characterization of Qualitative Morphological Traits

Data on qualitative morphological characters were collected from 51 genotypes of sugarcane according to the standardized sugarcane descriptor list developed jointly by Institute of plant Breeding (IPB), College of Agriculture, UP Los Baňos (UPLB) and Philippines Sugar Research Institute Foundation Inc. (PHILSURIN). Each trait was scored and grouped into different classes. The frequency distribution for each morphological character was estimated and presented in Table 4.2.

Plant Habit

Plant habit is one of the major identifying stem characters that also determine the suitability of the cane for commercial cultivation and use in the sugarcane industry. Three types of plant habit viz., erect, intermediate and reclining were found in the germplasm. Out of 51 genotypes, 35 genotypes (68.63%) showed erect type, 15 genotypes (29.41%) intermediate and only one genotype reclining type. In recent times, a straight stalk development is sought in new variety to facilitate machine harvest.

Plant Tops

Plant tops represent the weight of upper part of sugarcane plant due to the number of green leaves and their weight. Both heavy and light tops were

observed among the evaluated genotypes. As many as 36 genotypes (70.59 %) showed light tops while 15 genotypes (29.41 %) had heavy tops.

Leaf Characters

Sugarcane leaves possess a number of distinctive features being used for morphological characterization. As many as 13 characters of leaves were recorded. These are: leaf carriage, trashiness, leaf blade texture, leaf blade erectness, leaf margin pubescence, leaf sheath waxiness, presence of prickles on leaf sheath, trichome quality, persistence of prickles, outer auricle shape, inner auricle shape, dewlap waxiness, dewlap shape and legule shape etc.

Two types of leaf carriage were recorded. The open leaf carriage was most frequent (62.75 %) followed by compact leaf carriage (37.25 %). Trashiness tendency of senescent (dry) leaves of sugarcane is preferred character for breeders and farmers as well. Three types of trashing viz. free, intermediate and clinging type were recorded in the studied genotypes. As many as 25 genotypes (49.02 %) were free (self-trashing) followed by 14 genotypes (27.45 %) clinging and 12 genotypes (23.53 %) were intermediate type.

In respect of leaf blade texture, all the genotypes (100%) had smooth surface, which is the desirable texture of leaf. Presence of leaf margin pubescence was also recorded where most of the genotypes (88.24 %) had no pubescent while only six genotypes (11.76 %) had pubescence.

Erect leaves are more efficient in photosysnthesis. From the observation from all mature leaves from the third-fourth leaf onward, four types of leaf blade erectness were recorded. The leaf curved near middle was most frequent (43.14 %) followed by leaf blade curved near tip and bent near tip were present in equal frequencies (25.49 %) while erect to tip type of leaf blade was the lowest (5.88 %).

Table 4.2 Frequency distribution of qualitative morphological traits of 51 genotypes of sugarcane

Sl. No.	Character	Descriptor	Frequency	Percentage
1.	**Plant habit**	1. Erect	35	68.63
		2. Intermediate	15	29.41
		3. Reclining	1	1.96
		Total	**51**	**100**
2.	**Tops**	1. Light	36	70.59
		2. Heavy	15	29.41
		Total	**51**	**100**
3.	**Leaf carriage**	1. Open	32	62.75
		2. Compact	19	37.25
		Total	**51**	**100**
4.	**Trashiness**	1. Free	25	49.02
		2. Intermediate	12	23.53
		3. Clinging	14	27.45
		Total	**51**	**100**
5.	**Leaf blade texture**	1. Smooth	51	100
		2. Rough	-	-
		Total	**51**	**100**
6.	**Leaf blade erectness**	1. Erect to tip	3	5.88
		2. Curved near tip	13	25.49
		3. Bent near tip	13	25.49
		4. Curved near middle	22	43.14
		Total	**51**	**100**
7.	**Leaf margin pubescence**	1. Absent	45	88.24
		2. Present	6	11.76
		Total	**51**	**100**
8.	**Leaf sheath waxiness**	1. Absent	1	1.96
		2. Light	30	58.82
		3. Medium	17	33.33
		4. Heavy	3	5.89
		Total	**51**	**100**
9.	**Presence of prickles/trichomes**	1. Absent	38	74.51
		2. Few	8	15.69
		3. Many	5	9.80
		Total	**51**	**100**
10.	**Trichome quality**	1. Absent	38	74.51
		2. Soft	4	7.84
		3. Hard	9	17.65
		Total	**51**	**100**

Table 4.2 continued…

Sl. No.	Character	Descriptor	Frequency	Percentage
11.	**Persistence of prickles/trichomes**	1. Absent	38	74.51
		2. Deciduous	9	17.65
		3. Non-deciduous	4	7.84
		Total	**51**	**100**
12.	**Outer auricle shape**	1. Sloping transitional	13	25.49
		2. Straight transitional	18	35.29
		3. Ascending transitional	8	15.69
		4. Dentoid	2	3.92
		5. Deltoid	8	15.69
		6. Short Lanceolate	1	1.96
		7. Long lanceolate	1	1.96
		Total	**51**	**100**
13.	**Inner auricle shape**	1. Sloping transitional	13	25.49
		2. Straight transitional	8	15.69
		3. Ascending transitional	13	25.49
		4. Dentoid	3	5.88
		5. Deltoid	6	11.76
		6. Short Lanceolate	1	1.96
		7. Long lanceolate	7	13.73
		Total	**51**	**100**
14.	**Dewlap waxiness**	1. Light	35	68.63
		2. Medium	13	25.49
		3. Heavy	3	5.88
		Total	**51**	**100**
15.	**Dewlap shape**	1. Very sloping , more or less ligulate	2	3.92
		2. Tall, triangular, with convex upper and lower margins	18	35.29
		3. Squarish	5	9.80
		4. Deltoid	2	3.92
		5. Triangular	1	1.96
		6. Triangular with horizontal basal margin	8	15.69
		7. More or less triangular, sloping , with horizontal upper margin	14	27.45
		8. Typical ligulate, very narrow and practically horizontal	1	1.96
		Total	**51**	**100**

Table 4.2 continued…

Sl. No.	Character	Descriptor	Frequency	Percentage
16.	**Legule shape**	1. Crescent with narrow lozenge	1	1.96
		2. Crescent with lozenge	30	58.82
		3. Broad crescent	6	11.77
		4. Linear crescent	8	15.69
		5. Broad subarcuate	4	7.84
		6. Inverted crescent	1	1.96
		7. Arcuate	1	1.96
		Total=	**51**	**100**
17.	**Stalk waxiness**	1. Restricted to the wax band below the leaf scar	1	1.96
		2. Light, restricted to the upper half of internode	9	17.65
		3. light-throughout internode	23	45.10
		4. Heavy-throughout internode	18	35.29
		Total	**51**	**100**
18.	**Splits/growth cracks on cane**	1. Absent	48	**74.12**
		2. Present	3	**5.88**
		Total	**51**	**100**
19.	**Corky cracks on cane**	1. Absent	30	58.82
		2. Present	21	41.18
		Total	**51**	**100**
20.	**Corky patch on cane**	1. Absent	36	70.59
		2. Present	15	29.41
		Total	**51**	**100**
21.	**Internode shape**	1. Cylindrical shaped	25	49.02
		2. Barrel shaped	1	1.96
		3. Bobbin shaped	1	1.96
		4.Conoidal shaped	24	47.06
		Total	**51**	**100**
22.	**Stalk alignment**	1. Straight	48	94.12
		2.Zigzag	3	5.88
		Total	**51**	**100**
23.	**Node swelling**	1. Depressed	1	1.96
		2. Not swollen	22	43.14
		3. Swollen	28	54.90
		Total	**51**	**100**

Table 4.2 continued...

Sl. No.	Character	Descriptor	Frequency	Percentage
24.	**Growth ring width**	1. Narrow	6	11.76
		2. Broad	45	88.24
		Total	**51**	**100**
25.	**Number of rows of root primordia**	1. One row	1	1.96
		2. Two rows	43	84.31
		3. Three rows	4	7.84
		4. Five rows	3	5.88
		Total	**51**	**100**
26.	**Leaf scar prominence**	1. Prominent	51	100
		2. Not prominent	0	0
		Total	**51**	**100**
27.	**Root band shape**	1. Conoidal	1	1.96
		2. Straight	28	54.90
		3. Obconoidal	22	43.14
		Total	**51**	**100**
28.	**Bud shape**	1. Ovate with emarginated wings	12	23.53
		2. Ovate with secondary wings	1	1.96
		3. Simple ovate	3	5.88
		4. Ovate wing broadening	5	9.80
		5. Pentagonal	11	21.57
		6. Roundish with wing	10	19.61
		7. Round with central germ pore	9	17.65
		Total	**51**	**100**
29.	**Bud prominence**	1. Flat	21	41.18
		2. Bulging	30	58.83
		Total	**51**	**100**
30.	**Bud germ pore position**	1. Apical	25	49.02
		2. Sub-apical	22	43.14
		3. Median	4	7.84
		Total	**51**	**100**
31.	**Bud groove/furrow expression**	1. Absent	43	84.31
		2.Low	5	9.80
		3. Medium	3	5.88
		Total	**51**	**100**
32.	**Bud hair group (Hair group No. 9)**	1. Prominent	3	5.88
		2. Not prominent	48	94.12
		Total	**51**	**100**

Table 4.2 Continued….

Sl. No.	Character	Descriptor	Frequency	Percentage
33.	**Bud tip position**	1. Below growth ring	37	72.55
		2. Above growth ring	2	3.92
		3. On growth ring	9	17.65
		4. Combination of 1 & 2	3	5.88
		Total	**51**	**100**
34.	**Bud base position**	1. At leaf scar	32	62.75
		2. Above leaf scar	19	37.25
		Total	**51**	**100**
35.	**Solidness of stalk**	1. Solid	29	56.86
		2. Hollow	13	25.49
		3. Pithy	9	17.65
		Total	**51**	**100**
36.	**Internode cross section shape**	1. Round	51	100
		2. Oval	0	0
		Total	**51**	**100**

Leaf sheath waxiness was also recorded. Out of 51 genotypes, 30 genotypes (58.82 %) had light wax followed by 17 genotypes had medium wax (33.33%), and three genotypes (5.89 %) had heavy wax on the leaf sheath. Only one genotype had no wax on their leaf sheath.

Most of the genotypes (74.51 %) had no prickles (trichome) whereas 8 genotypes (15.69 %) had few prickles (trichome) and 5 genotypes (9.80 %) had many prickles on their sheath.In respect of trichome quality, soft and hard trichomes were recorded. As many as 9 genotypes showed hard trichome (17.65%) while 4 genotypes showed soft trichome (7.84%). The persistence of leaf sheath trichome was also verified. Out of 51 genotypes, 13 genotypes had trichome, where only nine genotypes possessed deciduous trichome (17.65%) and four genotypes had non-deciduous trichome (7.84%).

The auricle is an earlike appendage on the upper age of sheath margin. The shape of auricle is one of the important morphological traits being considered in varietal identification. Of the 10 types of outer auricle, seven types were observed during characterization of sugarcane genotypes. The most predominant type was straight transitional (35.29 %) followed by sloping transitional (25.49 %). Both

ascending transitional and deltoid type were in equal frequencies (15.69 %) whereas, only one genotype with short lanceolate (1.96%) and one genotype with long lanceolate types (1.96%) were observed. It was reported that, of the 569 sugarcane accessions of Southeast Asia, 217 accessions had straight transitional outer auricle while 194 accessions had sloping transitional type (Sugarcane Variety Improvement, 2007).

In case of inner auricle shape, both sloping transitional and ascending transitional types were most frequent (25.49 %) followed by straight transitional (15.69 %) and long lanceolate (13.73 %). Dentoid type was found in only three genotypes (5.88 %) whereas deltoid type was observed in six genotypes (11.76 %). Only one genotype (1.96 %) had short lanceolate type of inner auricle. Out of 569 sugarcane accessions, there were 146 accessions had sloping transitional inner auricle and 113 accessions had straight transitional inner auricle (Sugarcane Variety Improvement, 2007).

Dewlap waxiness character was also recorded. According to the intensity of waxiness, 51 genotypes were categorized as light, medium and heavy. Out of 51 genotypes, 35 genotypes had light wax (68.63%), 13 genotypes with medium (25.49 %) and only three genotypes had heavy wax (5.88%) on their dewlap.

Dewlap shape is one of the important leaf characters being used for varietal identification. Out of nine type's dewlap, eight types dewlap were found in 51 genotypes. Of the eight types, tall, triangular with convex upper and lower margins shaped was most frequent (35.29%) followed by more or less triangular sloping, with horizontal upper margin type (27.45 %) and triangular with horizontal basal margin type (15.69%) were most predominant types.

The ligule is a membranous appendage of sheath separating it from the blade. It is translucent and hyaline. The ligule is a diagnostic character for cultivars (Purseglove, 1988). Among 12 types of legule shape, seven types were observed in 51 genotypes with variable frequencies. The most common legule shape was

crescent with lozenge (58.82 %) followed by linear crescent (15.69 %) and broad crescent (11.77 %). In a sugarcane variety improvement study of Southeast Asia, it was found that approximately 50% (283 accessions) of 569 accessions had crescent shape with lozenge legule, 147 accessions had crescent and broad lozenge, while 111 accessions had crescent shape legule (Sugarcane Variety Improvement, 2007).

Stalk Characters

A number of stalk characters viz. stalk waxiness, splits/growth cracks, corky cracks, internode shape, stalk alignment, node swelling, number of rows of root primordia , solidness of stalk and internode cross section were recorded. Different intensities of wax layer were found in the different portion of the sugarcane stalks. Most of the genotypes (45.10%) had light wax throughout the internode followed by heavy wax (35.29 %) throughout the internode and light wax, restricted to the upper half of the internode (17.65 %).

Growth splits/growth cracks on cane are one of the identifying morphological characters of sugarcane variety. Growth cracks were absent in majority (74.12 %) of the genotypes while only 3 genotypes had growth cracks (5.88%). The corky cracks were also absent in most of the genotypes (58.82 %) and was present in 21 genotypes (41.18%). Most of the genotypes had no corky patch (70.59 %) on the cane while rest of the genotypes (29.41 %) had corky patch on the cane.

Internode Characters

Out of six different shapes of internode, four types were found in 51 genotypes. The cylindrical shaped internode was the most frequent (49.02 %) followed by Conoidal shape (47.06%) internode. The sugarcane stalk may be straight or zigzag shaped. Of the 51 genotypes, 48 genotypes had straight stalk (94.12 %) and rest 3 genotypes (5.88 %) had zigzag stalk.

Cross section of internode was done to observe the solidness and shape of cross section area. Solid internode was found in most of the genotypes (56.86 %) followed by hollow stalk (25.49%) i.e. round hole present at the centre of the stalk. Round shaped cross section was found in all the genotypes (100%).

Node Characters

Different characters of node viz. node swelling, growth ring width, number of rows of root primordia, leaf scar prominence and root band shape were also recorded for characterization of sugarcane. Three types of sugarcane node were found. Most of the genotypes had swollen node (54.90%) followed by not swollen node (43.14%) while only one genotype had depressed node (1.96%).

Majority of the genotypes (88.24%) had broad growth ring while only (11.76 %) genotypes had narrow growth ring. Out of 51 genotypes, 43 genotypes (84.31%) had two rows root primordia, 4 genotypes had three rows root primordia (7.84%), 3 genotypes with five rows root primordia (5.88%) while only one genotype (1.96%) had one row root primordia.

Leaf scar at the nodal region may be prominent or not prominent. Prominent leaf scar was found in all the genotypes (100%).The root band shape was also recorded at the nodal region. Root band shape may be conoidal, straight or obconoidal. The most common root band shape was Straight (54.90%) followed by obconoidal (43.14%). Only one genotype had conoidal shaped root band (1.96 %).

Bud Characters

At the node, a single bud is located which may vary in shape, size, color and hairiness according to cultivar. Bud shape is one of the major identifying characters of a variety /clone. Of the 12 types of bud shape, seven types of bud shape were recorded in 51 genotypes. Among them, ovate with emarginated wing was most frequent (23.53%) followed by pentagonal (21.57%), roundish with wing (19.61%) and round with central germ pore (17.65%) respectively. Only one genotype (1.96%) had ovate with secondary wings shaped bud.

Bud prominence was also recorded as flat or bulging. Of the 51 genotypes, 30 genotypes (58.83%) had bulging type bud while 21 genotypes (41.18%) had flat type bud. Germ pore may be present at apical, sub-apical or median position of the bud. Germ pore was found at apical position of the bud in 25 genotypes (49.02%) followed by sub-apical position of the bud in 22 genotypes (43.14%). Bud germ pore at median position was found in only four genotypes (7.84%).

Expression of bud groove/ furrow on the cane was observed. Most of the genotypes (84.31%) had no bud groove/furrow while low bud groove/furrow was found in five genotypes (9.80%). Medium bud groove/furrow was found in three genotypes (5.88%).Bud hair group number 9 was also recorded as prominent or not prominent. Most of the genotypes (94.12%) had no prominent hair group number 9. Only three genotypes (5.88%) had prominent hair group number 9 in the bud.

Sugarcane bud is categorized in to four types according to relative position of the bud tip on the growth ring. Bud tip below the growth ring was found in most of the genotypes (72.55%) while bud tip on the growth ring was found in 9 genotypes (17.65%).Bud tip above growth ring was found in two genotypes (3.92%), while combination of type 1 & 2 was found in only three genotypes (5.88%). Similar to bud tip, bud base may be present at leaf scar or above leaf scar position. Bud base at leaf scar position was found to be the highest (62.75%) while bud base position above leaf scar was found to be the lowest (37.25%).

4.1.4 Shannon-Weaver Diversity Index

Quantitative Traits

Study of genetic diversity in the available germplasm is an essential first step for varietal development program of any crop. The Shannon-Weaver diversity index (H') (Shannon and Weaver, 1949; Hutcheson, 1970) is one of the widely used methods of estimating genetic diversity of agromorphological traits of germplasm collections (Jaradat 1992; Bechere et al., 1996; Ayane and Bekele 1998; Muyco, 2002). Table 4.3 presents the standardized Shannon-Weaver diversity indices of 16 agromorphological traits of 51 genotypes of sugarcane of BSRI, Ishurdi, Pabna, Bangladesh.

All the quantitative agromorphological traits measured showed high diversity indices based on Shannon-Weaver indices ranged from 0.80 to 0.99. Mean diversity index for all characters among 51 genotypes was 0.94. The highest diversity index (0.99) was found for stalk length, bud length and width, internode length, number of internode/cane and single cane weight while the lowest value of diversity index (0.80) was observed for number of tiller/clump. The most diverse traits were plant height, stalk length, leaf length, bud length and width, number of internode/cane, single cane weight, juice purity percentage, number of millable cane and cane yield with diversity indices > 0.95. The average diversity index was slightly lower (H'=0.88), reported by Muyco (2002) who studied the diversity of 81 sugarcane cultivars of the Philippines. Mean Shannon-Weaver diversity index for quantitative traits was found 0.8967 from studying diversity of 569 sugarcane accessions collected from several countries of Southeast Asia (Sugarcane Variety Improvement, 2007).

Table 4.3 Standardized Shannon-Weaver diversity indices of quantitative agro-morphological traits in 51 sugarcane genotypes of BSRI

Sl. No.	Traits	Diversity Index
1.	Number of tiller/clump	0.80
2.	Plant height	0.96
3.	Stalk length	0.99
4.	Leaf length	0.98
5.	Leaf width	0.92
6.	Bud length	0.99
7.	Bud width	0.99
8.	Internode length	0.99
9.	Internode diameter	0.95
10.	Number of internode/cane	0.99
11.	Single cane weight	0.99
12.	Brix %	0.86
13.	Pol%	0.82
14.	Juice purity %	0.97
15.	Number of millable cane/10 m^{2*}	0.96
16.	Cane yield (t/ha)	0.98
	Mean Diversity Index	**0.94**
	Range	**0.80 - 0.99**

***** ($x10^3$ ha^{-1})

Qualitative Traits

The Shannon-Weaver diversity indices for 40 qualitative traits are presented in Table 4.4. Wide range of diversity was found for qualitative characters of 51 genotypes of sugarcane. The diversity index for qualitative traits ranged from 0 to 0.98. The mean diversity index was 0.59 for all traits and 0.71 when the seven traits, which were monomorphic, were excluded. High Shannon-Weaver diversity (>0.80) indices were found for 14 qualitative characters namely, plant tops (0.87), leaf carriage (0.95),trashiness (0.95), leaf erectness (0.89), outer auricle shape (0.81), inner auricle shape (0.90), stalk waxiness (0.80), corky cracks on stalk (0.97), corky patch on stalk (0.87), bud shape (0.91), bud prominance (0.98) bud germ pore position (0.83), bud base position (0.95), and stalk solidness(0.88). Zero diversity index was found for tillering habit, leaf blade texture, presence of genetic freckles on stem and stripes on cane, leaf scar prominence, and internode cross section shape and leaf blade color.

Moderate diversity indices (H'= > 0.50 to <0.80) were recorded for plant habit (0.63), leaf margin pubescence (0.52), leaf sheath waxiness (0.67), prickles on leaf sheath (0.67), trichome quality (0.67), persistence of trichome (0.57) dewlap waxiness (0.70), legule shape (0.66) and internode shape (0.62). Low diversity indices (H'= >0.0 to 0.50) were found for splits/growth cracks on stalk (0.32), stalk alignment (0.32), number of rows of root primordia (0.42), bud groove/furrow expression (0.49), and bud hair group (0.32). Mean diversity index for qualitative traits was found moderate (0.7509) from the diversity study of 569 sugarcane accessions of several Southeast Asian countries (Sugarcane Variety Improvement, 2007). This result is in accordance with the present experiment.

It was also observed that mean diversity index for quantitative traits (0.94) is higher than the qualitative traits (0.71), indicating that the quantitative traits in this sugarcane germplasm collection are still very diverse, although this magnitude of diversity could be due to genotype x environment interaction, since quantitative traits are greatly influenced by environmental factors.

Table 4.4 Standardized Shannon-Weaver diversity indices of qualitative morphological traits in 51 sugarcane genotypes of BSRI

Sl. No.	Traits	Diversity Index
1.	Plant habit	0.63
2.	Tillering habit	0.00
3.	Plant tops	0.87
4.	Leaf carriage	0.95
5.	Trashiness	0.95
6.	Leaf blade texture	0.00
7.	Leaf blade color	0.00
8.	Leaf erectness(3^{rd} & 4^{th} leaf)	0.89
9.	Leaf margin pubescence	0.52
10.	Presence of genetic freckles	0.00
11.	Leaf sheath waxiness	0.67
12.	Prickles on leaf sheath	0.67
13.	Trichome quality	0.67
14.	Persistence of trichome	0.57
15.	Outer auricle shape	0.81
16.	Inner auricle shape	0.90
17.	Dewlap waxiness	0.70
18.	Dewlap shape	0.79
19.	Legule shape	0.66
20.	Stalk waxiness	0.80
21.	Presence of stripes on cane	0.00
22.	Splits/growth cracks on stalk	0.32
23.	Corky cracks on stalk	0.97
24.	Corky patch on stalk	0.87
25.	Internode shape	0.62
26.	Internode cross section shape	0.00
27.	Stalk alignment	0.32
28.	Stalk solidness	0.88
29.	Node swelling	0.70
30.	Growth ring width	0.52
31.	No. of rows of root primordia	0.42
32.	Leaf scar prominence	0.00
33.	Root band shape	0.70
34.	Bud shape	0.91
35.	Bud prominence	0.98
36.	Bud germ pore position	0.83
37.	Bud groove/furrow expression	0.49
38.	Bud hair group	0.32
39.	Bud tip position	0.60
40.	Bud base position	0.95
Mean (including all traits)		0.59
Mean (excluding monomorphic traits, i.e. when H'=0.00)		0.71

4.1.5 Correlation Analysis between Agromorphological Traits

In living organism, most of the traits are associated with each other and such correlation may be product of some pleiotropic effects of a gene, existence of genes on the same chromosome, chromosomal segmental affiliation or due to environmental influences. Estimates of correlation between a pair of character indicate the inherent relationship that exists between the characters (Heinz, 1987). If there is a high correlation between two characters, selection for one of the characters should result in selection for the other character.

Sugarcane yield is a complex quantitative character which is the final expression and contributions of many components of sugarcane plants. Therefore, determining the most important influencing agromorphological traits to the total variability of sugarcane yield is a vital target to successfully achieve a breeding program. Great efforts have been made to develop proper models that can explain and predict the relationship between the sugarcane yield and its components. Indirect selection for a specific trait is restored to by plant breeders when a high association exists between two traits. Correlated traits should be highly heritable and relatively easier to measure. At the early stages of selection where thousands of genotypes are handled, selection for yield highly relies on visual estimates and less frequently on actual or direct yield measurements. Indirect selection for correlated traits simplifies and hastens the selection process during the early selection stages. The knowledge of correlation coefficient can provide some guide to breeders for selecting best parents for hybridization program.

Pearson's correlation coefficients were computed and measured for all possible combinations of 16 agromorphological traits of 51 genotypes of sugarcane and presented in Table 4.5.

Plant Height

Plant height was highly and significantly correlated with stalk length (0.892) and moderately correlated with single cane weight (0.668), number of internode/cane

(0.519) and cane yield (0.523). Although the correlation coefficient values were < 0.5, plant height was found to have positive and significant correlation with bud length (0.295), bud width (0.365) and leaf length (0.296). Negative but insignificant association was found between plant height and number of millable cane (-0.086), pol percent (-0.057) and juice purity percent (-0.067) while positive but insignificant correlation was observed between plant height and brix percent (0.005). Deng et al. (1995) showed that plant height is positively correlated with cane weight. This result is in accordance with the findings of this experiment. Soomro et al. (2006) also reported that there was positive correlation between plant height and cane weight. Chen et al. (1991) showed that there was positive significant correlation between plant height and brix percentage. This finding is not in full agreement with the result of this experiment. This may be due to different environmental conditions and genotypes used in the experiment.

Stalk Length

Positive and highly significant correlations were observed on the number of internode per cane (0.649), single cane weight (0.606) and cane yield (0.424). Positive and significant association was observed between stalk length and internode diameter (0.302). Positive but insignificant association was found between number of tiller per clump (0.061), leaf length (0.174) and leaf width (0.047), bud length (0.207) and width (0.311), internode length (0.051) and brix per cent (0.028) whereas negative but insignificant correlation was observed between stalk length and number of millable cane(-0.145), pol percent (-0.038) and juice purity percent (-0.036). Madhavi et al. (1991) and Singh et al. (2005) concluded that there was positive association of cane length with single cane weight. Ahmed et al. (2010) observed positive correlation of stalk length with single cane weight, millable cane and cane yield. All these findings support the results of the present experiment.

Number of Tiller per Clump

Number of tiller per clump was positively and highly correlated with the number of millable cane (0.750) and cane yield (0.440). This indicate that higher number of tiller per clump is responsible for increasing the number millable cane per unit area as well as high cane yield per hectare of land. Moderate value of correlation was found between the number of tiller per clump and cane yield. Positive but insignificant correlation was observed between the number of tiller per clump and leaf length (0.047), leaf width (0.197), bud length (0.272), bud width (0.190), internode length (0.268), brix percent (0.007), pol percent (0.064) and juice purity percent (0.088). On the contrary, negative but non-significant association was found between the number of tiller per clump and number of internode per cane (-0.019), internode diameter (-0.038) and single cane weight (-0.082). Ahmed et al. (2010) showed the negative correlation between the number of tiller and stalk diameter, and single cane weight. Reddy and Reddi (1986) concluded that number of tillers were the major constituent of yield.

Leaf Length

Most of the agromorphological traits had no significant relationship with leaf length of sugarcane except internode length. Positive and highly significant correlation was observed on leaf length and internode length (0.406).

Leaf Width

Leaf width was positively and highly correlated with bud length (0.390), internode diameter (0.553) and cane yield (0.370) and moderately correlated with bud width (0.301), internode length (0.338) and single cane weight (0.340). Negative but insignificant correlation was observed between leaf width with number of internode (-0.101), brix percent (-0.081), pol percent (-0.051) and juice purity percent (-0.053).

Bud Length

Positive and highly significant correlations were observed on bud length with bud width (0.860), internode diameter (0.423) and cane yield (0.408), although the value of correlations of internode diameter and cane yield with bud length were found moderate. Bud length was positively and significantly associated with single cane weight (0.373).

Bud Width

Bud width was positively and highly correlated with internode diameter (0.447), single cane weight (0.449) and cane yield (0.476). The correlation coefficient values were moderate and ranged from 0.447 to 0.476. Positive but insignificant association was found between bud width and number of internode (0.119), internode length (0.155), and number of millable cane (0.063), brix percent (0.083) and pol percent (0.020). Bud width had also negative but insignificant correlation with juice purity percent (-0.081).

Number of Internode per Cane

Highly significant positive association was observed between the number of internode per cane with plant height (0.519), stalk length (0.649) and single cane weight (0.534). Significantly positive association was also observed between the number of internode per cane with internode diameter (0.301) and cane yield (0.312). The number of internode per cane was negatively and highly correlated with internode length (-0.508). This suggests that increase in the number of internodes correspond to a decrease in internode length. This could indicate competition effects among stalks. Association of number of internode per cane with bud length (0.053), bud width (0.119) and brix percent (0.055) was found positive but insignificant. The findings of this experiment are in agreement with the results of Muyco (2002) who reported significant positive correlation coefficient values of number of internode per cane with plant height, stalk length, stalk diameter and stalk weight.

Table 4.5 Correlation coefficients among quantitative agro-morphological traits in sugarcane

Traits	Plant height	Stalk length	No. of tiller/ clump	Leaf length	Leaf width	Bud length	Bud width	No. of Internode/ cane	Internode length	Internode diameter	Single cane weight	No. of millable Cane	Brix %	Po l %	Juice purity %	cane Yield
Plant height	-	0.892**	0.0917^{NS}	0.2962*	0.218	0.295*	0.365**	0.519**	0.238^{NS}	0.383**	0.668**	-0.086^{NS}	0.005^{NS}	-0.057^{NS}	-0.067^{NS}	0.523**
Stalk length		-	0.061^{NS}	0.174^{NS}	0.047^{NS}	0.207^{NS}	0.311^{NS}	0.649**	0.051^{NS}	0.302*	0.606**	-0.145^{NS}	0.028^{NS}	-0.038^{NS}	-0.036^{NS}	0.424**
Tiller No./clump			-	0.047^{NS}	0.197^{NS}	0.272^{NS}	0.190^{NS}	-0.019^{NS}	0.268^{NS}	-0.038^{NS}	-0.082^{NS}	0.750**	0.007^{NS}	0.064^{NS}	0.088^{NS}	0.440**
Leaf length				-	0.213^{NS}	0.032^{NS}	0.038^{NS}	-0.040^{NS}	0.406**	0.204^{NS}	0.042^{NS}	0.202^{NS}	0.036^{NS}	0.048^{NS}	0.167^{NS}	0.161^{NS}
Leaf width					-	0.390**	0.301*	-0.101^{NS}	0.338*	0.553**	0.340*	0.102^{NS}	-0.081^{NS}	-0.051^{NS}	-0.053^{NS}	0.372**
Bud length						-	0.860**	0.053^{NS}	0.121^{NS}	0.423**	0.373*	0.085^{NS}	0.081^{NS}	0.024^{NS}	0.089^{NS}	0.408**
Bud width							-	0.119^{NS}	0.155^{NS}	0.447**	0.449**	0.063^{NS}	0.083^{NS}	0.020^{NS}	-0.081^{NS}	0.476**
No. of internode/cane								-	-0.508**	0.301*	0.534**	-0.196^{NS}	0.055^{NS}	-0.001^{NS}	-0.018^{NS}	0.312*
Internode length									-	0.050^{NS}	0.067^{NS}	0.238^{NS}	-0.162^{NS}	-0.144^{NS}	-0.037^{NS}	0.231^{NS}
Internode diameter										-	0.680**	-0.039^{NS}	0.003^{NS}	-0.011^{NS}	-0.054^{NS}	0.577**
Single cane weight											-	-0.206^{NS}	0.021^{NS}	-0.078^{NS}	-0.153^{NS}	0.735**
No. of millable cane												-	-0.101^{NS}	-0.055^{NS}	-0.017^{NS}	0.496**
Brix %													-	0.974**	0.889**	-0.115
Pol %														-	0.915**	-0.127^{NS}
Juice Purity %															-	-0.167^{NS}

* Significant at 5% level; ** Significant at 1% level; NS- Not significant

Internode Length

Internode length was positively and highly correlated with leaf length (0.406). Internode length had negative correlation with the number of internode (-0.508). Internode length had positive significant association with leaf width (0.338). Internode length had positive but insignificant correlation with plant height (0.238), stalk length (0.051), number of tiller per clump (0.268), bud length (0.121), bud width (0.155), internode diameter (0.050), single cane weight (0.067), and number of millable cane (0.238) and cane yield (0.231). Negative but insignificant association was found between internode length and brix percent (-0.162), pol percent (-0.144) and juice purity percent (-0.037). Muyco (2002) reported positive association of internode length with plant height, and stalk length. He also found negative relationship with the stalk diameter and number of internode per cane. Amalraj et al. (2011) reported that there was positive correlation between internode length and plant height. Khan et al. (2001) indicated positive association between internode length and plant height, and negative association between internode length and cane diameter at both genotypic and phenotypic levels.

Internode Diameter

Positive and highly significant correlation coefficient values were observed on internode diameter with plant height (0.383), leaf width (0.553), bud length (0.423), bud width (0.447), single cane weight (0.680), and cane yield (0.577). Positive and significant correlation values were also observed between internode diameter and stalk length (0.302) and number of internode per cane (0.301). Negative but insignificant association was observed between internode diameter and number of tiller per clump (-0.038), number of millable cane (-0.039), pol percent (-0.011) and juice purity percent (-0.054). Madhavi et al. (1991) and Verma et al. (1999) showed that positive association of cane diameter with cane weight. Positive correlation coefficient between cane girth and cane yield was observed by Khan et al. (2015). On the contrary, negative association was

observed between cane girth and number of canes per stool both at genotypic and phenotypic level (Khan et al., 2015). Muyco (2002) reported positive association between cane diameter and each of stalk weight, internode number and cane yield. He also reported negative correlation between stalk diameter and internode length, and number of stalk per m^2. Mariotti (1972) obtained high negative genotypic correlation between stalk diameter and number of stalks per plot. A higher stalk population reduces stalk diameter and stalk weight, thereby producing slender stalks. The negative association between stalk population vs. stalk diameter and between stalk populations vs. stalk weight can be the result of competition for water, soil nutrients and photosynthates production.

Single Cane Weight

Single cane weight was positively and highly correlated with plant height (0.668), stalk length (0.606), bud width (0.449), number of internode/cane (0.534) and internode diameter (0.680) and cane yield (0.735). Significantly positive association was also observed between single cane weight and leaf width (0.340), and bud length (0.373). The association of single cane weight with leaf length (0.042), internode length (0.067) and brix percent (0.021) was positive but insignificant. Whereas association of single cane weight with pol percent (-0.078) and juice purity percent (-0.153) was found negative but insignificant. From these results, it can be concluded that single cane weight can be increased by selecting for thick and tall stalks having higher number of internode per cane. Ahmed et al. (2010) reported positive correlation of single cane weight with tiller per clump, stalk length and stalk diameter. Tyagi and Lal (2007) obtained positive correlation between weight of millable stalks and stalk height. Significant positive association of stalk weight with plant height, stalk length, stalk diameter, number of internode per cane, leaf width and cane yield (TC/Ha) was reported by Muyco (2002). He also observed significant negative association between stalk weights with number of millable cane. All these findings support the results of this experiment.

Number of Millable Cane

Correlation studies showed that (Table 4.5) number of millable cane was positively and significantly correlated with number of tiller per clump (0.750) and cane yield (0.496). These findings indicate that increasing millable cane population per unit area might increase the cane yield per hectare of land. The association of number of millable cane with leaf length (0.202), leaf width (0.102), bud length (0.085), bud width (0.063) and internode length (0.238) was positive but insignificant. Whereas, the relationship of number of millable cane with plant height (-0.086), stalk length (-0.145), number of internode per cane (-0.196), internode diameter (-0.039), single cane weight (-0.206), brix percent (0.101), pol percent (-0.055) and juice purity percent (-0.017) was negative but non-significant. Ahmed et al. (2010) observed significantly positive correlation of number of millable cane with cane yield. Tyagi and Lal (2007) reported significant positive correlation of number of millable stalks with weight per stalk and stalk thickness. Highly significant positive correlation coefficient value was recorded of number of millable stalks with sugar yield (Al-Sayed et al. 2012). Muyco (2002) reported that number of millable stalks were positively correlated with cane yield but negatively correlated with stalk weight, stalk diameter, leaf width and juice purity percent. All these findings support the results of the present experiment.

Juice Quality Characters

High positive correlation values were observed on brix percent with pol percent (0.974) and juice purity percent (0.889). Negative but insignificant association of brix percent with leaf width (-0. 081), internode length (-0.162), number of millable cane (-0.101) and cane yield (-0.115) was observed. Positive but insignificant association of brix percent with each of plant height (0.005), stalk length (0.028), number of tiller per clump (0.007), leaf length (0.036), bud length (0.081), bud width (0.083), number of internode per cane (0.055), internode diameter (0.003) and single cane weight (0.021) was found. Although the strength of these association was very weak.

Highly positive and significant correlation was detected for pol percent with brix percent (0.974), and juice purity percent (0.915). Non-significant positive association of pol percent with the number of tiller per clump (0.064), leaf length (0.048) and bud length (0.024) was observed. Whereas, negative but insignificant association of pol percent with plant height (-0.057), stalk length (-0.038), number of internode per cane (-0.001), internode length (-0.144) and diameter (-0.011), single cane weight (-0.078), number of millable cane (-0.55) and cane yield (-0.115) was found.

Positive and high correlation values were recorded between juice purity percent and brix percent (0.889), and juice purity percentage and pol percent (0.915). Most of the traits viz. plant height (-0.067), stalk length (-0.036), leaf width (-0.053), bud width (-0.081), number of internode per cane (-0.018), internode length (-0.037), internode diameter (-0.054), single cane weight (-0.153), number of millable cane (-0.017) and cane yield (-0.167) had non-significant negative correlation values.

Significant positive and strong correlation between brix percent and pol percent was reported by Tyagi and Lal (2007) and Muyco (2002). Tyagi and Singh (2000), and Nosheen and Ashraf (2003) found positive and significant correlation of brix with sucrose contents. Kumar and Kumar (2014) concluded that there was negative correlation between brix value and yield components of sugarcane. Ahmed et al. (2010) showed negative but insignificant association of pol percent and juice purity percentage with cane yield. Negative but non-significant association of number of millable cane with brix percent and pol percent was also reported by Ahmed et al. (2010).

Cane Yield

Improvement of cane yield is the final goal of sugarcane breeding. Higher cane yield is the function of greater genetic potential of a variety (Nazir et al., 1997). Cane yield was positively and highly correlated with each of plant height (0.523), stalk length (0.424), number of tiller per clump (0.440), leaf width (0.372),

internode diameter (0.577), number of millable cane (0.496) and single cane weight (0.735). Cane yield also showed highly positive and significant association with bud length (0.408) and bud width (0.476). Cane yield showed insignificant positive association with leaf width (0.161). Cane yield also showed insignificant but negative association with brix percent (-0.115), pol percent (-0.127) and juice purity percent (-0.167). Chaudhary and Joshi (2005) reported that cane yield showed highly significant positive association with stalk length and stalk diameter. Chaudhry (1982) concluded that the increase in cane yield was done to combined effect of stalks per stool, length of the stalk and weight per stool. It has been observed that number of stalks per stool was major yield contributing factor followed by height and cane girth (Raman et al., 1985). Ton canes per hectare (TC/Ha) showed low to moderate correlation values with plant height, leaf width, stalk diameter and stalk length (Muyco, 2002). These results are in agreement with the findings of the present investigation.

4.1.6 Relationships between Sugarcane Genotypes based on Agromopological traits

4.1.6.1 Euclidean Distance Estimates

Mean Euclidean distance value was 87.33 between pairs for all possible combinations among 51 genotypes studied. It ranged from 6 for genotypes pair I 14-96/I 326-86 to 251 for SC 10d/ I 156-97 based on 16 agromorphological traits (Appendix Table 4.4). Phenotypic distance estimates between genotypes that ranged from >0.0 to 60 comprised 29.73%, estimates > 60 to 100 made up 36.16%, >100 to 160 accounted for 28.71 % and > 160 accounted for 5.41% of 1275 pair wise combinations (Figure 4.1).

Thirty seven pairs of closely related sugarcane genotypes with Euclidean distances ≤30 are shown in Table 4.6. Twenty three pairs of local genotypes and 14 pairs that involved an exotic and a local genotype showed Euclidean distances ranging from 6 to 30. In contrast, 14 pairs of genotypes had Euclidian distances ≥ 200. The most distant genotype pairs were SC 10d/I 156-97 (251), SC 10d/ I 111-03 (244),

Isd 40/ I 156-97 (228), Isd 40/ I 111-03 (227), Isd 38/ I 156-97 (224), SC 5b/ I 156-97 (219), Isd 38/ I 111-03 (218), SC 10d/Bo 43 (210), SC 5b/ I 111-03 (209), I 1-05/I 156-97(208), I 1-05/I 111-03 (206), SC 10d/I 112-01 (201), Co 642/I 156-97 (201), I 48-05/I 156-97 (201) (Appendix Table 4.4).

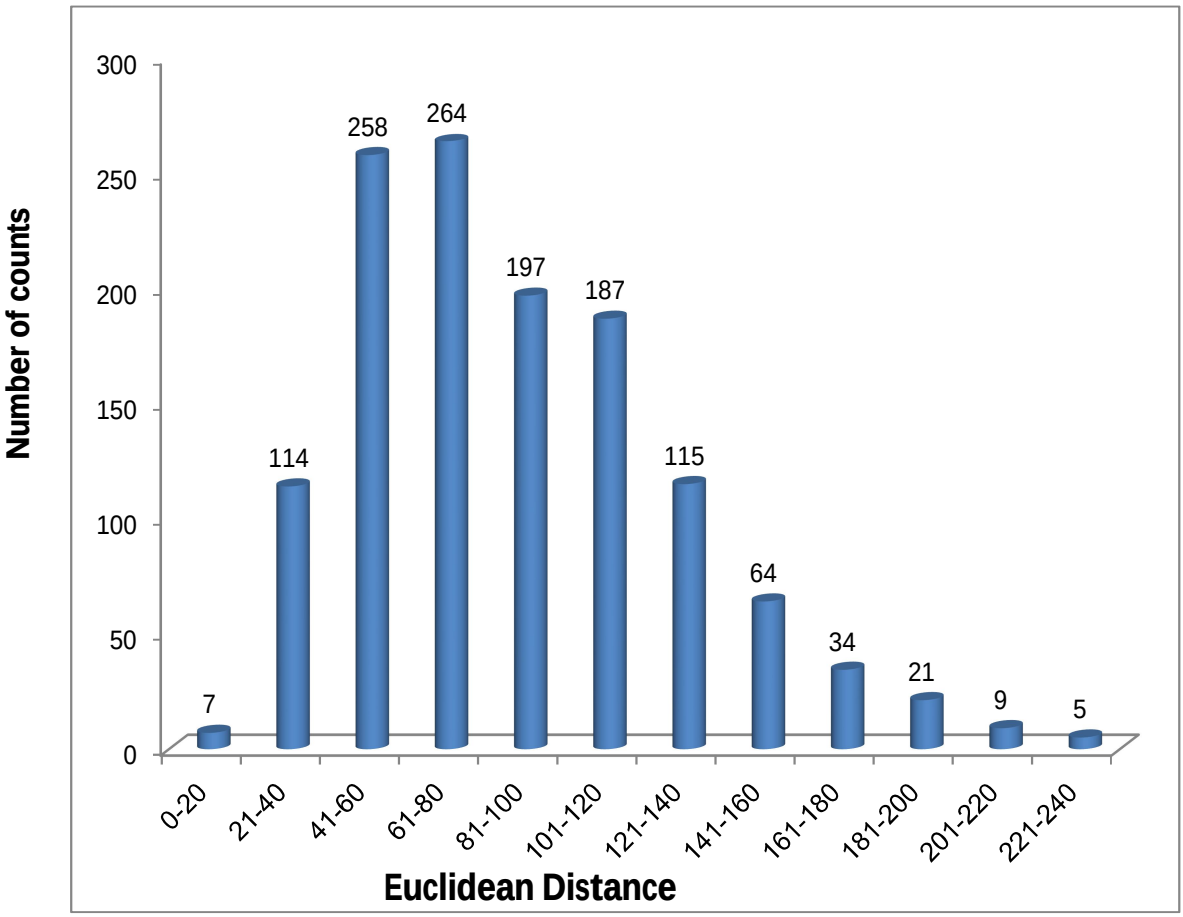

Figure 4.1 Distribution of Euclidean distances between sugarcane genotypes estimated from agromorphological data

Among the 51 genotypes, I 156-97 was the most distant genotypes with a mean Euclidean distance of 87.33 with other 50 genotypes. This genotype displayed Euclidean distances >100 in combination with other 43 genotypes ranged from 63.14 to 153.46. In case of exotic genotypes, mean Euclidean distances were generally low to medium and ranged from 62.92 to 115.24 (Appendix Table 4.4). The almost equal proportion of low and moderate to high distance values observed among the 51 genotypes of sugarcane and among pair wise comparisons (Appendix Table 4.4) indicates a decreasing level of diversity in the

active collection of germplasm as a consequence of crossing genetically related parents. In the segregating population, the level of genetic diversity present diminishes when closely related parents are utilized in the breeding program (Muyco, 2002).

To increase the level of diversity in the active core collection, sampling of parental clones should include genotypes with maximum contribution to the total diversity of the collection. Consequently, duplicates of closely related cultivars can be excluded in the core. In making cross combinations, selection of parents should be based on information of distance estimates among the parents as well as available germplasm. Cox et al. (1986) proposed that crosses between distantly related lines in an inbred improvement program would increase the number of segregating loci in the F_2 and subsequent inbred generations.

Diverse genotypes based on their mean Euclidean distance values can be utilized as parents in the hybridization program. Integrating available information on their good combining ability with other genotypes to the phenotypic distance data, as a criterion in parental selection, ensures a higher chance of generating better performing hybrids. Thus, cross combinations between genetically closely related genotypes should be avoided. Crosses between genetically distant sugarcane genotypes should produce higher variances for quantitatively inherited traits in segregating populations.

Table 4.6 Euclidean distances (≤30) between phenotypically closely related sugarcane genotypes

Sl. No.	Genotype pair		Euclidean distance
1.	I 39-04	I 134-70	15
2.	I 39-04	I 152-04	19
3.	I 6-04	I 326-86	28
4.	I 6-04	CP 69-105	30
5.	Co 635	I 562-85	26
6.	SC 2d	CP 69-1052	30
7.	SC 2d	Co 635	22
8.	SC 2d	CP 75-105	30
9.	SC 2d	I 137-96	28
10.	SC 2d	I 33-97	25
11.	SC 2d	I 26-04	27
12.	SC 2d	I 181-03	21
13.	SC 2d	SC 6d	17
14.	I 14-96	I 326-86	6
15.	I 181-03	CP 69-1052	14
16.	CP 75-361	I 98-98	30
17.	CP 75-361	I 181-03	27
18.	CP 75-361	I 48-05	28
19.	CP 75-361	Co 462	26
20.	CP 75-361	SC 6d	21
21.	I 98-98	I 64-98	24
22.	I 98-98	I 137-96	28
23.	I 98-98	SC 6d	28
24.	I 26-04	I 91-79	30
25.	I 26-04	I 33-97	29
26.	I 181-03	I 33-97	24
27.	I 108-01	Saipan 17	23
28.	I 26-04	I 181-03	30
29.	I 26-04	SC 6d	30
30.	I 26-04	I 108-01	30
31.	I 143-01	I 82-98	26
32.	I 181-03	SC 6d	28
33.	Co 642	I 1-05	30
34.	Co 642	I 48-05	18
35.	I 23-05	I 43-01	30
36.	Isd 38	I 48-05	27
37.	Isd 38	Isd 40	30

4.1.6.2 Cluster Analysis

The Euclidean distances computed from morphological quantitative traits are presented in Appendix Table 4.4.The relationships (dendrogram) among 51 sugarcane genotypes derived by UPGMA cluster analysis are presented in Figure 4.2. Sugarcane genotypes were clustered based on their mean Euclidean distance values. Truncating the line at an average linkage distance of 70 between clusters resulted to six major clusters. Among the different clusters, the cluster size ranged from 2 to 26. Cluster 1 with two genotypes, cluster 2 with seven genotypes, cluster 3 with 26 genotypes, cluster 4 with five genotypes, cluster 5 with only two genotypes and cluster 6 with nine genotypes. The cluster 3 is the largest cluster which is again sub-divided in to three sub-clusters viz. Sub-cluster 1(SC1), sub-cluster 2 (SC2) and sub-cluster3 (SC3).

Cluster 1 was composed of only two genotypes viz. one exotic genotype Co 630 and one BSRI developed Genotype I 21-00.This cluster was characterized by intermediate tillering habit and short internode length, medium tall cane and medium length and width of leaves. Other characteristics included moderate values for internode diameter, number of internode per stalk, single cane weight, brix percent, pol percent and juice purity percent, moderate number of millable cane and medium cane yield. Medium long and wide bud also observed in stalk of this cluster.

Cluster 2 comprised of one exotic genotype- Co 642 and two BSRI developed released varieties viz. Isd 38 and Isd 40 and four BSRI developed advanced clones viz. SC 5b, SC 10d, I 1-05 and I 48-05. These genotypes were tall in stature with longer stalk length, medium sized leaves and intermediate in width, and having moderate values for internode length, brix percent, pol percent, juice purity percent and number of millable cane. This cluster also exhibited high values for the number of internode per stalk, bud length, bud width, and the single cane were also heavy (robust). The cane yield was also high and the genotypes of this cluster showed the highest cane yield among six clusters.

The third cluster was found to be the largest cluster having 26 genotypes. This cluster was sub-divided into three sub-clusters viz. sub-cluster 1(SC1), sub-cluster 2 (SC2) and sub-cluster 3(SC3).

Sub-cluster 1 was composed of BSRI developed clones I 82-98 and I 143-01. This sub-cluster was composed of genotypes having light tillering habit with medium sized plant, short and narrow leaves, short with narrow bud, short internode, thin and light stalks, low brix percent, lowest number of millable cane and lowest cane yield. This sub-cluster also exhibited intermediate values for length and number of internodes, pol percent and juice purity percent.

Genotypes that grouped together in sub-cluster 2 included one exotic genotype Saipan 17 and five BSRI bred genotypes viz. IC 7a, I 91-79, I 108-01, I 26-04 and I 23-05. The distinctive features of these genotypes were light tillering habit, tall plant with long stalks, low number of millable cane and low cane yield. The genotypes of this sub-cluster showed moderate values for leaf length and width, bud length and width, internode length and diameter, number of internode per stalks and single cane weight. This sub-cluster also exhibited intermediate values for brix percent, pol percent and juice purity percent.

Third cluster was characterized by moderate values for most of the economically important traits related to yield. The genotypes under 4th cluster produced second highest yield having characteristic features of profuse tillering habit, long leaves and highest number of millable cane. The sub-cluster 3 comprised of 18 genotypes of which three were exotic genotypes (CP 69-1052, CP 75-361 and Co 635), and 14 were BSRI generated advanced clones (I 6-04, I 326-86, I 562-85, SC 2d, SC 6d, I 181-03, I 33-97, I 137-96, I 98-98, I 64-98, I 17-01, I 174-93, I 255-06, I 14-96 and one BSRI developed released variety Isd 39.

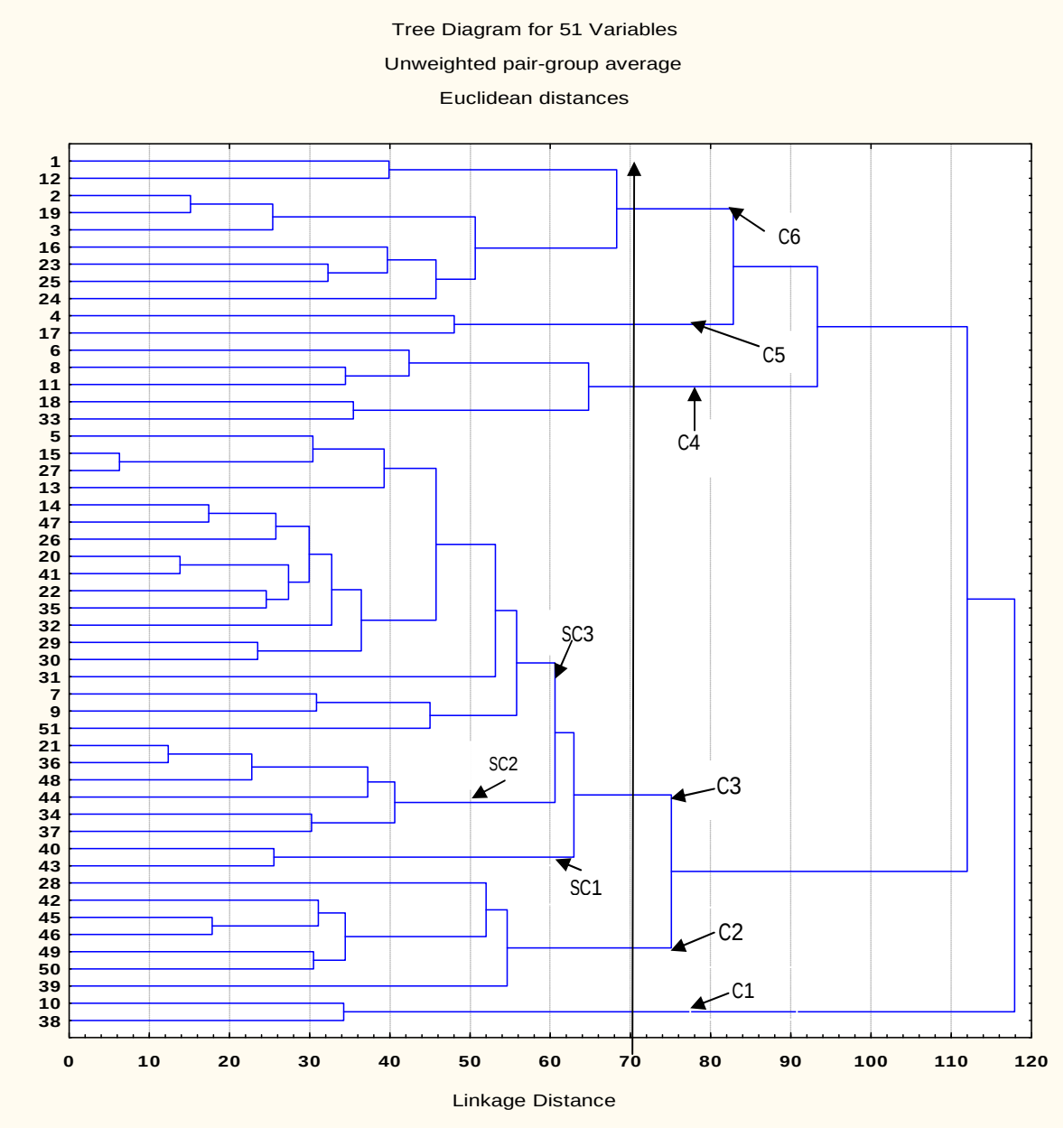

Figure 4.2 Dendrogram derived by UPGMA from agromorphological data using Euclidian distances.

Clusters-C1, C2, C3, C4, C5, C6; Sub-clusters-SC1, SC2, SC3 (1…..51 indicate serial number of genotypes mentioned in Appendix Table 4.2)

These genotypes were of intermediate tillering habit with medium sized canes and stalks having broad leaves. These genotypes were also exhibited moderate values for bud length and width, internode length and diameter, number of internodes per stalk, single cane weight, number of millable cane and cane yield. They also showed intermediate values for brix percent, pol percent and juice purity percent.

Cluster 4 included five genotypes of sugarcane of which two of them were exotic (CPI 96-80 and POJ 2878) and rest three were BSRI bred advanced clones (SC 5d, I 127-96 and I 189-04). The genotypes of this cluster attributed to profuse tillering habit, and having long leaves with moderate width, small bud, highest number of millable cane and high cane yield. The stalks of these genotypes were light in weight having short and medium thick internode but the number of internode per stalk was also low. These genotypes had intermediate values for bud width, brix percent, pol percent, and juice purity percent.

The 5th cluster composed of only two genotypes (I 156-97 and I 111-03).This cluster was characterized by poor tillering habit, short stature, short stalk with small leaves but medium width and medium sized narrow bud. Genotypes of this cluster had light cane with short but medium thick internode and having moderate values for brix percent and pol percent but high values for the purity of juice percentage, low number of millable cane and low cane yield.

The cluster 6 included four exotic genotypes (B 34-231, Bo 43, CL 41-229 and CP 36-105), and five BSRI bred advanced clones (I 112-01, I 39-04, I 134-70, I 152-04 and I 40-00). Genotypes of this cluster characterized by poor tillering habit, short stature and short stalk having thin and short internode. These genotypes had low cane yield, and having low values for leaf length and width, moderate values for bud length and width, number of internode per stalk, single stalk weight, brix percent, pol percent, juice purity percent and number of millable cane.

Table 4.7 Cluster means derived by UPGMA using Euclidean distances for agromorphological traits of 51 sugarcane genotypes

Cluster/ Sub-cluster	Genotype	No. of Tiller/clump	Plant height	Stalk length	Leaf length	Leaf width	Bud length	Bud width	Internode length	Internode diameter	No. of internode	Single cane weight	Brix%	Pol%	Juice Purity%	No. of millable cane/10m²*	Cane yield(t/ha)
C1	Co 630	6.15	430.84	310.66	130.22	4.41	7.07	5.80	10.44	1.75	25.48	0.88	18.99	13.27	87.55	93.44	82.34
	I 21-00	4.79	412.24	290.39	143.62	3.39	6.19	5.30	9.45	1.91	26.28	0.76	19.73	13.89	89.19	108.44	83.95
	Mean	**5.47**	**421.54**	**300.53**	**136.92**	**3.90**	**6.63**	**5.55**	**9.945**	**1.83**	**25.88**	**0.82**	**19.36**	**13.58**	**88.37**	**100.94**	**83.15**
C2	SC 10d	4.86	515.77	357.16	146.42	4.07	6.92	5.86	11.71	2.14	31.88	1.44	15.53	10.17	82.81	93.44	136.61
	I 1-05	4.16	495.44	336.69	137.29	3.57	8.35	7.09	11.14	1.82	26.28	1.10	18.79	12.17	83.95	85.44	93.51
	I 48-05	3.36	469.54	353.29	136.49	3.95	7.46	7.33	9.07	1.89	29.18	1.15	19.49	12.59	83.69	89.44	102.31
	Co 642	3.96	474.44	350.49	138.29	5.13	8.62	6.46	9.38	2.12	31.18	1.36	21.79	15.13	89.58	74.44	100.39
	Isd-38	5.30	477.70	371.90	142.92	3.82	7.07	6.83	11.10	2.10	29.73	1.24	22.38	15.94	89.65	94.00	116.63
	Isd-40	4.43	500.57	362.23	130.62	4.21	7.38	6.53	11.25	2.07	29.25	1.19	20.33	14.61	90.13	88.67	105.45
	SC 5b	6.79	465.44	357.39	136.82	4.18	8.24	7.33	12.12	1.81	27.18	1.10	19.93	14.06	89.40	121.44	134.89
	Mean	**4.69**	**485.56**	**355.59**	**138.41**	**4.13**	**7.72**	**6.78**	**10.82**	**1.99**	**29.24**	**1.23**	**19.75**	**13.52**	**87.03**	**92.41**	**112.83**
C3																	
SC1	I 82-98	3.79	422.74	320.99	113.42	3.23	5.57	4.73	10.01	1.68	26.68	0.74	19.33	14.02	91.86	64.44	48.37
	I 143-01	2.96	436.34	312.79	132.29	2.96	6.24	5.45	11.29	1.69	24.28	0.78	18.59	12.40	86.42	63.44	49.47
	Mean	**4.57**	**439.49**	**319.14**	**145.62**	**4.46**	**6.97**	**5.94**	**11.29**	**1.91**	**26.11**	**0.98**	**19.30**	**13.44**	**88.04**	**92.49**	**89.28**

Table 4.7 continued…

Cluster/ Sub-cluster	Genotype	No. of Tiller/clump	Plant height	Stalk length	Leaf length	Leaf width	Bud length	Bud width	Internode length	Internode diameter	No. of internode	Single cane weight	Brix%	Pol%	Juice Purity%	No. of millable cane/10m²*	Cane yield(t/ha)
C3																	
SC2	IC 7a	4.69	469.51	360.56	146.45	3.77	5.35	4.92	12.29	1.75	25.08	0.91	19.56	13.69	88.21	63.11	55.63
	Saipan 17	3.39	465.44	353.39	143.72	3.61	5.77	5.24	9.59	2.08	32.38	0.88	20.83	14.96	91.02	61.44	54.49
	I 108-01	3.56	469.54	343.59	159.29	4.06	7.82	6.44	11.90	1.76	24.98	1.00	18.79	12.82	88.33	63.44	63.10
	I 23-05	3.36	453.84	321.79	152.09	3.48	6.81	5.56	10.98	1.62	24.98	0.73	19.89	13.69	89.00	72.44	52.94
	I 91-79	4.19	458.34	363.39	159.62	3.62	6.40	5.28	11.86	1.84	23.88	0.87	19.23	13.28	87.51	89.44	78.81
	I 26-04	5.59	464.94	334.69	158.02	3.74	7.13	6.05	10.07	1.72	29.48	0.88	19.63	13.73	88.63	87.44	77.89
	Mean	**3.94**	**455.09**	**338.90**	**145.61**	**3.56**	**6.39**	**5.46**	**11.00**	**1.77**	**26.47**	**0.85**	**19.48**	**13.57**	**88.87**	**70.65**	**60.09**
C3																	
SC3	I 64-04	3.96	444.41	297.06	168.6	4.72	6.86	5.44	12.94	1.91	23.18	0.93	19.84	14.19	89.92	82.11	77.42
	I 326-86	4.33	439.14	304.06	163.82	4.10	5.52	5.00	12.18	1.66	23.68	0.70	18.79	13.27	88.49	108.44	75.74
	I 14-96	5.86	440.97	304.06	162.32	6.95	6.86	5.64	11.45	1.92	23.58	0.67	18.53	12.77	87.15	112.44	74.19
	I 562-85	4.93	420.94	309.66	143.72	3.16	5.04	4.51	12.59	1.40	20.78	0.60	20.59	14.86	90.50	95.44	56.94
	SC 2d	4.13	442.04	334.56	146.52	4.60	4.64	4.45	12.41	1.70	26.28	0.87	18.09	12.76	88.36	85.44	74.43
	SC 6d	4.56	449.24	337.89	133.89	4.52	7.41	6.26	9.75	1.88	27.28	1.00	18.59	12.39	86.41	80.44	80.11
	CP 75-361	4.06	452.17	337.36	139.02	4.10	7.20	5.85	9.73	2.14	29.98	1.26	21.53	15.46	90.83	77.44	99.35
	CP 69-1052	2.89	442.61	317.66	159.35	4.03	5.27	4.65	10.59	2.21	30.48	1.11	21.06	14.28	89.83	87.11	94.19
	I 181-03	4.96	442.84	321.69	151.09	3.92	8.71	6.79	11.07	1.86	22.68	1.04	20.49	14.01	88.36	86.44	89.51
	Co 635	4.89	430.01	322.16	140.45	3.80	7.18	5.85	10.91	1.64	28.78	0.82	19.56	13.75	88.6	96.11	75.98
	I 33-97	5.59	448.94	328.99	146.62	4.26	9.19	7.06	11.41	1.93	26.18	0.76	19.53	13.55	87.88	106.44	82.39

Table 4.7 continued ….

Cluster/ Sub-cluster	Genotype	No. of Tiller/clump	Plant height	Stalk length	Leaf length	Leaf width	Bud length	Bud width	Internode length	Internode diameter	No. of internode	Single cane weight	Brix%	Pol%	Juice Purity%	No. of millable cane/10m²*	Cane yield(t/ha)
C3																	
SC3	I 137-96	5.06	419.87	335.66	155.42	4.37	8.96	7.60	10.31	2.10	29.28	1.00	18.93	13.23	88.39	86.44	87.81
	I 98-98	4.66	424.57	332.76	129.62	4.25	6.31	6.09	10.27	1.95	27.28	1.09	18.83	13.13	88.21	84.44	93.55
	I 64-98	4.06	419.07	312.66	122.42	4.25	6.84	5.22	9.31	1.81	29.48	1.03	20.73	14.70	89.69	83.44	87.36
	I 17-01	5.26	462.67	329.76	171.32	3.82	6.42	5.95	11.71	1.76	26.48	0.98	20.13	14.27	89.66	108.44	106.89
	I 174-93	3.56	430.01	305.06	143.60	4.67	6.28	6.36	11.64	2.39	27.28	1.47	17.09	11.65	85.55	82.11	122.24
	I 255-06	4.73	440.04	303.46	126.02	6.05	7.97	6.10	13.31	2.27	22.18	1.25	13.69	8.41	76.54	101.44	127.49
	Isd-39	4.85	461.35	310.03	117.33	4.63	8.76	8.02	11.57	1.77	25.07	1.02	21.47	15.39	90.26	100.67	101.47
	Mean	**4.57**	**439.49**	**319.14**	**145.62**	**4.46**	**6.97**	**5.94**	**11.29**	**1.91**	**26.11**	**0.98**	**19.3**	**13.44**	**88.04**	**92.49**	**89.28**
C4	I 189-04	5.56	405.21	254.06	163.50	4.66	8.14	6.54	13.35	1.89	20.88	0.78	20.24	14.4	89.43	101.11	79.98
	SC-5d	4.76	411.21	287.26	158.80	3.92	6.36	6.48	10.86	1.99	23.38	0.93	19.59	14.01	89.85	108.11	101.86
	CPI 96-80	5.33	415.64	272.56	146.92	4.14	5.51	4.28	10.28	1.66	24.38	0.67	20.39	14.67	90.25	133.44	89.19
	POJ 2878	9.29	427.01	295.06	147.55	4.35	7.64	5.90	11.74	1.83	22.88	0.76	19.86	14.30	90.80	159.11	117.37
	I 127-96	5.79	398.64	303.89	153.42	3.05	5.31	4.47	10.64	1.72	27.58	0.60	18.03	12.28	86.24	166.44	102.79
	Mean	**6.15**	**411.54**	**282.57**	**154.04**	**4.02**	**5.59**	**5.53**	**11.37**	**1.82**	**23.82**	**0.75**	**19.62**	**13.93**	**89.31**	**133.64**	**98.24**
C5																	
	I 111-03	3.36	323.21	230.56	141.30	4.03	7.40	5.64	10.46	1.77	21.58	0.64	20.19	14.40	89.62	87.11	56.60
	I 156-97	4.29	330.51	231.46	104.25	3.91	5.70	4.63	9.73	1.76	20.78	0.59	21.56	15.37	89.91	65.11	36.93
	Mean	**3.83**	**326.86**	**231.01**	**122.78**	**3.97**	**6.55**	**5.14**	**10.10**	**1.77**	**21.18**	**0.62**	**20.88**	**14.885**	**89.77**	**76.11**	**46.77**

Table 4.7 continued…

Cluster/ Sub-cluster	Genotype	No. of Tiller/clump	Plant height	Stalk length	Leaf length	Leaf width	Bud length	Bud width	Internode length	Internode diameter	No. of internode	Single cane weight	Brix%	Pol%	Juice Purity%	No. of millable cane/10m²*	Cane yield(t/ha)
C6																	
	I 112-01	4.36	359.61	244.36	141.5	4.58	7.53	6.55	11.61	2.02	20.38	0.77	19.59	14.05	90.16	112.11	87.54
	B 34-231	7.13	362.84	263.76	117.02	3.24	6.43	5.5	11.04	1.39	21.28	0.53	19.09	13.31	87.35	120.44	63.4
	I 39-04	3.76	404.21	280.66	132.4	3.93	7.67	5.32	9.82	2.06	27.68	0.94	19.29	13.65	86.33	76.11	72.55
	I 134-70	3.49	394.81	289.06	126.45	2.74	4.73	3.88	8.27	1.10	28.08	1.02	19.06	13.20	87.32	76.11	76.95
	I 152-04	3.56	417.21	281.56	143.00	5.47	5.92	5.39	11.26	1.83	24.68	0.83	20.29	14.51	89.93	78.11	65.76
	Bo 43	3.53	365.74	254.16	129.82	2.46	5.26	4.85	10.76	1.39	22.68	0.42	16.89	10.99	81.87	81.44	33.60
	CL 41-229	3.09	380.71	287.26	139.95	3.67	6.39	5.36	8.85	1.47	28.58	0.52	17.26	11.72	85.54	77.11	38.45
	CP 36-105	3.26	370.87	282.96	121.42	2.63	6.11	6.08	9.99	1.60	28.18	0.59	18.73	12.78	86.30	93.44	56.05
	I 40-00	2.69	386.41	256.46	154.35	3.98	6.12	5.65	10.9	1.84	22.48	0.82	20.86	15.01	90.72	63.11	50.05
	Mean	**3.87**	**332.49**	**271.14**	**133.99**	**3.63**	**6.24**	**5.40**	**10.28**	**1.63**	**24.89**	**0.72**	**19.00**	**13.25**	**87.28**	**86.44**	**60.48**

	Low	Medium	High
No. of tiller/clump:	<4	4-6	>6
Plant height (cm):	<410	410.02 -450.66	> 450.66
Stalk length (cm):	<293.22	293.22-328.17	>328.17
Leaf length (cm):	<134.78	134.78-149.54	>149.54
Leaf width (cm):	<3.65	3.65- 4.44	>4.44
Bud length (mm):	<6.21	6.21- 7.36	> 7.36
Bud width (mm):	<5.33	5.33-6.25	>6.25
No. of internode:	<24	24-28	>28

	Low	Medium	High
Internode length (cm):	<10.34	10.34-11.48	>11.48
Internode diameter(cm):	<1.71	1.71-1.95	>1.95
Single cane weight(kg):	<0.78	0.78-1.02	>1.02
Brix %:	<19	19-21	>21
Pol%:	<13	13-15	>15
Juice purity%	<86.74	86.74-89.46	>89.46
No. of millable cane/10m²*	<81	81-103	>103
Cane yield (t/ha)	<69.36	69.36-94.28	>94.28

* ($x10^3$/ha)

In the present study, an attempt has been made to determine the extent of diversity among 51 genotypes using agro-morphological traits. These easily observable morphological traits are useful tool for preliminary evaluation, because they offer a fast and useful approach for accessing the extent of diversity. Diversity studies based on agronomic characters using Euclidian distances have been done in many crops viz., sugarcane (Muyco, 2002, Zhou et al., 2015), soybean (Dayaman, 2007), and wheat (Mishra et al., 2015).

Different clusters showed no distinct classification of the 51 genotypes. Exotic and locally bred genotypes were loosely distributed in the different clusters suggesting that they are distantly related. Only a few exotic or BSRI developed genotypes clustered together with other genotypes indicating they are phenotypically related or similar. The first cluster was characterized by medium values for most of the economically important traits like juice quality and yield contributing characters except internode length. The 2nd cluster was the highest yielding among the six clusters with characteristic features of high values for most of the yield component characters viz. number of tiller per clump, plant height, stalk length, internode diameter, number of internode per stalk, single cane weight and moderate value for internode length and number of millable cane.

All the sub-clusters showed low to moderate number of tillers per clump, medium to tall plant, medium to long stalk, small to medium leaf, thin to medium stalk diameter and low to moderate values for single cane weight and number of millable cane. Among the three clusters, sub-cluster 3 was highest yielding while the sub-cluster 1 produced the lowest cane yield.

The exotic and locally bred genotypes that clustered together are phenotypically related based on traits being assessed. Such case is not unexpected because these genotypes had undergone several years of selection for desirable traits required by plant breeders from different breeding programs. However, morphological similarities or differences in the population could be a result of different allele combinations producing similar phenotypes that were not proportional to the underlying genetic differences (Johns et al., 1997).

Tai et al. (1996) studied the diversity of four juice quality characters (sucrose, glucose, fructose and Brix) and five morphological characters (fiber content, stalk diameter, leaf length and leaf width and leaf module) of 125 S*. spontaneum* clones collected from World Collection of Sugarcane and Related Grasses. Their results indicated that a considerable high variation present in the collection for the characters studied. The clones from India were the most diverse and were scattered in nine clusters. The clones from Indonesia, the Philippines and Taiwan were also diverse and were scattered in more than five clusters.

Genetic diversity studies based on agronomic characters (Kanwal et al., 1983, Shamsuddin, 1985; Sidhu and Menhdiratta, 1981) assumed that differences of measured characters approximate the genetic divergence of loci throughout the genome. Classification using multiple agronomic characters identifies a genotype's relationship with other genotypes. Cluster analysis based on quantitative characters can be a useful tool for classifying numerous genotypes and in parental selection because it provides information on specific traits of genotypes from different clusters.

4.1.6.3 Mahalanobis D^2 statistic

Genetic divergence arises due to geographical separation or due to genetic barriers to cross ability or due to different patterns of evolution could be measured following D^2 statistic that measure cluster distance based on multiple traits (Mahalanobis, 1928) and it has become one of the important techniques to estimate genetic diversity on the basis of multiple characters. With this technique, one can easily predict genotypes which have high index scores and fall into different clusters can be crossed to have maximum variability of good combinations of traits. Application of Mahalanobis D^2 statistics for estimating genetic divergence had been emphasized by many workers (Vavilov, 1951; Murty and Aurunachalam, 1966; Singh and Bains, 1968; Singh and Gupta, 1968), because it permitted precise comparison among all possible pairs of population in any group before affecting actual crosses. In addition to helping in the selection of diverse parents for crossing, D^2 statistics also measures the degree of

diversification and determines the relative contribution of each component character to the total divergence (Singh, 1990). Rao (1952) suggested the application of this technique for the assessment of genetic divergence in crop improvement program. Diversity analysis is being considered a powerful tool in quantifying the degree of divergence at genotypic level based on phenotypic data in different crops.

Sixteen morphological characters were used to calculate Mahalanobis D^2 statistic (Mahalanobis, 1936) to study the divergence among 51 genotypes of sugarcane on multivariate scale. Assuming the D^2 values as χ^2, it appears that there were significant variations among all the genotypes. Fifty one genotypes were grouped in to six clusters, which are presented in Table 4.8. The cluster IV was the largest cluster containing 17 genotypes while cluster VI was the second largest cluster containing 10 genotypes. Each of the clusters III and V contained 7 genotypes. Similarly, both the clusters I and II were the smallest and contained 5 genotypes of each (Table 4.8). Appendix Table 4.5 shows entry name (genotype name) mentioned against each entry number used for estimation of D^2 statistic followed by clustering.

Average intra and inter-cluster distance of six clusters are presented in Table 4.9. The magnitude of intra-cluster distances indicated the extent of genetic diversity among genotypes within the same cluster. The distances between clusters were more than intra-clusters distances indicating that diversity in between clusters was more than within clusters. Singh and Singh (1980) used Mahalanobis's statistical distance to group 48 sugarcane varieties collected from different geographical sources on the basis of eight agronomic characters which include millable cane population, stalk and internode characteristics.

Table 4.8 Distribution of 51 sugarcane genotypes in six clusters based on D^2 statistic

Cluster	No. of genotypes	Entry number	Genotypes
Cluster I	5	4,7,15,19,20	I 112-01, I 111-03, B 34-231, Bo 43, I 156-97
Cluster II	5	11,14,21,36,41	SC 5d, CPI 96-80, Poj 2878, I 127-96, I 21-00
Cluster III	7	5,6,9,22,26,27,28	I 39-04, I 152-04, I 189-04, I 174-70, CL 41-229, I 40-00, CP 36-105
Cluster IV	17	2,8,10,12,13,16,18,23, 25,29,30,32,33,34,35, 38, 44	Isd 39, I 64-04, I 174-93, I 255-06, Co 630, I 562-85, I 326-86, CP 69-1052, Co 630, CP 75-361, I 14-96, I 98-98, I 64-98, I 17-01, I 137-96, I 33-97, I 181-03
Cluster V	7	1,3,31,42,45,48,49	Isd 38, Isd 40, SC 10d, SC 5b, I 1-05, I 48-05, Co 642
Cluster VI	10	17,24,37,39,40,43,46, 47,50,51	SC 2d, IC 7a, I 91-79, Saipan 17, I 26-04, I 82-98, I 143-01, I 23-05, SC 6d, I 108-01

Gill and Tripathi (1983) also used the same technique to investigate the nature of divergence among 30 foreign sugarcane varieties using twelve agronomic and juice quality characteristics. In both the studies, authors reported that the distribution of clones into the different clusters was not on the basis of their geographical origin.

Table 4.9 Average intra and inter cluster distances based on D^2 statistic in 51 sugarcane genotypes

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster I	**0.8506**	6.403	3.839	8.086	12.358	10.648
Cluster II		**0.6030**	6.132	6.533	9.595	9.285
Cluster III			**0.7498**	4.532	8.797	6.872
Cluster IV				**0.6433**	4.287	2.868
Cluster V					**0.5373**	2.628
Cluster VI						**0.5726**

The highest distance was obtained between the cluster I and cluster V (12.358) indicating the wider genetic divergence between these two clusters. It was followed by the distance between the clusters I and VI, and II and V. Cluster I had the highest distance from the rest of the clusters indicating that the genotypes in this cluster I was distantly related from others. Thus, genotypes with

high index for specific character that fall into different clusters could be intercrossed to have maximum hybrid vigor and good number of useful segregants. The distance between cluster V and cluster VI was minimum (2.628) followed by the distance between the clusters IV and VI (2.868) indicating that the genotypes belonging to these clusters were comparatively less diverse. Thus crossing of genotypes from these two clusters may not produce high level of heterotic expression in the F_1's and broad spectrum variability in segregating populations. Clusters with comparatively less magnitude of divergence showed instability, while widely divergent clusters remained distinct in different environments (Somayajulu et al., 1970; Raut et al., 1985 and Singh et al., 1980). Parents for hybridization could be selected on the basis of large inter-cluster distance for isolating useful recombinants in the segregating generations. Increasing parental distance implies a greater number of contrasting alleles at the desired loci, and then to the extent that these loci recombine in the F_2 and F_3 generations, following a cross of distantly related parents, the greater will be the opportunities for successful selection for any character of yield of interest (Ghaderi et al., 1984). Principal component analysis was done on 16 agromorphological traits.

Principal Component Analysis (PCA) also helps in assessment of diversity in multivariate scales. In PCA, the first five components were found to contribute 81.31% of the total variation. Therefore, scores obtained for the first two components were plotted against two main axes and then superimposed with clustering (Figure 4.3). This clustering pattern confirmed the results obtained by D^2 analysis. (Number 1…..51 indicate entry number of genotypes, Appendix Table 4.5 shows entry name (Genotype).

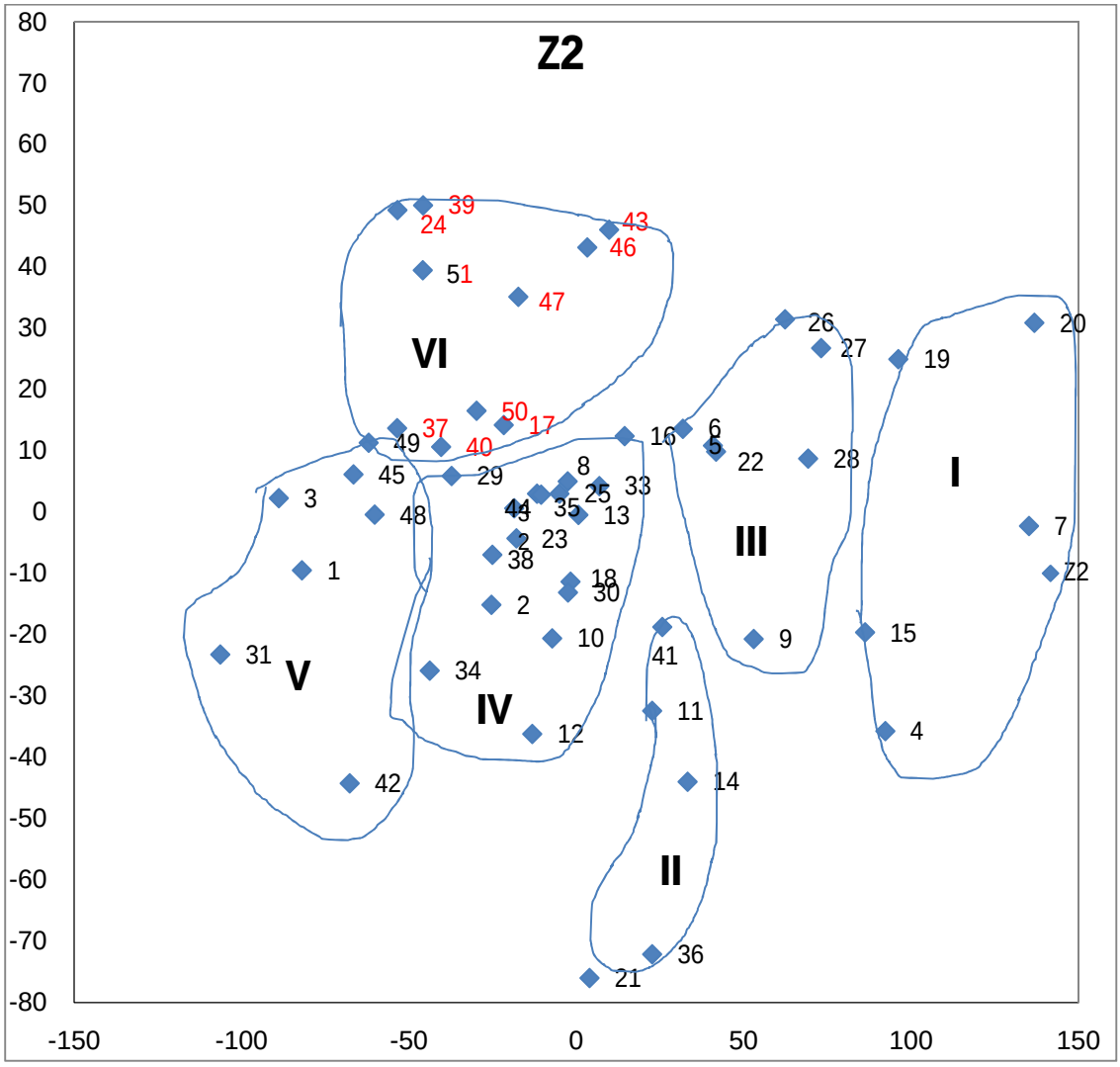

Figure 4.3 Scatter distribution of 51 sugarcane genotypes based on their principal component scores super imposed with clustering

4.1.6.4 Principal Component Analysis (PCA)

The PCA methodology was used to reduce the dimension and attempt to find patterns in the data. Principal Component Analysis (PCA) also helps in assessment of diversity in multivariate scales. Principal component analysis (PCA) was done on 16 agromorphological traits of sugarcane. Applying PCA to data removes the colinearity between characters and residual variability (Hamon et al., 1995). New uncorrelated variables (Eigenvectors) and new coordinates of each individual were obtained. Residual variability is eliminated when an axis is with an eigenvalues greater than 1 is considered. Thus, the different original

variables were reduced in to new sets of uncorrelated variables that represent the variation of the collection. The matrix of correlation coefficients among the 16 morphological traits served as the input data for this analysis.

The proportion of variance criterion determines the number of principal components with cumulative variation of 80% to be retained. The first five components that accounted for 81.31% of total variation were retained (Table 4.10). Initial eigenvalues presented in Table 11 were above 1 for components 1, components 2, components 3, components 4 and components 5.

Table 4.10 Eigenvalues and cumulative variance of the five principal components retained following the proportion of variance criterion

Principal components	Eigenvalue	Percent	Cumulative
PC1	4.61	28.82	28.82
PC2	3.00	18.11	46.93
PC3	2.59	16.18	63.11
PC4	1.50	9.39	72.50
PC5	1.41	8.81	81.31

The PC1 showed the highest contribution of 28.82% to the total variability present in the 51 genotypes. The PC2 contributed 18.11% while PC3 and PC4 accounted for 16.18% and 9.39% contribution, respectively to total variation with a cumulative contribution of 72.50% for the first four principal components. The PC5 explained 8.81% of the total variation. The cumulative variance explained by the first five components was 81.31 %. In the contrary, Muyco (2002) found first four principal components giving rise to 76% variation in the data while Tahir et al., (2013) used only two components which account for 88% variation. Table 4.11 shows the component loadings of the first five components. Characters with largest absolute value close to unity within the first principal components influences the clustering more than those with low values.

Traits separating across the first five components (absolute loadings in parenthesis) were plant height (0.732) and stalk length (0.621). Plant height and stalk length are commercially important traits used as selection criteria to evaluate outstanding clones. The high correlation value of plant height and stalk length was supported by the principal component (PC) analysis.

Table 4.11 Component loadings of the five principal components retained following the proportion of variance criterion

Character	PC1	PC2	PC3	PC4	PC5
No. of tiller/clump	-0.00283	-0.02877	0.00283	0.02909	0.02699
Plant height	0.73231	0.05621	0.22503	-0.34263	0.53910
Stalk length	-0.62148	0.19843	-0.17604	0.61862	-0.39195
Leaf length	-0.07322	-0.10412	0.83275	-0.13110	-0.52007
Leaf width	-0.00270	-0.00726	0.00333	-0.02363	-0.00232
Bud length	-0.00622	-0.00873	-0.00998	-0.01977	0.00024
Bud width	-0.00644	-0.00676	-0.01086	-0.01393	-0.00714
Internode length	-0.00386	-0.01077	0.02772	-0.01756	0.01280
Internode diameter	-0.00181	-0.00186	-0.00215	-0.00791	-0.00798
No. of internode/stalk	-0.03411	0.01621	-0.05595	0.02792	-0.06665
Single cane weight	-0.00311	-0.00088	-0.00598	-0.00878	-0.00708
Brix %	-0.00005	0.00723	0.000720	0.00479	-0.00446
Pol %	0.00154	0.00444	0.00863	0.00507	-0.00484
Juice purity %	0.00342	0.00825	0.03989	0.02337	-0.02221
No. of millable cane/10 m^2 *	0.02027	-0.72515	0.21832	0.54410	0.35541
Cane Yield (t/ha)	-0.26537	-0.64751	-0.41393	-0.42763	-0.39103

* ($x10^3$ ha^{-1})

Traits separating across the first five components (absolute loadings in parenthesis) were plant height (0.732) and stalk length (0.621). Plant height and stalk length are commercially important traits used as selection criteria to evaluate outstanding clones. The high correlation value of plant height and stalk length was supported by the principal component (PC) analysis.

In the second principal component (PC2), traits causing the separation of genotypes were the number of millable cane (0.725) and yield (0.647) i.e. these two traits are the major contributors for the diversity. These two characters-number of millable cane and cane yield are highly correlated with each other. The combined sets of PC1 and PC2 characters explained 46.93% of the variation existing in 51 genotypes. These characters are plant height, stalk length and number of millable cane.

In PC3, separation of genotypes was mainly due to leaf length (0.832) and cane yield (0.413). High component loadings for stalk length (0.618), number of millable cane (0.544) and cane yield (0.427) attributed to the variation in the 4th principal component (PC4). In the 5th principal component (PC5), plant height

(0.539), stalk length (0.391), leaf length (0.520), number of millable cane (0.355) and yield (0.391) contributed to the total variation.

From the total of 16 principal component axes, the first 7 axes accounted for 91.38% of the multivariate variation among genotypes (data not shown) indicating a high degree of correlation among characters for these genotypes. Muyco (2002) found 93% variation from first 7 axes of PCA of 16 agromorphological traits of sugarcane. Findings of Muyco (2002) are in accordance with this investigation. Contribution of characters towards divergence was estimated through canonical variate analysis. In this method, vectors of canonical roots were calculated to represent the genotypes in the graphical form (Rao, 1952). The coefficients pertaining to the different characters in the first two Canonical roots are presented in Table 4.12.

Table 4.12 Contribution of characters towards divergence in sugarcane genotypes

Traits	Vector I	Vector II
No. of tiller/clump	-0.1757	0.1651
Plant height	0.3089	0.2327
Stalk length	0.1493	-1.8046
Leaf length	-0.1244	-0.1905
Leaf width	0.2550	-0.5343
Bud length	0.0182	-0.5459
Bud width	0.4621	-0.3639
Internode length	0.0715	0.6954
Internode diameter	0.4876	0.1412
No. of internode/stalk	0.1211	0.7617
Single cane weight	0.0348	1.0829
Brix %	-1.5090	-2.1828
Pol %	1.0949	0.3091
Juice purity %	-0.8139	0.6176
No. of millable cane/10m^2*	-1.3068	-0.0893
Cane yield (t/ha)	1.3912	-0.6594

*:(x10^3 ha^{-1})

The positive absolute values of the two vectors revealed that plant height, internode length, internode diameter, number of internode/cane, single cane weight and pol percent had the greatest contribution to genetic divergence (Table 4.12). The negative absolute values for two vectors for leaf length, brix percent and number of millable cane (x10^3 ha^{-1}) indicated the least responsibility of both

the primary and secondary differentiations. However, the positive absolute values of vector-1 and negative absolute value for vector-2 for the characters like stalk length, leaf width, bud length, bud width and cane yield indicated the responsibility of primary differentiation.

Table 4.13 Cluster (based on D^2 statistic) means of 16 agromorphological traits of sugarcane genotypes

Sl. No.	Traits	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster V I
1.	No. of tiller/clump	4.53	5.99	3.63	4.69	4.69	4.02
2.	Plant height (cm)	348.38	412.95	394.20	438.26	485.56	453.20
3.	Stalk length (cm)	244.86	289.83	276.00	316.63	355.59	338.36
4.	Leaf length (cm)	126.78	150.06	140.15	145.35	138.41	144.53
5.	Leaf width(cm)	3.64	3.77	3.87	4.44	4.13	3.76
6.	Bud length (mm)	6.46	6.20	6.44	7.08	7.72	6.31
7.	Bud width (mm)	5.43	5.29	5.46	6.00	6.78	5.44
8.	Internode length (cm)	10.72	10.59	10.35	11.26	10.82	11.02
9.	Internode diameter (cm)	1.67	1.82	1.68	1.91	1.99	1.77
10.	No.of internode/cane	21.34	24.90	25.79	25.99	29.24	26.53
11.	Single cane weight (kg)	0.59	0.74	0.79	0.98	1.23	0.87
12.	Brix %	19.46	19.52	19.39	19.40	19.75	19.25
13.	Pol %	13.62	13.83	13.61	13.54	13.52	13.37
14.	Juice purity %	87.78	89.27	87.94	88.08	87.03	88.57
15.	No. of millable cane/10m ^{2}*	93.24	135.11	80.73	93.67	92.41	73.11
16.	Cane yield (t/ha)	55.61	99.03	62.83	90.29	112.83	63.52

*: ($x10^3$ ha $^{-1}$)

Mean values of cluster (based on D^2 statistic) for 16 agromorphological traits of sugarcane genotypes are presented in the Table 4.13. It appears that genotypes with high tillering capacity were included in the cluster II followed by cluster IV and cluster V. The tall genotypes having highest stalk length grouped in the cluster V followed by cluster VI and dwarf genotypes included in the cluster I. The genotypes under cluster I had also smallest stalk length. The genotypes possess largest leaf were included in cluster II while shortest leaves were found in the genotypes under cluster I. It was revealed that broad leaved genotypes formed cluster in the cluster IV followed by cluster V whereas genotypes having narrow leaves were found in the cluster I. The genotypes having largest internode were found in the cluster IV and smallest in the cluster III. The thickest internodes were also observed in the cluster V followed by cluster IV and the thinnest internodes

were found in the cluster I. The genotypes with highest number of internode per cane were included in the cluster V while cluster I contains genotypes with lowest number of internode. The genotypes with heaviest cane were formed cluster in the cluster V while genotypes with lightest cane were grouped in the cluster I. The genotypes with high brix percent were included in the cluster V and lowest brix percent were found in the cluster VI. The genotypes having high percentage of pol were included in the cluster II and low pol percent were grouped in cluster VI. Similarly, cluster II contains sugarcane genotypes having highest percentage of juice purity while cluster V constellate with lowest percentage juice purity. The genotypes with the highest number of millable cane ($x10^3ha^{-1}$) were included in the cluster II whereas the cluster VI contains genotypes having lowest number of millable cane. The highest yielding genotypes were grouped in the cluster V. On the contrary, lowest yielding genotypes were included in the cluster I followed by cluster III. Considering all the traits, it appears that the genotypes in the cluster V had good performance. The genotypes in this cluster had moderate tillering capacity, tallest plant, largest stalk and thickest stalk, highest number of internode per cane, highest single cane weight, highest percentage of brix, moderate pol percentage, moderate number of millable cane and the highest cane yield. On the other hand, genotypes of the cluster I showed poor performance in respect of almost all important yield contributing characters.

4.2 Genetic Diversity Analysis Based on Microsatellite Markers

Twenty three microsatellite markers i.e, SSR markers were used to investigate genetic diversity of 51 sugarcane genotypes available at “Field Gene Bank” of Bangladesh Sugarcrop Research Institute, Ishurdi, Pabna, Bangladesh.

Polymorphism of Microsatellite Markers

Molecular diversity was analyzed in 51 sugarcane genotypes using 23 microsatellite markers. Out of 23 SSR primers, 13 SSR primers were chosen from gSSR series developed by International Consortium of Sugarcane Microsatellite (ICSM) and 10 microsatellite markers were selected from unigene-

derived microsatellite markers (UGMS) series. PCR products were electrophoresed in 2% agarose gel and DNA bands were documented by using gel documentation system (FluorChem FC2, Cell Biosciences, USA) and band size was measured with the help of Alpha view3.1 software.

Number of Allele

All the 23 SSR primer pairs used amplified a total of 619 alleles from 51 genotypes of sugarcane. Representative electrophoregrams are shown in Figure 4.4 (a,b,c) and Figure 4.5 (a,b,c). For each primer pair, the number of alleles varied from 7 to 66 (Table 4.14). The primer pair SMC 226CG identified the highest number of alleles (66) followed by SMC 278CS (64) and SMC 336BS (54). The lowest number of alleles was amplified by both the primer pairs UGMS 316 (7) and SMC 703 BS (7). The average number of allele per locus was 26.91. A total 402 alleles were amplified by 13 gSSR series of primer pairs. In this Series, the highest number of allele was amplified by SMC 226 CG (66) while lowest number of allele was produced by primer pair SMC 703BS (7). The average number of allele was 30.92 amplified by 13 gSSR series of primers. In the UGMS series, 217 alleles were produced by 10 microsatellite markers. The number of alleles ranged from 7 to 39 with a mean of 21.7. The primer pair UGMS 504 amplified the highest number of alleles (39), while the lowest number of allele was produced by the primer pair UGMS 316 (7). The number effective allele ranged from 4.80 to 48.36 with a mean of 21.23. The primer pair SMC 226 CG produced the highest number of effective allele (48.36), while primer pair SMC 703 BS generated lowest number of effective alleles (4.80). Muyco (2002) reported 5 to 19 bands per SSR locus in 81 sugarcane cultivars of the Philippines. At SASEX, Natal, South Africa, worked on the application of 36 sugarcane microsatellites and identified from 1 to 18 alleles per marker across four varieties (Bester, 2000).

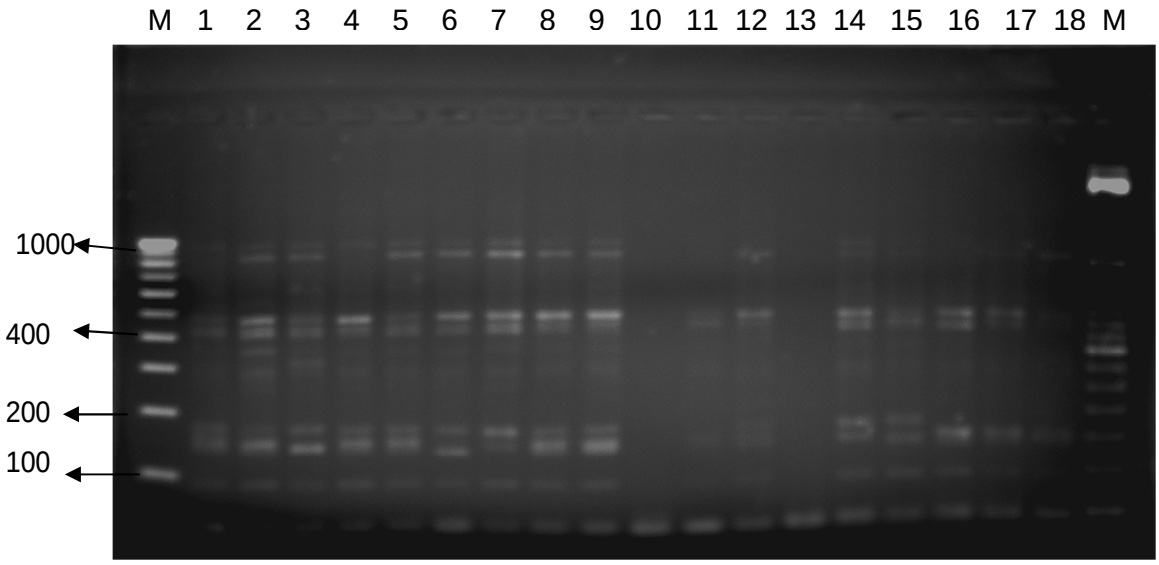

Figure 4.4a SSR banding pattern of 51 sugarcane genotypes with SMC 226 CG

Lane1: I 112-01
Lane2: I 39-04
Lane 3: I 152-04
Lane 4: I 111-03
Lane 5: I 6-04
Lane 6: I 189-04
Lane 7: I 174-93
Lane 8: SC 5d
Lane 9: I 255-06
Lane 10: CPI 96-80
Lane 11: B 34-231
Lane 12: I 562-85
Lane 13: SC 2d
Lane 14: I 325-86
Lane 15: Bo 43
Lane 16: I 156-97
Lane17: POJ 2878
Lane 18: I 134-70

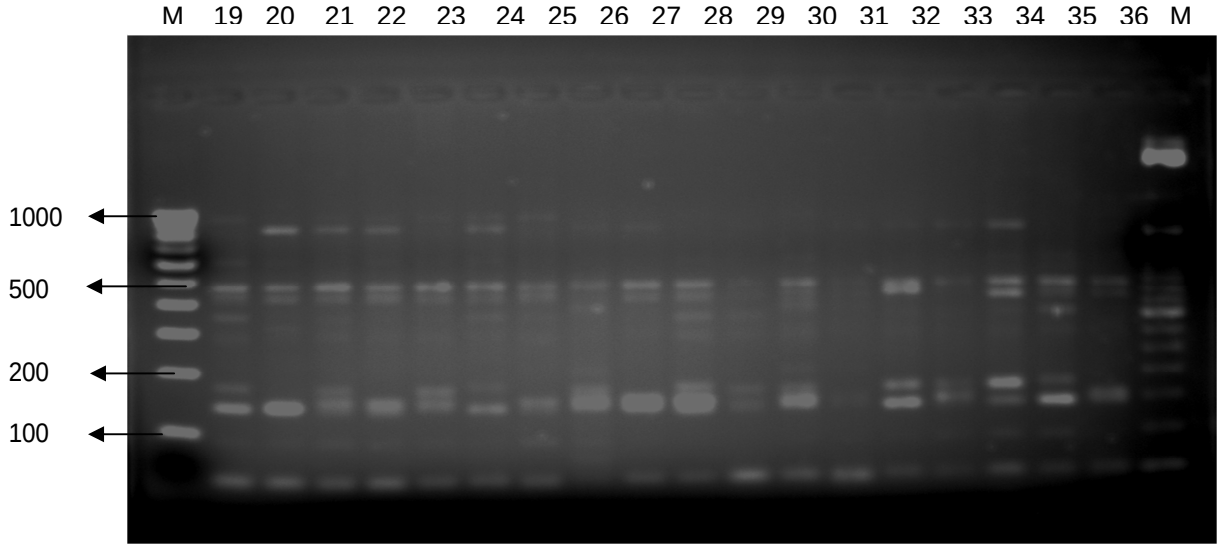

Figure 4.4b SSR banding pattern of 51 sugarcane genotypes with SMC 226 CG

Lane19:CP 69-1052
Lane20:I C 7a
Lane 21: Co 635
Lane 22: CL 41-229
Lane23: I 40-00
Lane 24: CP 36-105
Lane 25: CP 75-361
Lane 26: I 14-96
Lane 27: SC 10d
Lane 28: I 98-98
Lane 29: I 64-98
Lane 30: I 17-01
Lane 31: I 137-96
Lane 32: I 127-96
Lane 33: I 91-79
Lane 34: I 33-97
Lane35: Saipan 17
Lane 36: I 26-04

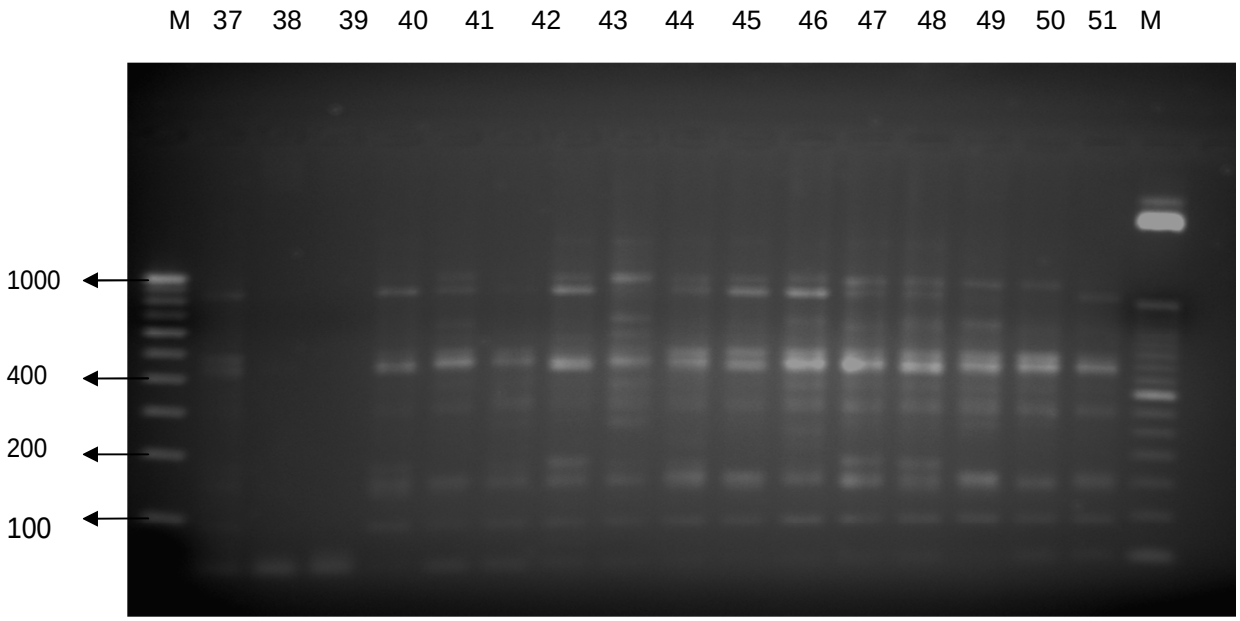

Figure 4.4c SSR banding pattern of 51 sugarcane genotypes with SMC 226 CG

Lane 37:Co 630
Lane 38:I 21-00
Lane 39: SC 5b
Lane 40:I 82-98
Lane 41: I 181-03
Lane 42: I 1-05
Lane 43: I 143-01
Lane 44: I 23-05
Lane 45: I 48-05
Lane 46: Co 642
Lane 47: SC 6d
Lane 48: I 108-01
Lane 49: Isd 38
Lane 50: Isd 40
Lane 51: Isd 39

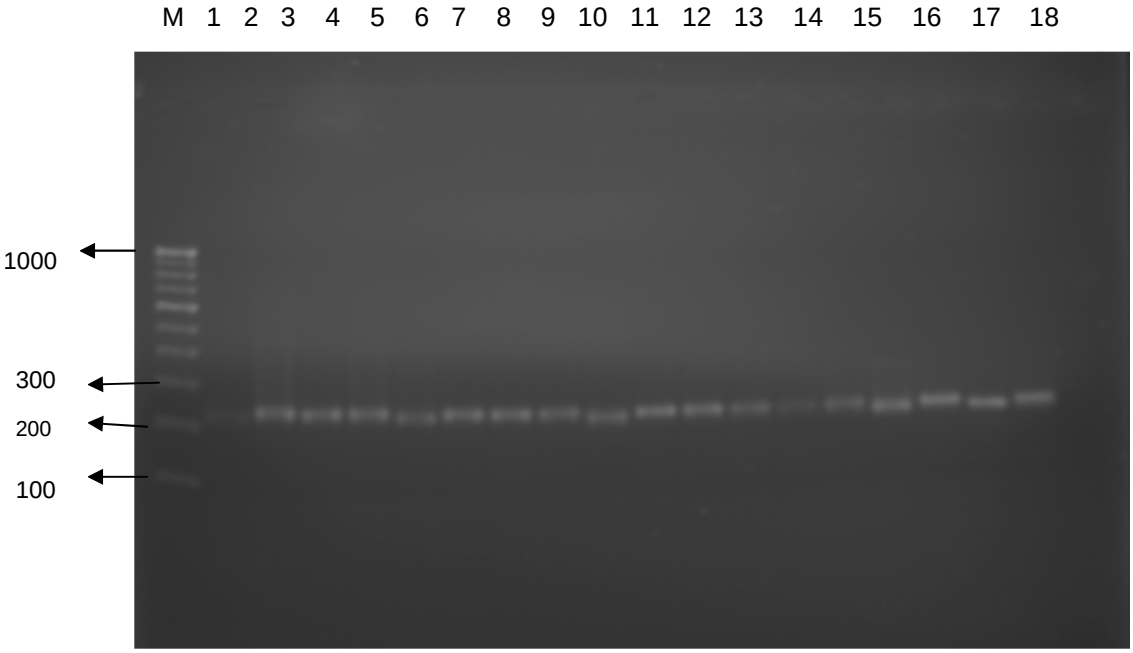

Figure 4.5a SSR banding pattern of 51 sugarcane genotypes with UGMS 302

Lane1:I 112-01
Lane2:I 39-04
Lane 3: I 152-04
Lane 4: I 111-03
Lane5: I 6-04
Lane 6: I 189-04
Lane 7: I 174-93
Lane 8: SC 5d
Lane 9: I 255-06
Lane 10: CPI 96-80
Lane 11: B 34-231
Lane 12: I 562-85
Lane 13: SC 2d
Lane 14: I 325-86
Lane 15: Bo 43
Lane 16: I 156-97
Lane17: POJ 2878
Lane 18: I 134-70

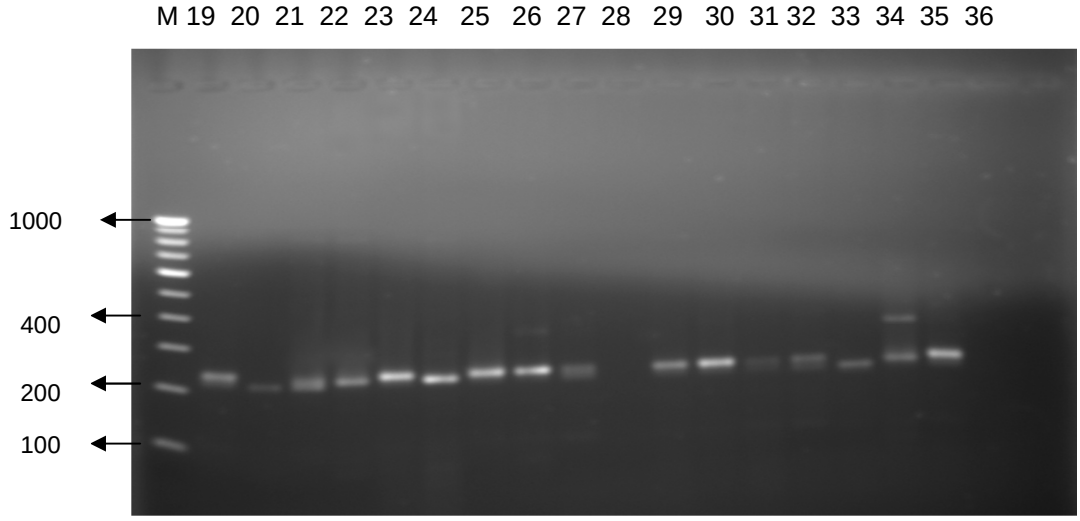

Figure4.5b SSR banding pattern of 51 sugarcane genotypes with UGMS 302

Lane19: CP 69-1052	Lane 25: CP 75-361	Lane31: I 137-96
Lane 20: IC 7a	Lane 26: I 14-96	Lane 32: I 127-96
Lane 21: Co 635	Lane 27: SC 10d	Lane 33: I 91-79
Lane22: CL 41-229	Lane 28: I 98-98	Lane34: I 33-97
Lane 23: I 40-00	Lane 29: I 64-98	Lane 35: Saipan 17
Lane 24: CP 36-105	Lane 30: I 17-01	Lane 36: I 26-04

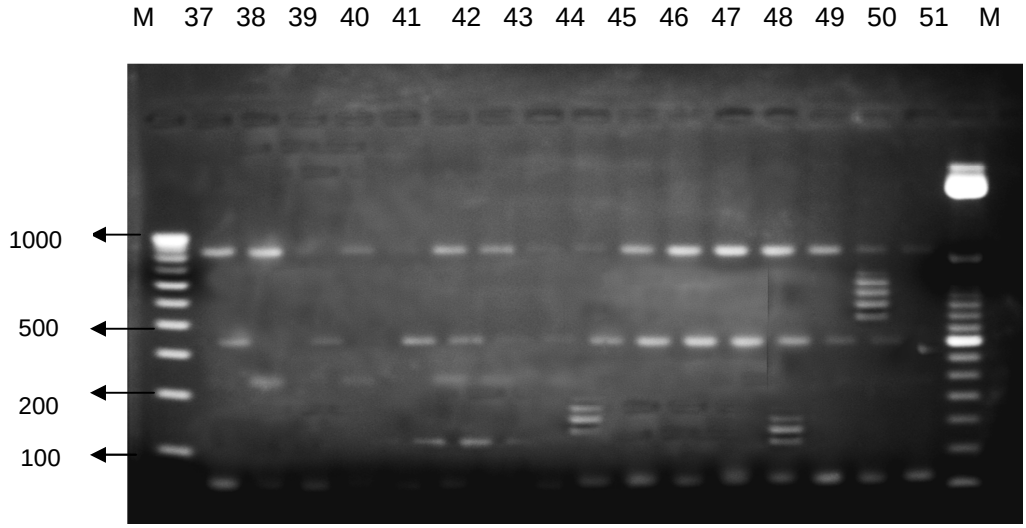

Figure 4.5c SSR banding pattern of 51 sugarcane genotypes with UGMS 302

Lane 37:Co 630	Lane 42: I 1-05	Lane 47: SC 6d
Lane 38:I 21-00	Lane 43: I 143-01	Lane 48: I 108-01
Lane 39: SC 5b	Lane 44: I 23-05	Lane 49: Isd 38
Lane 40: I 82-98	Lane 45: I 48-05	Lane 50: Isd 40
Lane 41: I 181-03	Lane 46: Co 642	Lane 51: Isd 39

Table 4.14 Number and size of alleles, polymorphic allele, % polymorphism polymorphism information content (PIC) and number of effective allele of 23 microsatellite markers used in the analysis of 51 sugarcane genotypes

Sl. No.	Primer	Allele size range(bp)	Total allele	Polymorphic allele	% Polymo-rphism	PIC	No. of effective allele
	a) gSSR (ISMC)						
1.	SMC 334	132-248	29	9	31.03	0.947	19.11
2.	SMC 36 BUQ	100-256	18	9	50.00	0.937	16.13
3.	SMC 238 MS	120-262	24	7	29.17	0.957	23.15
4.	SMC 687	164-205	11	5	45.45	0.896	9.62
5.	SMC 336 BS	151-624	54	21	38.89	0.974	38.09
6.	SMC278 CS	157-652	64	29	45.31	0.977	43.60
7.	mSSCIR74	195-657	21	5	23.81	0.939	16.51
8.	SMC 703 BS	109-238	7	5	71.43	0.920	4.80
9.	SMC 569 CS	137-321	43	17	39.53	0.972	36.38
10.	SMC 597CS	154-1000	29	9	31.03	0.964	27.68
11.	SMC 477CG	128-383	18	5	27.78	0.936	15.78
12.	mSSCIR 43	142-1110	18	7	38.89	0.925	13.39
13.	SMC 226 CG	100-1389	66	28	42.42	0.979	48.36
	Sub-Total =	-	402	156	-	--	-
	Average	-	30.92	12.0	39.60	0.948	24.06
	b) UGMS type SSR						
14.	UGMS 60	394-565	18	10	55.56	0.939	16.62
15.	UGMS 302	201-1036	22	9	40.91	0.945	18.45
16.	UGMS 312	209-385	18	6	33.33	0.942	17.36
17.	UGMS 316	658-718	7	2	28.57	0.850	6.76
18.	UGMS 504	308-2219	39	28	71.79	0.968	31.36
19.	UGMS 567	258-981	18	12	66.67	0.934	15.24
20.	UGMS 575	315-2207	19	9	47.37	0.943	17.63
21.	UGMS 585	330-1751	29	18	62.07	0.942	17.52
22.	UGMS 671	223-273	14	3	21.43	0.928	13.89
23.	UGMS 681	133-425	33	15	45.45	0.952	20.94
	Sub-Total=	-	217	112	-	-	-
	Average	-	21.7	11.2	47.32	0.934	17.58
	Total (a+b)=	-	619	268	-	-	-
	Overall average	-	26.91	11.65	42.95	0.942	21.23

In MISRI, Mauritius, the number of alleles generated per primer pair ranged from 9 to 20 using 5 primer pairs on 96 sugarcane cultivars (Jannoo et al., 2000). At CPCG, SCU, in NSW, Australia 3 to 12 alleles per primer pair were recorded across the sugarcane genotypes using 91 primer pairs (Cordeiro et al. 2000). You et al. (2013) recorded 11 to 26 alleles per locus amplified by five gSSR

across 115 Chinese sugarcane germplasm. In other crop species, the number of alleles amplified per primer pair ranged from 3 to 25 for rice (Yang et al., 1994), 11 to 26 for soybean (Rongwen et al. 1995), 3 to 16 for wheat (Plaschke et al., 1995), 2 to 19 for potatoes (Provan et al. 1996), 2 to 23 for maize (Senior et al., 1998) and 2 to 9 for spelt (Bertin et al., 2001).

Allele Size

The overall sizes of amplified alleles in the 51 genotypes ranged from 100 bp to 2219 bp (Table 4.14). SSR primer pairs, both SMC 36BUQ and SMC 226 CG amplified smallest allele (100bp), while largest allele was amplified by the primer UGMS 504 (2219 bp). The SSR primer pair UGMS 504 revealed allele sizes that ranged from 308 bp to 2219 bp, from 315 bp to 2207 bp for locus UGMS 575 and allele sizes ranging from 100 bp to 1389 bp for SMC 226 CG. The smallest allele size difference (41bp) was found in the primer pair SMC 687, while the largest allele size difference (1911bp) was found in UGMS 504. In the gSSR series of marker, the largest allele size difference was recorded for the primer pair SMC 226 CG (1289bp), whereas smallest allele size difference was found for the primer pair SMC 287 (41bp). In the UGMS series of microsatellite markers, the largest allele size difference was noticed for the primer pair UGMS 504 (1911bp) followed by primer pair UGMS 575 (1892 bp), while the smallest allele size difference (50 bp) was revealed by the primer pair UGMS 671. Yang et al. (1994) pointed out that range in allele sizes can be influenced by the large number of samples screened.

Number of Polymorphic Allele

Out of 619 alleles amplified by 23 SSR primers in 51 genotypes of sugarcane, 268 alleles were found polymorphic. All markers were found to be polymorphic in nature. The number of polymorphic alleles ranged from 2 to 29 with a mean of 11.65. The highest number of polymorphic allele was amplified by the primer pair SMC 278 CS (29), whereas lowest number of polymorphic allele generated by the primer pair UGMS 316 (2). In the gSSR series of microsatellite markers, the

primer pairs SMC 278 CS amplified the highest number of polymorphic allele (29), while lowest number of polymorphic allele (5) was found for each of the primer pairs SMC 687, mSSCIR74 , SMC 703 BS and SMC 477CG. The average number of polymorphic allele per locus for gSSR series was 12. In the UGMS series of microsatellite markers, the highest number of polymorphic allele was amplified by the primer pair UGMS 504 (28), while the lowest number of polymorphic allele was generated by the primer pair UGMS 671(3). The mean number of polymorphic allele per locus of this series was 11.2.

Percentage of Polymorphism

The percentage of polymorphism showed by 23 microsatellite markers varied from 21.43 to 71.79 with a mean of 42.95. The primer pair UGMS 504 depicted maximum (71.79%) polymorphism followed by primer SMC 703BS with 71.43% polymorphism. The primer UGMS 671 was the least polymorphic with 21.43% polymorphism. Among the 13 gSSR primers, SMC 703 BS showed highest (71.43%) polymorphism, while the lowest (23.81%) polymorphism exhibited by the primer mSSCIR 74. The average polymorphism of gSSR markers was 39.60%. The range of UGMS series of markers was 21.43% (UGMS 671) to 71.79 % (UGMS 504) with a mean of 47.32%.

Polymorphism Information Content (PIC)

Polymorphism Information Content (PIC) is directly correlated with the allelic diversity at a given locus that can be detected per marker in a set of individuals. The PIC value is the discriminatory power of the primer used and it describes the capacity of the primer to exploit polymorphism. All the 23 SSR primer pairs amplified multiple fragments among 51 sugarcane genotypes. The genetic diversity or polymorphism information content (PIC) per primer pair or locus ranged from 0.850 to 0.979 with a mean value of 0.942 for all loci across the 51 genotypes evaluated (Table 4.14). The primer pair SMC 226 CG showed the highest PIC value of 0.979 followed by SMC 569 CS (0.972). The SSR primer pair UGMS 316 had the lowest PIC value of 0.850.

The PIC value for International Sugarcane Microsatellite Consortium (ISMC) developed gSSR markers ranged from 0.896 to 0.979 with a mean value of 0.948.The PIC value of SMC 226 CG locus was the highest (0.979) while the lowest PIC value (0.896) was recorded from SMC 687 locus. On the contrary, in unigene-derived microsatellite marker series, PIC value of 10 UGMS primers pair ranged from 0.850 to 0.968 with a mean value of 0.934. The locus UGMS 504 showed the highest PIC value of (0.968), while the lowest PIC value (0.850) was recorded for UGMS 316 locus.

4.2.1 Unique Alleles for Fingerprinting Sugarcane Genotypes

SSR genotypic data from a number of loci have the potential to provide unique allelic profiles, or DNA fingerprints for specific alleles, present in only one genotype for a given primer combinations. For varietal identification, each individual or genotype/line assessed must generate its own unique banding pattern or fingerprint. In the SSR approach, these banding patterns are composed of microsatellite-based PCR amplicons or markers. In general, the more unique patterns a microsatellite primer pair generates for a set of genotypes, the greater its efficacy for fingerprinting. To examine the suitability of the SSR approach for fingerprinting or varietal (genotype) identification, a set of 51 genotypes (Table 3.1) were fingerprinted using 23 primer pairs (Appendix Table 3.3). A total of 76 (12.28%) unique alleles, specific to only one genotype were observed with an average of 3.30 unique alleles per primer combinations (Table 4.15) The highest number of unique alleles (17) were detected by the primer pairs SMC 226 CG in I 326-86, I 156-97, IC 7a, Co 635, SC 10d, I 98-98, I 17-01, Saipan 17, I 23-05, I 48-05, SC 6d and Isd 39 genotypes followed by 14 unique alleles in CL 41-229, CP 36-105, SC 10d, I 137-96, I 21-00, I 1-05, I 143-01, I 23-05, I 48-05 and I 108-01 genotypes by the primer pairs SMC 278 CS. Out of 23 primer pairs, two primer pairs namely SMC 334 and UGMS 575 failed to amplify any unique allele in 51 sugarcane genotypes.

It was revealed that most of the unique allele producer markers in this study had high PIC value. Twenty one SSR markers generated unique allele in 45 sugarcane genotypes. Therefore, it could be said that, 88.24% sugarcane genotypes were

distinguished. On the contrary, only two primer pairs viz. SMC 226 CG and SMC 278 CS produced unique allele in 19 genotypes i.e. 37.25% of genotypes were identified. The number of SSR markers required to characterize germplasm collections or provide cultivar identification is based on the combined discrimination power provided by several markers. This ability to discriminate genotypes depends on the assumption that alleles of one marker are not linked to alleles of other markers. The discrimination power of the marker increases exponentially with each additional marker provided there is no linkage disequilibrium (Brown et al., 1996).

Jannoo et al., (2000) reported that one primer pair discriminated 88 out of 66 sugarcane cultivars evaluated and a minimum of two primers produced the same results as the five primers. In other crops, cultivar identification was successful using 7 microsatellites in soybean (Rongwen et al., 1995), 11 in barley (Russell et al., 1997b), 5 in maize (Senior et al., 1998) and 10 in rice (Garland et al., 1999).

Table 4.15 Number of unique allele generated by SSR markers

Sl. No.	Marker	No. of unique allele
1.	SMC 334	-
2.	SMC 36BUQ	2
3.	SMC 238 MS	2
4.	SMC 687	1
5.	SMC 336 BS	4
6.	SMC 278 CS	14
7.	mSSCIR74	2
8.	SMC 703BS	2
9.	SMC 569 CS	4
10.	SMC 597CS	1
11.	SMC 477CG	2
12.	mSSCIR 43	1
13.	SMC 226 CG	17
14.	UGMS 60	4
15.	UGMS 302	3
16.	UGMS 312	1
17.	UGMS 316	1
18.	UGMS 567	6
19.	UGMS 671	2
20.	UGMS 504	4
21.	UGMS 575	-
22.	UGMS 585	1
23.	UGMS 681	2
	Range	1-17
	Mean	3.30

4.2.2 Relationships between Sugarcane Genotypes based on Microsatellite Markers

4.4.2.1 Genetic Distance

Euclidean distance between pair of genotypes using SSR profile data were calculated to estimate genetic distance between genotypes at genotypic level.

4.2.2.2 Euclidean Distance Estimates from SSR Profile Data

Euclidean distances of 1275 pairs of combinations among 51 genotypes were estimated. Mean Euclidean distance value was 7.61 between pairs for all possible combinations among 51 genotypes studied. It varied from 5.66 for pair POJ2878/ I 156-97 to 8.77 for I 33-97/I 6-04, I 33-97/ I 174-93 estimated from microsatellite data arranged in binary matrix form. Genotypic distance estimates between genotypes that ranged from > 0.0 to <6.5 comprised of 1.57%, estimates, 6.5 to <7.0 made up 7.06 %, 7.0 to <7.5 consisted of 31.29 %, 7.5 to <8.0 comprised of 36.71%, 8.0 to <8.5 accounted for 22.27 % and >8.5 accounted for 1.10 % of 1275 pair wise combinations (Appendix Table 4.6 & Figure 4.6).

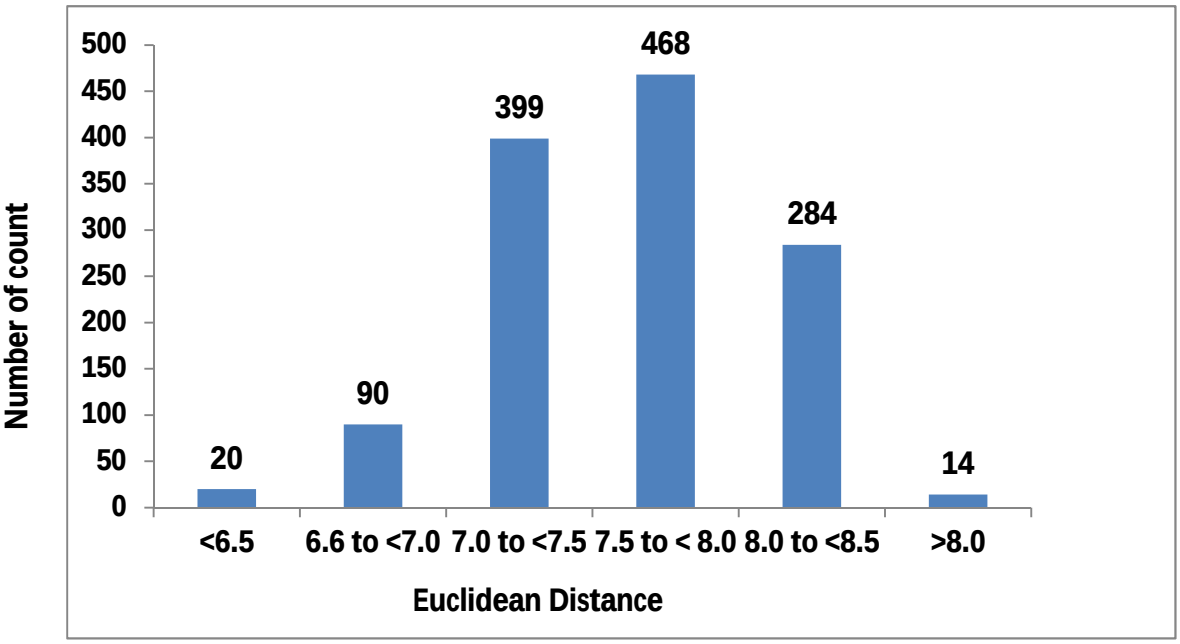

Figure 4.6 Distribution of Euclidean distances (based on SSR profile data) between genotypes

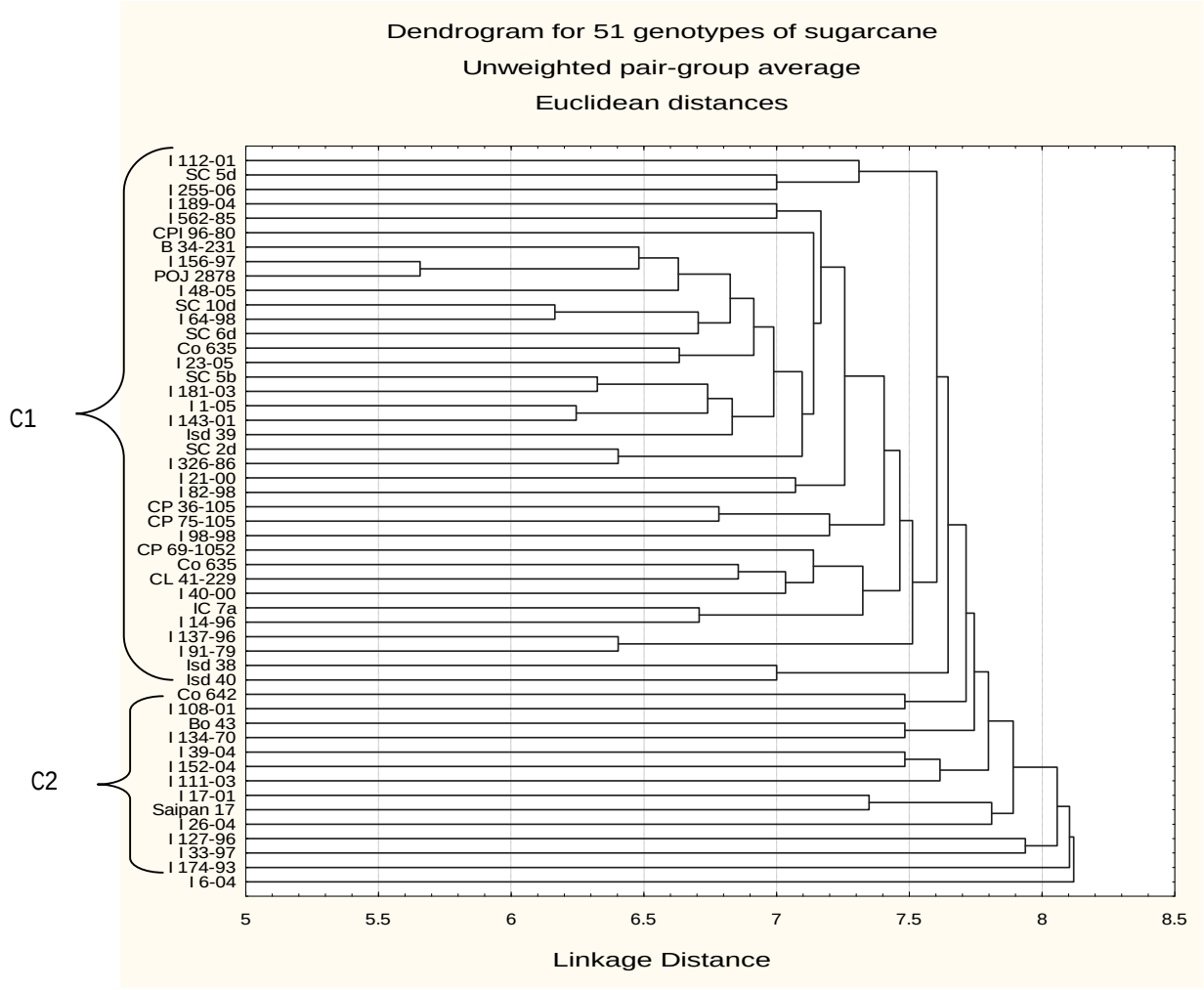

Figure 4.7 Dendrogram derived by UPGMA method from Euclidean distance valuesgenerated through SSR-PCR analysis

Table 4.16 Euclidean distances (≥8.30) between genotypically distant sugarcane genotypes based on SSR marker data

Sl. No.	Genotype pair		Euclidean distance	Sl. No.	Genotype pair		Euclidean distance
1.	I 39-04	I 33-97	8.31	41.	I 255-06	I 33-97	8.72
2.	I 152-04	I 14-96	8.31	42.	I 255-06	I 26-04	8.54
3.	I 152-04	I 33-97	8.54	43.	CPI 96-80	I 33-97	8.31
4.	I 152-04	I 26-04	8.49	44.	Bo 43	I 127-96	8.37
5.	I 152-04	I 108-01	8.37	45.	Bo 43	I 91-79	8.31
6.	I 111-03	I 108-01	8.31	46.	Bo43	I 33-97	8.31
7.	I 111-03	I 33-97	8.49	47.	B0 43	I 26-04	8.49
8.	I 111-03	I 127-96	8.43	48.	I 134-70	I 14-96	8.31
9.	I 111-03	I 17-01	8.49	49.	I 134-70	I 17-01	8.31
10.	I 111-03	I 134-70	8.31	50.	I 134-70	I 33-97	8.54
11.	I 6-04	I 255-06	8.43	51.	CPI 69-1052	I 17-01	8.37
12.	I 6-04	I 562-85	8.31	52.	CPI 69-1052	I 33-97	8.37
13.	I 6-04	I 326-86	8.31	53.	IC 7a	I 17-01	8.43
14.	I 6-04	Bo 43	8.37	54.	IC 7a	I 33-97	8.31
15.	I 6-04	Co 635	8.31	55.	CP 75-361	I 33-97	8.37
16.	I 6-04	CP 75-361	8.43	56.	I 14-96	I 17-01	8.37
17.	I 6-04	I 14-96	8.54	57.	I 14-96	I 33-97	8.37
18.	I 6-04	I 98-98	8.43	58.	I 14-96	I 26-04	8.31
19.	I 6-04	I 91-79	8.54	59.	I 14-96	I 108-01	8.31
20.	I 6-04	I 33-97	8.77	60.	I 14-96	Isd 40	8.31
21.	I 6-04	Saipan 17	8.43	61.	I 98-98	I 33-97	8.37
22.	I 6-04	Co 642	8.37	62.	I 17-01	I 127-96	8.43
23.	I 6-04	I 108-01	8.37	63.	I 17-01	I 33-97	8.37
24.	I 6-04	Isd 40	8.37	64.	I 17-01	Co 642	8.43
25.	I 174-93	I 326-86	8.43	65.	I 17-01	Isd 40	8.43
26.	I 174-93	Bo 43	8.49	66.	I 127-96	Co 642	8.49
27.	I 174-93	IC 7a	8.49	67.	I 127-96	Isd 40	8.37
28.	I 174-93	Co 635	8.43	68.	I 91-79	Co 642	8.31
29.	I 174-93	I 14-96	8.43	69.	I 33-97	I 26-04	8.54
30.	I 174-93	I 17-01	8.66	70.	I 33-97	Co 642	8.54
31.	I 174-93	I 91-79	8.54	71.	I 33-97	I 108-01	8.66
32.	I 174-93	I 33-97	8.77	72.	I 33-97	Isd 40	8.31
33.	I 174-93	I 26-04	8.49	73.	I 26-04	I 108-01	8.37
34.	I 174-93	Co 642	8.49	74.	I 326-86	I 33-97	8.37
35.	I 174-93	I 108-01	8.37	75.	I 127-96	I 174-93	8.60
36.	I 174-93	Isd 38	8.31	76.	I 26-04	I 6-04	8.60
37.	SC 5d	I 17-01	8.31	77.	I 255-06	I 152-04	8.31
38.	I 255-06	I 17-01	8.49	78.	SC 10 d	I 189-04	8.35
39.	I 255-06	I 127-96	8.43				
40.	I 255-06	I 91-79	8.37				

Seventy eight pairs of distantly related genotypes with Euclidean distances ≥ 8.30 are shown in Table 4.16. Fifty eight pairs of local (BSRI developed) genotypes and 20 pairs that involved exotic and local (BSRI developed) genotypes showed Euclidean distances ranging from 8.31 to 8.77. In contrast, 110 pairs of genotypes had Euclidean distances <7.0. The most distant genotype pairs were I 33-97/ I 6-04 (8.77), I 33-97/I 174-93 (8.77) followed by I 33-97/I 255-06 (8.72). Among the 51 genotypes, I 33-97 was the most distant genotype with a mean Euclidean distance of 8.01 with other 50 genotypes.

Information on the genetic relationships among accessions in the germplasm collection based on molecular markers can help germplasm managers to take decision which accessions that highly contribute to the total variability present in the germplasm pool should be retained. Thus, duplicates and closely related genotypes can be discarded. This will mean savings on labour and maintenance cost. Similarly, for plant breeders, the goal in the breeding program can be directed towards the selection of diverse parents to produce heterotic hybrids.

Reduced values of distance estimates between genotypes seemed to suggest a downward trend on the level of genetic diversity present in the genotypes evaluated. This can be further enhanced by the use of related or limited number of parents in the hybridization program. Low level of genetic diversity in the germplasm collection is not beneficial to breeding because the use of genetically related parents can boost the effects of inbreeding depression like susceptibility to biotic and abiotic stresses, narrow adaption and decreased productivity. When the level of genetic diversity available in the germplasm materials is low, progress in selection must be low (Muyco, 2002).

4.2.2.3 Cluster Analysis Based on Euclidean Distance

In this study, a UPGMA dendrogram was constructed based on Euclidean distance (Figure 4.7) showing the genetic relationships among 51 sugarcane genotypes. Fifty one genotypes were grouped into two major clusters viz. cluster 1 (C1) and cluster 2 (C2). The cluster 1 was the largest cluster consisted of 37

genotypes while cluster 2 comprised of only 14 genotypes. Genotypes were clustered based on their mean genetic distances. Cluster 1 can be sub-divided into two sub-clusters e.g., sub-cluster1 (SC1) and sub-cluster-2 (SC2). Between two sub-clusters, SC1 was the smallest, which contained only three genotypes namely- I 112-01, SC 5d and I 255-06. The second sub-cluster (SC2) consisted of 34 genotypes viz. I 189-04, I 562-85, CPI 96-80, B 34-231, I 156-97, POJ 2878, I 48-05, SC 10d, I 64-98, SC 6d, Co 630, I 23-05, SC 5b, I 181-03, I 1-05, I 143-01, Isd 39, Sc 2d, I 326-86, I 21-00,I 182-98, CP 36-105, CP 75-361, I 98-98, CP 69-1052, Co 635, CL 41-229, I 40-00, IC 7a, I 14-96, I 137-96, I 91-79, Isd 38, and Isd 40 . The second major cluster (C2) comprised of 14 genotypes namely, Co 642, I 108-01, Bo 43, I 134-70, I 39-04, I 152-04, I 111-03,I 17-01, Saipan 17, I 26-04, I 127-96, I 33-97, I 174-93 and I 6-04.

In choosing parental clones for crossing program, selection criteria should be based on genetic distances between genotypes and information on their relationships in cluster analysis. Crossing cultivars based only on their genetic distances limits the number of parents and reduces the level of genetic variability in the population derived from them. Cross combinations involving parents that are distantly related and coming from different clusters are expected to produce heterotic offsprings.

4.2.2.4 Cluster Analysis based on Jaccard's Similarity Coefficients

The binary data from polymorphic 23 SSR primers were used for computing Jaccard's similarity indices. The similarity values obtained for each pair-wise comparison of 23 SSR markers among 51 sugarcane genotypes were used to construct dendrogram based on Jaccard's coefficient and the results are presented in Figure 4.8 The cluster analysis grouped 51 sugarcane genotypes into five major clusters. Among different clusters, the cluster size ranged from 9 to 11 genotypes. Cluster1, cluster 2 and cluster 4 are the largest clusters, each consisted of 11 genotypes. Cluster 1 composed of two exotic genotypes (CPI 96-80 and B 34-231) and 9 BSRI developed advanced clones viz. I 112-01, I 39-04, I 152-04, I 111-03, I 6-04, I 189-04, I 174-93, SC 5d and I 255-06.

Cluster 2 consisted of 8 BSRI developed advanced clones namely I 562-85, SC 2d, I 326-86, I 156-97, I 134-70 , I 23-05, I 48-05 and 4 exotic genotypes viz. Bo 43, POJ 2878, Co 630 and Co 642. These four genotypes were developed from Barbados, Indonesia and Coimbatore, India. In cluster 3, five BSRI generated genotypes and four exotic genotypes grouped together. In cluster 4, one exotic genotype-Saipan 17 and 10 BSRI developed genotypes viz. SC 10d, I 64-98, I 17-01, I 137-96, I 127-96, I 91-79, I 33-97, I 26-04, SC 6d and I 108-01 grouped together. Three released varieties e.g. Isd 38, Isd 39 and Isd 40, and BSRI generated six advanced clones viz. I 21-00, Sc 5b, I 82-98, I 181-03, I 1-05 and I 143-01 has formed cluster 5. Clustering pattern could not be explained due to incomplete pedigree information about parentages of all genotypes (Table 3.1).

The low level of correlation between genetic similarity based on pedigree and DNA profiles has been reported by several workers (Graner et al. 1995; Barrett et al. 1998) in different crop species. This could be due to the fact that the pedigree records do not take into account during selection and genetic drift which play pivotal role in variety development (Selvi et al. 2003). It is also possible that DNA markers employed in these investigations are insufficient to assay a significant proportion of the genome. The genome of sugarcane is complex and very large (2500-4000 Mb) (Selvi et al. 2003; Lima et al. 2002) and would therefore require a large number of markers to substantially cover unlinked genomic regions, enabling establishment of meaningful association between pedigree and molecular diversity, since the consistency of genetic similarity estimates depends upon number and location of markers in the genome (Lima et al. 2002). In this situation, the sugarcane breeders should select the parents with high genetic distance (GD) so as to create more variability for effective selection.

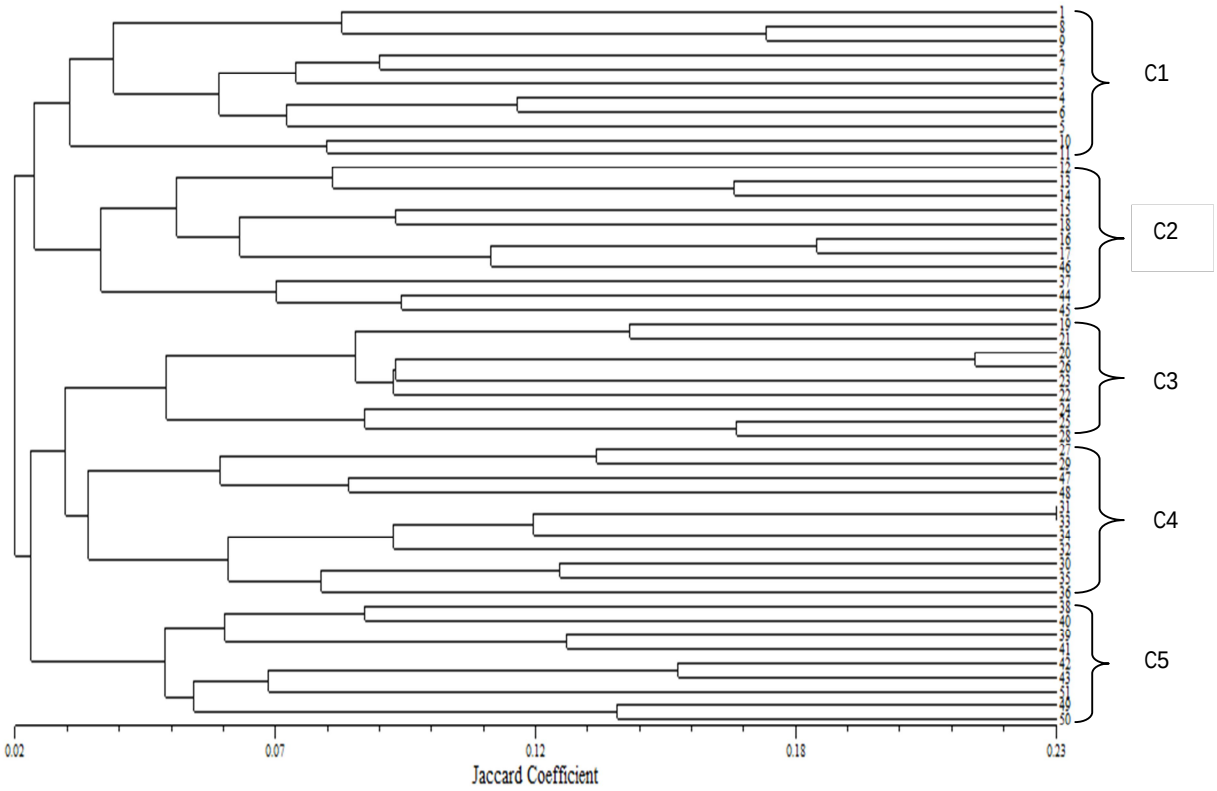

Figure 4.8 Dendrogram generated from Jaccard's Coefficients values of SSR markers data following UPGMA method showing clustering of 51 sugarcane genotypes

Chapter V

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Genetic diversity is essential for the continued progress in breeding of any crop as well as adaptation to cope up with upcoming climate change challenges. Assessment of genetic diversity and identification of superior genotypes are important prerequisites for a successful crop improvement program. Genetic diversity in crop plants can be measured using various tools such as morphological, biochemical and DNA based markers.

The present study was conducted with the aims of assessing genetic diversity among 51 genotypes of sugarcane at phenotypic and genotypic levels using morphological and microsatellite markers, finding association of quantitative morphological characters with cane yield, determining relationship among the genotypes studied and classifying them into different clusters, fingerprinting of sugarcane genotypes, and also to evaluate the utility and efficiency of 23 microsatellite markers.

To assess the variability for morphological traits, observations were recorded on 16 quantitative agromorphological traits and 36 qualitative morphological characters were studied. Analysis of variance of quantitative traits revealed highly significant differences among the genotypes studied indicating the existence of sufficient genetic variation among the genotypes for all the traits studied. Coefficient of variability ranged from 3.08 to 30.44, which indicated the consistency of the experimental conditions.

Estimation of descriptive statistics revealed wide range of variability and variance for number of tillers per clump, single cane weight, and number of millable cane and cane yield indicated the existence of considerable morphological diversity in the selected germplasm accessions.

High Shannon-Weaver diversity indices were exhibited by 16 quantitative agromorphological traits of sugarcane and it were ranged from 0.80 to 0.99 with a mean of 0.94. The diversity index for 36 qualitative characters ranged from 0.0 to

0.98 with a mean diversity index of 0.59 for all traits and 0.71 when seven monomorphic traits were excluded. Some desirable traits like erect tillering habit, straight stalks, bud groove/furrow expression, absence of stripes on cane, smooth and green leaves, absence of splits/growth cracks on cane were predominant and had zero to very low diversity indices. This suggests the success of plant breeders in incorporating these traits in the population.

Correlation studies showed that plant height, stalk length, number of tiller per clump, leaf width, internode diameter, number of millable cane and single cane weight had significant and strong positive association with cane yield. Qualitative traits like juice purity percentage showed significant positive association with brix percent and pol percent. On the other hand, very strong positive relationship existed between brix percent and pol percent i.e. sugarcane juice containing higher percentage of brix must produce higher amount of sucrose from that juice. This study indicated that higher length of stalk, number of tiller per clump, internode diameter, single cane weight and number of millable cane were the most important characters which should be considered while selection to be made for higher cane yield in sugarcane genotypes.

Mean Euclidean distances between genotypes generally were from low to high with a few genotype combinations that were phenotypically distant. It ranged from 6.0 to 251 based on 16 agromorphological traits. Among the BSRI bred genotypes, mean Euclidean distances were generally low to high and ranged from 63.14 to 153.46. In case of exotic genotypes, mean Euclidean distances were generally low to medium and ranged from 62.92 to 115.24. An almost equal proportion of low and moderate to high distance values among all possible pairs were observed. The overall mean phenotypic distance among the genotypes was moderate.

Cluster analysis based on Euclidean distances measured from quantitative agromorphological data grouped 51 genotypes in to six major clusters. Cluster 3 was the largest one and was sub-divided into three sub-clusters viz. sub-cluster1, sub-cluster2 and sub-cluster3. The exotic and locally bred genotypes were

loosely distributed in the different clusters. The exotic and locally bred genotypes that clustered together are phenotypically similar based on traits being assessed.

Diverse genotypes based on their mean Euclidean distance values can be utilized as parents in the hybridization program. Integrating available information on their good combining ability with other genotypes to the phenotypic distance data, as a criterion in parental selection, ensures a higher chance of generating better performing hybrids. Thus, cross combinations between genetically closely related genotypes should be avoided. Crosses between genetically distant sugarcane genotypes should produce higher variances for quantitatively inherited traits in segregating populations.

Mahalanobis D^2 statistic was also used to study the divergence among 51 genotypes of sugarcane on multivariate scale. Fifty one genotypes were grouped into six clusters. The cluster VI was the largest containing 17 genotypes while both cluster I and II were the smallest and contained five genotypes each. The distances between clusters were more than intra-cluster distances indicating that divergence among the clusters were more than within clusters. The highest inter-cluster distance (12.358) was found between clusters I and cluster V. The distance between cluster V and cluster VI was minimum (2.628) followed by the distance between the clusters IV and VI (2.868). The crosses between genotypes in cluster I with genotypes in cluster V might produce a good hybrid which would exhibit highest heterosis. On the contrary, sugarcane breeders should not select sugarcane parents from cluster V and cluster VI because lowest inter-cluster distance was found between these two clusters. Similarly, crossing between the genotypes from cluster IV and cluster VI may not produce satisfactory hybrid. Considering cluster mean of different agromorphological traits, genotypes of cluster V were found to be superior. The genotypes in this cluster had moderate tillering capacity, tallest plant, largest stalk and thickest stalk, highest number of internode per cane, highest single cane weight, highest percentage of brix, moderate pol percentage, moderate number of millable cane and the highest

cane yield. On the contrary, genotypes of cluster I showed poor performance in respect of almost all important yield contributing characters.

Principal component analysis showed first five components that accounted for 81.31 % of total variation. It was also revealed that plant height, stalk length, number of millable cane and cane yield contributed to the variation accounted by the first and second principal components. Canonical variate analysis indicated the contribution of characters towards divergence. The positive absolute values of the two vectors revealed that plant height, internode length and diameter, number of internode per cane, single cane weight and pol percent had the greatest contribution to genetic divergence.

The 23 SSR markers were used to fingerprint 51 sugarcane genotypes. It was revealed that high PIC values in the genotypes surveyed and was able to distinguish 88.24 % of the genotypes as unique genotypes. The polymorphic information content (PIC) per primer pair or locus ranged from 0.850 to 0.979 with a mean value of 0.942 for all loci across 51 genotypes evaluated. The primer pair SMC 226 CG showed the highest PIC value (0.979) while the primer pair UGMS 316 exhibited the lowest PIC value (0.850). The level of polymorphism indicates that distinction between any two genotypes is possible with appropriate SSR primer pair. This supports to the use of SSR markers, as an excellent tool, for diversity analysis and loci mapping in sugarcane. As many as 619 alleles were amplified by 23 SSR primer pairs in 51 genotypes of sugarcane. For each primer pair, the number of alleles ranged from 7 to 66 with a mean value of 26.91. All markers were found to be polymorphic in nature. About 43.30% alleles were recorded as polymorphic. The number of polymorphic alleles ranged from 2 to 29 with a mean of 11.65. The highest number of polymorphic alleles was amplified by the primer pair SMC 278 CS (29) while the lowest number of polymorphic allele (2) was amplified by the primer pair UGMS 316.

A total of 76 unique alleles (specific to only one genotype) were observed with an average of 3.30 unique alleles per primer combinations. The highest number of unique allele (17) was detected for the primer pair SMC 226 CG. Out of 23 primer

pairs, two primer pairs namely SMC 334 and UGMS 575 failed to amplify any unique allele in 51 genotypes of sugarcane studied. It was revealed from this study that most of the unique allele producer marker had high PIC value. These 21 markers distinguished 88.24% sugarcane genotypes. Only two primer pairs viz. SMC 226 CG and SMC 278 CS produced unique allele in 19 genotypes i.e. 37.25% genotypes were distinguished.

Genetic diversity at genotypic (genetic) level was also estimated by Euclidean distance using SSR marker data. Euclidean distance varied from 5.66 for genotype pair POJ 2878/I 156-97 to 8.77 for genotype pairs I 6-04/ I 33-97 and I 174-93/ I 33-97. Fifty eight pairs locally bred genotypes and 20 pairs that involved local and exotic genotypes showed Euclidean distances ranging from 8.31 to 8.77. The most distant genotype pairs were I 6-04/ I 33-97 (8.77) and I 174-93 I 33-97 (8.77) followed by I 255-06/I 33-97 (8.72). Among 51 genotypes, I 33-97 was the most distantly related genotype with a mean Euclidean distance of 8.01 with other 50 genotypes. The difference between the lowest and the highest Euclidean distances indicated the presence of low to moderate level of genetic diversity among the studied sugarcane genotypes at genotypic level. Dendrogram based on UPGMA using Euclidean distances computed from SSR data revealed two major clusters viz. cluster 1 and cluster 2. The cluster 1 was the largest cluster consisting of 37 genotypes while cluster 2 comprised of 14 genotypes. Dendrogram also constructed from molecular data using Jaccard's similarity coefficient. Fifty one genotypes were grouped into five clusters based on SSR markers profile data. In choosing parental clones for crossing program, selection criteria should be based on genetic distances between genotypes and information on their relationships in cluster analysis. Crossing cultivars based only on their genetic distances limits the number of parents and reduces the level of genetic variability in the population derived from them. Cross combinations involving parents that are distantly related and coming from different clusters are expected to produce heterotic offspring.

Sugarcane is a polyploid and aneoploid with a ploidy level ranges from 5x to 16x. Its genome is highly complex in nature and it has very large (2500-4000 Mb) genome, for that reason it requires a large number of markers to substantially cover unlinked genomic regions, enabling establishment of meaningful association between pedigree and molecular diversity, since the consistency of genetic similarity estimates depends upon number and location of markers in the genome. In this situation, the sugarcane breeders should select the parents with high genetic distance (GD) so as to create more variability in the segregating generations for effective selection.

It was noticed from pedigree information of clones/ genotypes used in this experiment that several female parents from *Saccharum officinarum* L. had been used repeatedly from a long time. Therefore, to increase diversity of active collection for some characters like red rot resistance, drought resistance, salt tolerance and ratoonability, incorporation of *S. spontaneum, S. barberi, S. robustum* and *Erianthus spp*. in to the population should be initiated. *Saccharum* species other than *officinarum* can be utilized as female parents to widen the cytoplasmic base.

The information obtained from this investigation should be considered in designing future breeding program. Further extensive investigation should be done to assess genetic diversity of whole germplasm collection using agro-morphological traits coupled with more number of SSR markers having high discriminating power. Selection of genotypes to be retained in the active collection or as parents for hybridization program should integrate phenotypic and SSR data as selection criteria.

Chapter VI

REFERENCES

REFERENCES

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APPENDIX

APPENDIX

Appendix Table 3.1 List of equipments used during the experiment

Sl. No.	Name of equipment
1.	Digital Electronic Balance (Capacity: 210 g Readability: 0.1mg)
2.	pH Meter (Thermo Fisher Scientific, UK)
3.	Water Bath (Pharmacia Biotech, UK)
4.	Digital Micropipettes of different sizes/capacities (Eppendorf Co.)
5.	Centrifuge machine (RPM: 14,000; Sigma Co. UK)
6.	Refrigerator
7.	Freezer (- 20 ^{o} C) (Siemens Co., Germany)
8.	Digital Vortex Machine (IKA Vortex)(Genius 3, UK)
9.	Ice Maker (Ziegra EIS, Germany)
10.	Magnetic Stirrer (with hotplate)
11.	Oven
12.	Microwave oven
13.	Nanodrop 2000 (Spectrophotometer), (Thermo Fisher, UK)
14.	Thermal Cycler (Genius Techne, Cambridge Ltd. UK)
15.	My Cycler (PCR Machine, Bio-Rad, USA)
16.	Horizontal Electrophoresis Apparatus with power supply (CBS Scientific, USA)
17.	Gel Documentation System (FluorChem FC2, Cell Biosciences, USA)
18.	Polariscope
19.	Autoclave
20.	Digital Brix Meter
21.	Digital slide Calipers
22.	Digital Top load Balance (Capacity: 30 kg)

Appendix Table 3.2 List of chemicals used in the experiment

Sl. No.	Chemicals	Sl. No.	Chemicals
1	Absolute Ethanol Purity > 99.0%, Sigma, Germany	13.	Bromophenol Blue Mol. Biol. Grade, Sigma, Germany
2.	Isopropanol Purity>99.0%, Sigma, Germany	14.	RNase A (DNase free) High purity, pancreatic, lyophilized powder ; Purity>99.0%;Genei, India
3.	Isoamylalcohol Purity>99.0% ;ACS Grade, Sigma, Germany	15.	Xylene Cyanol FF Moisture<10.0%; Mol. Biol. Grade; Sigma, Germany
4.	Chloroform Mol. Biol. grade, Sigma, Germany	16.	Diethyl pyrocarbonate (DEPC) Purity: >98.5%, Refractive Index (20 ^{o} C): 1.3973
5.	Phenol (Saturated) Mol. Biology Grade (Mini. 90%); (DNase, RNase, Protease free), Sigma Co. Germany	17.	Taq DNA Polymerase 5 x1000 U (5U/µl);B.Genei, India
6.	Sodium Chloride (NaCl) Mol. Biol. grade, DNase, RNase & Protease free	18.	PCR buffer B.Genei, India
7.	CTAB (N,N,N,N-Cetyl trimethylammonium bromide) Mol. Biol. Grade, Mini. Assay: 98.0%;Sigma, Germany	19.	dNTPs B.Genei, India
8.	EDTA Mol. Biol. grade, DNase, RNase & Protease free	20.	23 SSR primers (Forward and Reverse), Sigma Co. Germany
9.	Trisma Base Mol. Biol. Grade; Sigma, Germany	21.	Agarose B; Low EEO;Sigma Co., Germany
10.	PVP Mol. Biol. Grade; Sigma, Germany	22.	6x Loading Buffer (Blue/Green color), Sigma, Germany
11.	SDS Mol. Biol. Grade; Sigma, Germany	23.	100 bp DNA ladder Size: 50 mg/vial; Fermentas
12.	Tris HCl Mol. Biol. Grade; Sigma, Germany	24.	50 bp DNA ladder Size: 50 mg/vial; Invitrogen

Appendix Table 3.3 SSR primer sequences (Forward and Reverse) and their annealing temperatures used in sugarcane genotyping /fingerprinting

Sl. No.	Primer	Primer sequence		Annealing Temp.(°C)
		Forward	Reverse	
1.	SMC 334	5´- CAA TTC TGA CCG TGC AAA GAT-3´	5´- CGA TGA GCT TGA TTG CGA ATG-3'	50
2.	SMC 36BUQ	5 ´-GGGTTTCATCTCTAGCCTACC-3´	5-´TCAGTAGCAGAGTCAGACGCTT-3 ´	50
3.	SMC 238 MS	5´-TTGGATTGGATTATAGTGCCAA-3´	5-´AGGAAATGGATTGCTCAGGTGT-3'	55
4.	SMC 687	5 ´-AGCCATGCAGGCAGGCAT -3´	5-´CGCACAATCTGCAAGTGCATCA-3 ´	55
5.	SMC 336 BS	5'- ATT CTA GTG CCA ATC CAT CTC A -3'	5'-CAT GCC AAC TTC CAA ACA GAC-3'	50
6.	SMC 278CS	5´-TTC TAG TGC CAA TCC ATC TCA GA-3'	5´CAT GCC AAC TTC CAA ACA GAC T-3´	50
7.	mSSCIR 74	5´- GCG CAA GCC ACA CTG AGA -3´	5´- ACG CAA CGC AAA ACA ACG -3´	54
8.	SMC 703 BS	5´-GCC TTT CTC CAA ACC AAT TAG T -3´	5´- GTT GTT TAT GGA ATG GTG AGG A -3´	50
9.	SMC 569 CS	5´- GCG ATG GTT CCT ATG CAA CTT-3´	5´-TTC GTG GCT GAG ATT CAC ACT A-3´	50
10.	SMC 597 CS	5´-GCA CAC CAC TCG AAT AAC GGA T-3´	5´- AGT ATA TCG TCC CTG GCA TTC A-3´	50
11.	SMC 477 CG	5´- CCA ACA ACG AAT TGT GCA TGT -3´	5´-CCT GGT TGG CTA CCT GTC TTC A -3´	55
12.	mSSCIR 43	5´-ATT CAA CGA TTT TCA CGA G-3´	5´- ACC CTA GCA ATT TAC AAG AG-3´	55
13.	SMC 226 CG	5´-GAG GCT CAG AAG CTG GCA T-3´	5´-ACC CTC TAT TTC CGA GTT GGT -3´	50
14.	UGMS 302	5´- GAAGAAGAAGAAGAAGAAGAAGAA -3'	5´- ACTCGTCCTACAACCACGACTAC -3´	50
15.	UGMS 312	5´- AACGTATCTTTATTTCCATTCTTC-3´	5´- CTTTCAGTTCAACTTTGGATAAAT-3´	58
16.	UGMS 316	5´- AGTTGAAATTAAGAGAACCATACC-3'	5´- TAAAGCCACTATCATATGCTGAC-3'	53
17.	UGMS60	5´- CGACTCCACACTCCACTC-3´	5´- CCGAACACCACCTTCTTG -3´	55
18.	UGMS 567	5´- CTTCATACGCCACCTTCTC-3´	5´- CAAATGTTCACTCGCATCA-3'	54
19.	UGMS 671	5´-TCCCTACTTCTATGAATATCCTTC-3'	5´-TTGACAAATTGCTTGATGTAGT-3'	53
20.	UGMS 504	5´- TAG AGG AAA TAG CAG AAC AGG-3´	5´- AGA CTG ACA CCT TTG AGA TGA-3´	56
21	UGMS 575	5´-CTG TTT CCT TCC TTC TCG T -3´	5´- CAA TCA TAG CCC AGA CAC C -3´	53
22.	UGMS 585	5´-GAA GAG GAG GAG AGG AGA AG-3´	5´-TGG GAT GGT TGT TGA CTG -3´	53
23.	UGMS 681	5´- ACA CAT CGC TTT CCC ACA -3´	5´- GCA TAC CTG TCG TCG TCT-3´	55

Appendix Table 4.1 ANOVA of 16 agromorphological traits of sugarcane genotypes

Source of variation	D F	No. of tiller/clump		Plant height (cm)		Stalk length(cm)	
		MS	F	MS	F	MS	F
Block (adj.)	5	0.0206	0.837	299.29	1.92	455.75	1.87
Treatment (adj.)	50	1.5385	62.65**	1969.78	12.63**	1436.98	5.90**
Error	10	0.0245		155.97		243.64	

Source of variation	D F	Leaf length (cm)		Leaf width (cm)		Bud length (mm)	
		MS	F	MS	F	MS	F
Block (adj.)	5	8.49	1.12	0.008	1.48	0.0176	3.10
Treatment (adj.)	50	274.27	36.24**	0.599	115.86**	1.44	253.23**
Error	10	7.57		0.005		0.0057	

Source of variation	D F	Bud width (mm)		Internode length (cm)		Internode diameter (cm)	
		MS	F	MS	F	MS	F
Block (adj.)	5	0.0075	0.887	0.0904	1.822	0.0016	0.502
Treatment (adj.)	50	1.196	141.58**	1.186	23.92**	0.0512	16.12**
Error	10	0.0085		0.0496		0.0032	

Source of variation	D F	No. of internode /stalk		Single cane weight (kg)		Brix %		Pol %	
		MS	F	MS	F	MS	F	MS	F
Block (adj.)	5	0.353	1.13	0.0004	0.032	0.010	0.787	0.077	1.144
Treatment (adj.)	50	9.953	31.94**	0.067	5.88**	3.269	260.40**	2.585	38.40**
Error	10	0.311		0.114		0.013		0.067	

Source of variation	D F	Juice purity %		No. of millable Cane/10m^2 (x10^3/ha)		Cane yield (t/ha)	
		MS	F	MS	F	MS	F
Block (adj.)	5	0.050	0.776	6.62	0.149	6.78	0.120
Treatment (adj.)	50	7.555	117.21**	440.14	9.88**	752.04	13.18**
Error	10	0.064		44.56		57.06	

** Significant at 1% level; DF: Degree of freedom; MS: Means square

Appendix Table 4.2 Means (adjusted) of 16 agromorphological traits of 51 sugarcane genotypes

Genotype	NTCP	PHT	STL	L L	LW	B L	B W	INT. L	INT. D	N. INT./ST	SCW	Brix %	Pol %	Juice P. %	NMC/ 10m²*	Cane yield
1. I 112-01	4.36	359.61	244.36	141.50	4.58	7.53	6.55	11.61	2.02	20.38	0.77	19.59	14.05	90.16	112.11	87.54
2. I 39-04	3.76	404.21	280.66	132.40	3.93	7.67	5.32	9.82	2.06	27.68	0.94	19.29	13.65	86.33	76.11	72.55
3. I 152-04	3.56	417.21	281.56	143.00	5.47	5.92	5.39	11.26	1.83	24.68	0.83	20.29	14.51	89.93	78.11	65.76
4. I 111-03	3.36	323.21	230.56	141.30	4.03	7.40	5.64	10.46	1.77	21.58	0.64	20.19	14.40	89.62	87.11	56.60
5. I 6-04	3.96	444.41	297.06	168.60	4.72	6.86	5.44	12.94	1.91	23.18	0.93	19.84	14.19	89.92	82.11	77.42
6. I 189-04	5.56	405.21	254.06	163.50	4.66	8.14	6.54	13.35	1.89	20.88	0.78	20.24	14.40	89.43	101.11	79.98
7. I 174-93	3.56	430.01	305.06	143.60	4.67	6.28	6.36	11.64	2.39	27.28	1.47	17.09	11.65	85.55	82.11	122.24
8. SC-5d	4.76	411.21	287.26	158.80	3.92	6.36	6.48	10.86	1.99	23.38	0.93	19.59	14.01	89.85	108.11	101.86
9. I 255-06	4.73	440.04	303.46	126.02	6.05	7.97	6.10	13.31	2.27	22.18	1.25	13.69	8.41	76.54	101.44	127.49
10. Co 630	6.15	430.84	310.66	130.22	4.41	7.07	5.80	10.44	1.75	25.48	0.88	18.99	13.27	87.55	93.44	82.34
11. CPI 96-80	5.33	415.64	272.56	146.92	4.14	5.51	4.28	10.28	1.66	24.38	0.67	20.39	14.67	90.25	133.44	89.19
12. B 34-231	7.13	362.84	263.76	117.02	3.24	6.43	5.50	11.04	1.39	21.28	0.53	19.09	13.31	87.35	120.44	63.40
13. I 562-85	4.93	420.94	309.66	143.72	3.16	5.04	4.51	12.59	1.40	20.78	0.60	20.59	14.86	90.50	95.44	56.94
14. SC 2d	4.13	442.04	334.56	146.52	4.60	4.64	4.45	12.41	1.70	26.28	0.87	18.09	12.76	88.36	85.44	74.43
15. I 326-86	4.33	439.14	304.06	163.82	4.10	5.52	5.00	12.18	1.66	23.68	0.70	18.79	13.27	88.49	108.44	75.74
16. Bo 43	3.53	365.74	254.16	129.82	2.46	5.26	4.85	10.76	1.39	22.68	0.42	16.89	10.99	81.87	81.44	33.60
17. I 156-97	4.29	330.51	231.46	104.25	3.91	5.70	4.63	9.73	1.76	20.78	0.59	21.56	15.37	89.91	65.11	36.93
18. POJ 2878	9.29	427.01	295.06	147.55	4.35	7.64	5.90	11.74	1.83	22.88	0.76	19.86	14.30	90.80	159.11	117.37
19. I 134-70	3.49	394.81	289.06	126.45	2.74	4.73	3.88	8.27	1.10	28.08	1.02	19.06	13.20	87.32	76.11	76.95
20.CP 69-1052	2.89	442.61	317.66	159.35	4.03	5.27	4.65	10.59	2.21	30.48	1.11	21.06	14.28	89.83	87.11	94.19
21. IC 7a	4.69	469.51	360.56	146.45	3.77	5.35	4.92	12.29	1.75	25.08	0.91	19.56	13.69	88.21	63.11	55.63
22. Co 635	4.89	430.01	322.16	140.45	3.80	7.18	5.85	10.91	1.64	28.78	0.82	19.56	13.75	88.60	96.11	75.98
23. CL 41-229	3.09	380.71	287.26	139.95	3.67	6.39	5.36	8.85	1.47	28.58	0.52	17.26	11.72	85.54	77.11	38.45

Appendix Table 4.2 continued….

Genotype	NTCP	PHT	STL	L L	LW	B L	B W	INT. L	INT. D	N. INT./ST	SCW	Brix %	Pol %	Juice P. %	NMC/ 10m²*	C. Yield
24. I 40-00	2.69	386.41	256.46	154.35	3.98	6.12	5.65	10.9	1.84	22.48	0.82	20.86	15.01	90.72	63.11	50.05
25. CP 36-105	3.26	370.87	282.96	121.42	2.63	6.11	6.08	9.99	1.60	28.18	0.59	18.73	12.78	86.30	93.44	56.05
26. CP 75-361	4.06	452.17	337.36	139.02	4.10	7.20	5.85	9.73	2.14	29.98	1.26	21.53	15.46	90.83	77.44	99.35
27. I 14-96	5.86	440.97	304.06	162.32	6.95	6.86	5.64	11.45	1.92	23.58	0.67	18.53	12.77	87.15	112.44	74.19
28. SC 10d	4.86	515.77	357.16	146.42	4.07	6.92	5.86	11.71	2.14	31.88	1.44	15.53	10.17	82.81	93.44	136.61
29. I 98-98	4.66	424.57	332.76	129.62	4.25	6.31	6.09	10.27	1.95	27.28	1.09	18.83	13.13	88.21	84.44	93.55
30. I 64-98	4.06	419.07	312.66	122.42	4.25	6.84	5.22	9.31	1.81	29.48	1.03	20.73	14.70	89.69	83.44	87.36
31. I 17-01	5.26	462.67	329.76	171.32	3.82	6.42	5.95	11.71	1.76	26.48	0.98	20.13	14.27	89.66	108.44	106.89
32. I 137-96	5.06	419.87	335.66	155.42	4.37	8.96	7.60	10.31	2.10	29.28	1.00	18.93	13.23	88.39	86.44	87.81
33.I 127-96	5.79	398.64	303.89	153.42	3.05	5.31	4.47	10.64	1.72	27.58	0.60	18.03	12.28	86.24	166.44	102.79
34. I 91-79	4.19	458.34	363.39	159.62	3.62	6.40	5.28	11.86	1.84	23.88	0.87	19.23	13.28	87.51	89.44	78.81
35. I 33-97	5.59	448.94	328.99	146.62	4.26	9.19	7.06	11.41	1.93	26.18	0.76	19.53	13.55	87.88	106.44	82.39
36. Saipan 17	3.39	465.44	353.39	143.72	3.61	5.77	5.24	9.59	2.08	32.38	0.88	20.83	14.96	91.02	61.44	54.49
37. I 26-04	5.59	464.94	334.69	158.02	3.74	7.13	6.05	10.07	1.72	29.48	0.88	19.63	13.73	88.63	87.44	77.89
38. I 21-00	4.79	412.24	290.39	143.62	3.39	6.19	5.30	9.45	1.91	26.28	0.76	19.73	13.89	89.19	108.44	83.95
39. SC 5b	6.79	465.44	357.39	136.82	4.18	8.24	7.33	12.12	1.81	27.18	1.10	19.93	14.06	89.40	121.44	134.89
40. I 82-98	3.79	422.74	320.99	113.42	3.23	5.57	4.73	10.01	1.68	26.68	0.74	19.33	14.02	91.86	64.44	48.37
41. I 181-03	4.96	442.84	321.69	151.09	3.92	8.71	6.79	11.07	1.86	22.68	1.04	20.49	14.01	88.36	86.44	89.51
42. I 1-05	4.16	495.44	336.69	137.29	3.57	8.35	7.09	11.14	1.82	26.28	1.10	18.79	12.17	83.95	85.44	93.51

Appendix Table 4.2 continued….

Genotype	NTCP	PHT	STL	L L	LW	B L	B W	INT. L	INT. D	N. INT./ST	SCW	Brix %	Pol %	Juice P. %	NMC/ 10m²*	C. Yield
43. I 143-01	2.96	436.34	312.79	132.29	2.96	6.24	5.45	11.29	1.69	24.28	0.78	18.59	12.40	86.42	63.44	49.47
44. I 23-05	3.36	453.84	321.79	152.09	3.48	6.81	5.56	10.98	1.62	24.98	0.73	19.89	13.69	89.00	72.44	52.94
45. I 48-05	3.36	469.54	353.29	136.49	3.95	7.46	7.33	9.07	1.89	29.18	1.15	19.49	12.59	83.69	89.44	102.31
46. Co 642	3.96	474.44	350.49	138.29	5.13	8.62	6.46	9.38	2.12	31.18	1.36	21.79	15.13	89.58	74.44	100.39
47. SC 6d	4.56	449.24	337.89	133.89	4.52	7.41	6.26	9.75	1.88	27.28	1.00	18.59	12.39	86.41	80.44	80.11
48. I 108-01	3.56	469.54	343.59	159.29	4.06	7.82	6.44	11.90	1.76	24.98	1.00	18.79	12.82	88.33	63.44	63.10
49. Isd 38	5.30	477.70	371.90	142.92	3.82	7.07	6.83	11.10	2.10	29.73	1.24	22.38	15.94	89.65	94.00	116.63
50. Isd 40	4.43	500.57	362.23	130.62	4.21	7.38	6.53	11.25	2.07	29.25	1.19	20.33	14.61	90.13	88.67	105.45
51. Isd 39	4.85	461.35	310.03	117.33	4.63	8.76	8.02	11.57	1.77	25.07	1.02	21.47	15.39	90.26	100.67	101.47
Mean	4.53	430.34	311.00	142.16	4.04	6.78	5.80	10.91	1.83	25.95	0.90	19.44	13.55	88.10	91.71	81.82
C D*	0.26	20.39	25.49	4.49	0.12	0.12	0.15	0.36	0.09	0.91	0.17	0.18	0.42	0.41	10.90	12.33

NTCP: No. of tiller/clump;
PHT: Plant height (cm);
STL: Stalk length (cm);
LL: Leaf length (cm);
LW: Leaf width (cm);
BL: Bud length (mm);
BW: Bud width (mm);
INT. L: Internode length (cm);
INT.D: Internode diameter (cm)
N. INT./ST: No. of internode/stalk;
SCW: Single cane weight (kg)
Juice P.%: Juice purity %
NMC/10 m²*: No. of millable cane/10 m²(x'000/ha)
C. Yield: Cane Yield (t/ha)

CD*: Critical difference between two treatments (among different blocks)

Appendix Table 4.3 Descriptors and descriptors states used in the characterization of sugarcane germplasm

Sl. No.	Descriptor	Descriptor states	
1.	Plant habit	1- Erect 2-Intermediate	3-Reclining
2.	Tillering habit	1- Compact 2-Intermediate	3-Spreading
3.	Tillering density	1-Poor (1-2 tillers/ stool) 5- Intermediate (5-8 tillers/stool)	3-Light (3-5 tillers / stool) 7- Profuse (>8 tillers/ stool)
4.	Tops	1- Light	2- Heavy
6.	Leaf carriage	1-Open	2- Compact
7.	Trashiness	1- Free 2- Intermediate	3- Clinging
8.	Leaf blade colour		
9.	Leaf blade texture	1-Smooth	2- Rough
10.	Leaf blade erectness	1-Erect to tip 2-Curved near tip	3- Bent near tip 4- Curved near middle
11.	Leaf length		
12.	Leaf width		
13.	Leaf margin pubescence	0- Absent	1- Present
14.	Mid rib color		
15.	Presence of genetic freckles	0-Absent	1-Present
16.	Leaf sheath waxiness	0-Absent 3- Light	5- Medium 7- Heavy
17.	Leaf sheath primary colour		
18.	Leaf sheath secondary color		
19.	Presence of prickles/trichomes	0- Absent 3- Few	7- Many
20.	Prickle/trichome quality	0- Absent 1-Soft	2- Hard
21.	Persistence of prickles/ trichomes	0-Absent 1- Deciduous	2- Non- deciduous
22.	Outer auricle shape	0- Absent 1- Sloping transitional 2- Straight transitional 3- Ascending transitional 4- Dentoid	5- Deltoid 6- Short Lanceolate 7-Unciform 8-Calcarate 9- Falcate 10- Long lanceolate
23.	Inner auricle shape	0- Absent 1- Sloping transitional 2- Straight transitional 3- Ascending transitional 4- Dentoid	5- Deltoid 6- Short Lanceolate 7-Unciform 8-Calcarate 9- Falcate 10- Long Lanceolate

Appendix Table 4.3 continued….

Sl. No.	Descriptor	Descriptor states	
24.	Dewlap waxiness	0- Absent	5-Medium
		3- Light	7-Heavy
25.	Dewlap primary color		
26.	Dewlap secondary colour		
27.	Dewlap shape	1- Very sloping narrow, triangular-ligulate	6-Triangular (*S. spontaneum* types)
		2- Very sloping, more or less ligulate	7- Triangular with horizontal basal margin
		3-Tall, triangular, with convex upper and lower margins	8- More or less triangular, sloping, with horizontal upper margin
		4-Squarish	9- Typical ligulate, very narrow and practically horizontal
		5- Deltoid	
28.	Dewlap margin undulation	0-Absent	
		1- Present	
29.	Legule shape	1-Orbicular crescent	7- Deltoid
		2-Flat crescent	8-Linear crescent
		3-Crescent with broad lozenge	9-Broad subarcuate
		4- Crescent with narrow lozenge	10- Inverted crescent
			11-Narrow crescent
		5- Crescent with lozenge	12- Arcuate
		6- Broad crescent	
30.	Stalk waxiness	1- Restricted to the wax band below the leaf scar	
		2- Light, restricted to the upper half of internode	
		3- Light, throughout internode	
		4- Heavy, throughout internode	
31.	Color of the exposed internode		
32.	Color of unexposed internode		
33.	Stripes on cane	0- absent	1-Present
34.	Splits/growth cracks on cane	0- absent	1-Present
35.	Corky cracks on cane	0- absent	1-Present
36.	Corky patch on cane	0- absent	1-Present
37.	Internode shape	1-Cylindrical	4-Conoidal
		2-Barrel-shaped	5-Obconoidal
		3-Bobbin-shaped	6-Curved
38.	Stalk alignment	1- Straight	2-Zigzag
39.	Node swelling	1-Depressed	3-Swollen
		2-Not swollen	
40.	Growth ring width	1-Narrow	2-Broad
41.	Number of rows of root primordia	1-One row	4-Four rows
		2-Two rows	5- Irregular
		3-Three rows	
42.	Leaf scar prominence	1-Prominent	2- Not prominent

Appendix Table 4.3 continued…

Sl. No.	Descriptor	Descriptor states	
43.	Root band shape	1-Conoidal 2-Straight	3-Obconoidal
44.	Bud shape	1-Ovate with emarginated wings 2-Ovate with secondary wings 3- Simple ovate 4-Narrow ovate 5-Ovate with wing broadening toward apex 6- Squat rhomboid	7-Pentagonal 8- Tall deltoid 9-Short deltoid 10- Squarish pentagonal with wing set high 11- Roundish with wings 12-Round with central germ pore
45.	Bud prominence	1-Flat	2-Bulging
46.	Bud length		
47.	Bud width		
48.	Bud germ pore position	1-Apical 2-Sub-apical	3-Median
49.	Bud groove/furrow expression	0- Absent 3- Low	5-Medium 7-High
50.	Bud hair group (Hair group 9)	1-Prominent	2-Not prominent
51.	Bud tip position	1-Below growth ring 2- Above growth ring	3-On growth ring 4- Combination of 1 and 2
52.	Bud base position	1=At leaf scar	2-Above leaf scar
53.	Lodging tendency	1-Lodging	2-Non-lodging
54.	Plant height		
55.	Stalk length		
56.	Number of internode		
57.	Internode length		
58.	Internode diameter		
59.	Solidness of stalk	1-Solid 2- Hollow	3-Pithy
60.	Internode cross section shape	1-Round	2-Oval
61.	Internode cross section color		
62.	Brix %		
63.	% Pol		
63.	Juice purity %		

Appendix Table 4.4 Euclidean distances between sugarcane genotypes based on agromorphological quantitative data

Genotype	1. I 112-01	2. I 39-04	3. I 152-04	4. I 111-03	5. I 6-04	6. I 189-04	7. I 174-93	8. SC 5d	9. I 255-06	10. Co 630	11. CPI 96-80	12. B 34-231	13. I 562-85	14. SC 2d	15. I 326-86	16. Bo 43	17. I 156-97	18. POJ 2878	19. I 134-70	20. CP 69-105	21. IC 7a	22. Co 635	23. CL 41-229	24. I 40-00	25. CP 36-105	26. CP 75-361	27. I 14-96	28. SC 10d	29. I 98-98	30. I 64-98	31. I 17-01	32. I 137-96
1. I 112-01	0																															
2. I 39-04	76	0																														
3. I 152-04	84	19	0																													
4. I 111-03	60	98	108	0																												
5. I 6-04	111	57	42	143	0																											
6. I 189-04	56	49	46	92	62	0																										
7. I 174-93	107	63	63	146	54	76	0																									
8. SC 5d	72	52	50	117	51	41	45	0																								
9. I 255-06	112	76	77	157	71	87	31	56	0																							
10. Co 630	140	106	105	168	106	118	106	107	107	0																						
11. CPI 96-80	64	63	61	116	68	43	71	34	69	112	0																					
12. B 34-231	40	66	76	67	110	69	109	79	111	129	68	0																				
13. I 562-85	98	44	35	126	44	66	68	55	78	100	63	83	0																			
14. SC 2d	128	68	60	159	44	92	58	68	68	101	84	116	39	0																		
15. I 326-86	103	62	50	142	28	61	58	42	66	103	51	100	36	42	0																	
16. Bo 43	70	62	69	56	107	73	122	97	131	139	95	52	85	120	108	0																
17. I 156-97	90	101	112	48	153	113	157	137	166	171	135	78	131	164	155	53	0															
18. POJ 2878	98	100	98	155	91	85	79	57	67	120	46	102	89	95	70	138	175	0														
19. I 134-70	76	15	32	97	66	59	63	55	76	107	68	63	48	69	68	64	99	101	0													
20. CP 69-105	118	65	56	154	30	77	38	50	55	103	72	115	48	30	33	121	164	82	68	0												
21. IC 7a	173	106	97	197	77	134	97	114	107	122	129	158	78	48	83	152	195	138	108	70	0											
22. Co 635	109	54	48	143	43	77	52	52	60	97	66	96	26	22	34	105	148	81	54	31	68	0										
23. CL 41-229	82	43	47	84	81	68	99	80	113	122	85	63	54	86	82	39	85	124	43	92	118	74	0									
24. I 40-00	76	46	47	74	80	53	100	79	117	130	88	74	72	102	89	42	80	130	54	98	134	91	40	0								
25. CP 36-105	62	43	55	74	92	67	95	73	103	120	74	36	62	94	87	40	76	110	37	97	132	76	32	55	0							
26. CP 75-361	139	79	74	173	56	104	47	75	57	105	94	131	63	30	59	138	177	98	80	32	55	40	107	117	110	0						
27. I 14-96	104	64	52	144	32	63	61	45	67	102	51	100	38	44	6	109	157	68	71	36	84	35	84	91	88	61	0					
28. SC 10d	201	152	144	244	114	163	102	132	97	147	146	198	134	100	114	210	251	129	154	95	99	111	182	189	183	79	114	0				
29. I 98-98	117	61	61	150	59	91	43	61	52	99	80	105	48	31	56	116	154	89	57	38	70	26	85	102	84	30	58	105	0			
30. I 64-98	100	40	44	132	56	76	44	54	53	98	69	87	40	42	55	97	133	88	37	45	83	29	70	84	66	47	57	121	24	0		
31. I 17-01	139	98	88	182	55	100	58	68	63	113	84	139	75	52	47	152	196	76	101	37	79	57	123	130	126	47	48	74	62	75	0	
32. I 137-96	116	64	61	147	50	85	49	56	66	102	80	108	44	28	46	115	157	89	63	31	69	27	82	97	86	39	49	111	28	41	55	0
33. I 127-96	87	101	101	140	101	87	93	63	87	128	51	89	89	101	77	127	164	35	99	93	146	85	114	126	99	110	75	152	95	93	92	90
34. I 91-79	159	104	95	192	69	122	80	94	88	117	111	149	71	36	65	153	198	110	104	51	39	54	118	135	127	42	66	84	56	75	50	49
35. I 33-97	124	75	66	163	47	89	56	61	59	100	71	115	45	25	33	126	171	75	77	30	64	25	96	110	99	36	33	92	38	48	38	38
36. Saipan 17	167	99	89	190	73	128	93	110	104	120	124	152	72	44	79	145	187	136	101	65	12	63	110	126	124	52	81	102	66	76	80	66
37. I 26-04	144	86	75	178	45	102	66	79	75	107	93	136	58	27	46	139	184	99	90	33	44	42	107	117	116	33	47	82	51	63	39	46
38. I 21-00	120	105	104	149	109	105	111	99	113	34	101	115	103	113	104	129	159	111	106	110	143	104	117	122	113	120	103	166	110	105	121	109
39. SC 5b	162	125	122	209	100	136	76	98	66	125	110	158	105	78	89	181	219	85	123	74	99	81	153	166	148	62	89	59	73	90	54	80
40. I 82-98	124	56	54	140	73	99	84	90	93	108	100	101	46	53	76	93	130	125	54	72	70	51	63	85	72	68	78	141	54	45	102	64
41. I 181-03	119	63	55	154	33	80	41	54	54	99	74	113	43	21	34	119	162	84	66	14	63	24	89	98	94	27	37	95	31	39	41	28
42. I 1-05	168	110	100	206	74	127	79	103	76	120	114	160	89	58	76	165	208	112	114	61	57	70	137	145	142	45	76	54	71	82	55	78
43. I 143-01	124	53	44	142	53	88	77	82	89	107	94	107	39	43	62	95	137	120	57	58	60	45	64	79	79	60	64	131	54	48	89	58
44. I 23-05	136	70	57	160	41	93	76	82	89	109	95	124	43	31	50	116	161	115	76	46	43	43	83	94	99	51	52	113	57	59	69	53
45. I 48-05	159	103	97	197	75	124	66	93	66	115	108	150	81	45	72	160	201	103	103	51	56	58	128	142	131	28	73	59	50	69	47	58
46. Co 642	164	103	97	199	73	126	68	98	73	116	115	155	84	47	77	161	201	114	104	52	49	62	129	140	134	26	78	60	54	71	54	62
47. SC 6d	136	73	67	168	54	102	58	77	65	102	92	123	50	17	55	127	169	101	74	37	45	31	95	111	100	21	56	91	28	42	56	38
48. I 108-01	162	96	84	187	59	118	85	100	97	118	117	151	70	40	69	145	189	128	100	55	23	60	111	121	126	48	70	94	66	77	66	61
49. Isd 38	179	127	121	218	95	145	84	111	83	128	127	171	104	68	92	184	224	113	126	71	70	80	152	165	153	49	92	47	71	91	56	76
50. Isd 40	188	131	124	227	99	151	94	122	90	131	134	178	108	74	98	188	228	124	133	79	66	87	157	169	160	57	98	40	82	98	67	90
51. Isd 39	126	76	72	168	63	94	50	69	40	103	75	117	66	53	59	132	170	78	79	50	84	49	110	118	105	43	58	87	48	49	58	65

	33. I 127-96	34. I 91-79	35. I 33-97	36. Saipan 17	37. I 26-04	38. I 21-00	39. SC 5b	40. I 82-98	41. I 181-03	42. I 1-05	43. I 143-01	44. I 23-05	45. I 48-05	46. Co 642	47. SC 6d	48. I 108-01	49. Isd 38	50. Isd 40	51. Isd 39
1. I 112-01																			
2. I 39-04																			
3. I 152-04																			
4. I 111-03																			
5. I 6-04																			
6. I 189-04																			
7. I 174-93																			
8. SC 5d																			
9. I 255-06																			
10. Co 630																			
11. CPI 96-80																			
12. B 34-231																			
13. I 562-85																			
14. SC 2d																			
15. I 326-86																			
16. Bo 43																			
17. I 156-97																			
18. POJ 2878																			
19. I 134-70																			
20. CP 69-105																			
21. IC 7a																			
22. Co 635																			
23. CL 41-229																			
24. I 40-00																			
25. CP 36-105																			
26. CP 75-361																			
27. I 14-96																			
28. SC 10d																			
29. I 98-98																			
30. I 64-98																			
31. I 17-01																			
32. I 137-96																			
33. I 127-96	0																		
34. I 91-79	117	0																	
35. I 33-97	85	42	0																
36. Saipan 17	143	43	61	0															
37. I 26-04	111	30	29	42	0														
38. I 21-00	115	132	109	139	121	0													
39. SC 5b	103	69	65	101	73	138	0												
40. I 82-98	126	82	69	62	73	121	120	0											
41. I 181-03	94	47	24	59	30	108	73	64	0										
42. I 1-05	132	53	54	59	41	138	66	93	57	0									
43. I 143-01	124	73	60	53	58	119	116	26	51	81	0								
44. I 23-05	121	53	46	37	35	121	104	51	41	63	30	0							
45. I 48-05	117	37	43	57	38	133	47	86	47	32	79	65	0						
46. Co 642	129	41	51	49	38	136	60	84	48	30	76	61	18	0					
47. SC 6d	110	39	31	41	30	118	73	52	28	49	45	38	35	36	0				
48. I 108-01	137	38	55	23	30	135	96	71	50	51	55	31	54	46	41	0			
49. Isd 38	128	47	64	74	58	146	39	110	68	48	104	88	27	34	60	71	0		
50. Isd 40	143	58	70	70	60	153	57	109	76	31	102	86	34	33	63	68	30	0	
51. Isd 39	98	73	42	80	55	115	65	76	44	51	71	68	50	55	46	76	71	68	0

Appendix Table 4.5 List of entry (Genotype) against each entry number used in D^2 statistic estimation

Entry No.	Entry (Genotype)	Entry No.	Entry (Genotype)
1.	Isd 38	27.	I 40-00
2.	Isd 39	28.	CP 36-105
3.	Isd 40	29.	CP 75-361
4.	I 112-01	30.	I 14-96
5.	I 39-04	31.	SC 10d
6.	I 152-04	32.	I 98-98
7.	I 111-03	33.	I 64-98
8.	I 6-04	34.	I 17-01
9.	I 189-04	35.	I 137-96
10.	I 174-93	36.	I 127-96
11.	SC 5d	37.	I 91-79
12.	I 255-06	38.	I 33-97
13.	Co 630	39.	Saipan 17
14.	CPI 96-80	40.	I 26-04
15.	B 34-231	41.	I 21-00
16.	I 562-85	42.	SC 5b
17.	SC 2d	43.	I 82-98
18.	I 326-86	44.	I 181-03
19.	Bo 43	45.	I 1-05
20.	I 156-97	46.	I 143-01
21.	POJ 2878	47.	I 23-05
22.	I 134-70	48.	I 48-05
23.	CP 69-1052	49.	Co 642
24.	IC 7a	50.	SC 6d
25.	Co 635	51.	I 108-01
26.	CL 41-229		

Appendix Table 4.6 Euclidean distances between sugarcane genotypes based on SSR profile data

Genotype	1. I 112-01	2. I 39-04	3. I 152-04	4. I 111-03	5. I 6-04	6. I 189-04	7. I 174-93	8. SC 5d	9. I 255-06	10. CPI 96-80	11. B 34-231	12. I 562-85	13. SC 2d	14. I 326-86	15. Bo 43	16. I 156-97	17. POJ 2878	18. I 134-70	19. CP 69-105	20. IC 7a	21. Co 635	22. CL 41-229	23. I 40-00	24. CP 36-105	25. CP 75-361	26. I 14-96	27. SC 10d	28. I 98-98	29. I 64-98	30. I 17-01	31. I 137-96	32. I 127-96	33. I 91-74	34. I 33-97	35. Saipan 1	36. I 26-04	37. Co 630
1. I 112-01	0																																			
2. I 39-04	7.48	0																																			
3. I 152-04	7.75	7.48	0																																		
4. I 111-03	7.68	7.55	7.68	0																																	
5. I 6-04	8.00	8.12	8.00	8.06	0																																
6. I 189-04	7.35	7.48	7.62	7.14	7.62	0																															
7. I 174-93	8.12	7.62	8.00	8.06	8.12	7.87	0																														
8. SC 5d	7.07	7.75	7.87	7.81	8.12	7.07	7.87	0																													
9. I 255-06	7.55	7.94	8.31	8.12	8.43	8.06	8.06	7	0																												
10. CPI 96-8	7.48	7.48	7.87	7.81	7.75	7.07	7.87	7.35	7.42	0																											
11. B 34-23	7.21	7.35	7.62	7.55	7.62	7.07	7.62	7.21	7.55	6.63	0																										
12. I 562-85	7.55	7.68	8.06	8.00	8.31	7.00	8.06	7.55	7.62	7.28	7	0																									
13. SC 2d	7.21	7.21	7.75	7.55	8.00	7.07	8.00	7.48	7.81	7.07	6.63	6.71	0																								
14. I 326-86	7.81	7.81	8.19	7.87	8.31	7.68	8.43	7.94	7.87	7.42	7.42	7.35	6.4	0																							
15. Bo 43	7.87	7.62	8.12	8.19	8.37	7.62	8.49	8.00	8.19	7.87	7.62	7.68	7.21	7.28	0																						
16. I 156-97	7.21	7.07	7.62	7.55	7.75	7.07	7.87	7.35	7.81	6.93	6.48	7.14	6.63	7.14	7.07	0																					
17. POJ 287	6.78	6.78	7.35	7.42	7.62	6.78	7.62	7.07	7.14	6.63	6.48	6.40	6.48	6.71	6.78	5.66	0																				
18. I 134-70	7.75	7.87	8.25	8.31	8.12	7.75	8.12	7.87	8.19	7.62	6.93	7.68	7.35	7.55	7.48	6.93	7.07	0																			
19. CP 69-1(	7.81	7.81	7.68	8.00	8.19	7.55	8.19	7.81	7.87	7.55	7.28	7.62	7.28	8.00	8.19	7.42	7.14	8.06	0																		
20. IC 7a	7.87	7.87	7.75	7.94	8.00	7.48	8.49	8.00	8.19	7.75	7.48	7.81	7.62	8.06	8.12	7.48	7.21	8.12	7.00	0																	
21. Co 635	7.81	7.94	7.94	8.25	8.31	7.68	8.43	7.81	8.12	7.81	7.42	7.75	7.42	8.00	8.19	7.42	7.00	7.81	6.93	7.28	0																
22. CL 41-2	7.35	7.62	7.75	8.06	8.00	7.35	8.00	7.48	7.81	7.48	7.07	7.42	7.35	7.81	8.00	7.35	7.07	7.62	7.42	6.93	6.86	0															
23. I 40-00	7.14	7.68	7.81	8.00	8.06	7.55	8.19	7.55	7.75	7.14	6.86	7.48	7.28	7.75	7.94	7.28	6.86	7.55	7.07	7.00	7.21	6.86	0														
24. CP 36-1(	7.68	7.55	7.81	8.00	8.19	7.55	7.68	7.68	8.00	7.55	7.14	7.48	7.28	7.87	8.06	7.14	7.00	7.81	7.87	7.14	7.48	7.14	6.93	0													
25. CP 75-1(	7.55	7.94	8.19	8.25	8.43	7.68	7.94	7.81	7.87	7.42	7.14	7.75	7.00	8.00	8.06	7.42	7.14	7.81	7.75	7.81	7.48	7.42	7.35	6.78	0												
26. I 14-96	8.06	8.06	8.31	8.25	8.54	8.06	8.43	8.06	7.87	7.81	7.68	8.12	7.68	8.00	8.19	7.68	7.42	8.31	7.62	6.71	7.87	7.55	7.35	7.48	7.62	0											
27. SC 10d	7.62	7.62	7.87	7.94	8.12	7.35	8.12	7.62	7.94	7.35	7.07	7.55	7.35	7.55	7.75	7.07	6.93	7.75	7.81	7.07	7.68	7.35	7.42	7.00	7.28	7.42	0										
28. I 98-98	7.55	7.81	8.06	8.00	8.43	7.68	8.06	7.94	7.75	7.68	7.14	7.75	7.42	7.75	7.68	7.14	7.00	7.81	8.00	7.94	7.62	7.55	7.35	7.62	6.78	7.75	7.42	0									
29. I 64-98	7.07	7.21	7.21	7.55	7.62	6.78	7.62	7.21	7.55	6.63	6.63	6.86	6.78	7.00	7.21	6.48	6.32	7.21	7.28	7.21	7.28	7.07	6.71	7.00	7.28	7.42	6.16	7	0								
30. I 17-01	8.19	7.94	8.19	8.49	8.19	8.06	8.66	8.31	8.49	7.55	7.42	8.12	7.68	8.12	8.06	7.94	7.55	8.31	8.37	8.43	8.37	8.19	8.00	8.12	8.25	8.37	7.68	8.12	7.28	0							
31. I 137-96	7.62	7.75	8.00	7.94	8.25	7.75	8.00	8.00	8.19	7.48	6.93	7.55	7.21	7.42	8.12	7.48	7.21	7.75	7.81	7.48	7.68	7.35	7.14	7.42	7.55	7.68	7.21	7.42	6.93	7.14	0						
32. I 127-96	7.75	8.12	8.25	8.43	8.25	8.00	8.60	8.12	8.43	8.12	7.62	8.06	7.75	8.06	8.37	7.75	7.62	8.25	7.94	8.12	7.94	7.75	7.68	8.06	8.06	8.19	7.87	8.06	7.21	8.43	7.75	0					
33. I 91-79	7.68	7.94	8.06	8.25	8.54	7.81	8.54	8.19	8.37	7.81	7.42	7.87	7.28	7.87	8.31	7.55	7.28	8.19	7.87	7.81	7.87	7.42	7.35	7.62	7.62	7.87	7.68	7.87	7.00	8.12	6.40	7.42	0				
34. I 33-97	7.94	8.31	8.54	8.49	8.77	8.19	8.77	8.19	8.72	8.31	7.81	8.25	7.81	8.37	8.31	8.06	7.68	8.54	8.37	8.31	8.37	7.94	7.87	8.12	8.37	8.37	7.94	8.37	7.55	8.37	7.55	7.94	7.48	0			
35. Saipan 1	7.55	7.55	8.06	8.00	8.43	7.68	8.19	7.81	8.25	7.68	7.28	7.75	7.28	7.87	7.94	7.55	7.28	7.94	7.75	7.94	7.87	7.55	7.62	7.62	7.87	8.12	7.55	7.75	6.86	7.35	7.55	7.81	7.62	7.87	0		
36. I 26-04	8.12	8.12	8.49	8.19	8.60	8.12	8.49	8.25	8.54	7.62	7.48	7.94	7.48	7.94	8.49	7.75	7.48	8.12	8.19	8.12	8.19	8.00	7.68	7.81	7.94	8.31	7.62	7.94	7.35	7.81	7.35	8.25	7.94	8.54	7.81	0	
37. Co 630	7.07	7.35	7.62	7.42	7.62	6.93	7.62	7.21	7.42	7.21	6.78	7.14	6.63	7.14	7.35	6.78	6.63	7.35	7.14	6.93	7.14	7.21	7.00	7.14	7.28	7.55	7.07	7.00	6.48	7.81	6.93	7.75	7.42	7.81	7.28	7.75	0
38. I 21-00	7.42	7.55	7.94	8.12	8.06	7.42	8.19	7.81	8.12	7.55	7.28	7.62	7.14	7.75	7.94	7.42	7.14	7.81	7.48	7.81	7.62	7.68	7.62	7.62	7.62	7.75	7.55	7.75	7.00	8.12	7.68	7.68	7.75	8.25	7.87	8.06	6.71
39. SC 5b	7.00	7.14	7.55	7.48	7.81	7.00	7.68	7.28	7.35	6.86	6.71	6.93	6.56	7.07	7.42	6.86	6.56	7.42	7.07	7.28	7.07	7.00	7.07	7.07	7.21	7.35	7.00	7.35	6.40	7.48	7.00	7.55	7.35	7.87	7.21	7.55	6.56
40. I 82-98	7.55	7.68	8.06	7.87	7.94	7.55	7.81	7.28	7.87	7.42	7.28	7.48	7.14	7.75	8.06	7.28	7.00	7.81	7.62	7.81	7.62	7.28	7.48	7.75	7.48	7.87	7.55	7.87	7.00	7.87	7.68	8.06	7.87	8.25	7.62	8.06	6.86
41. I 181-03	7.55	7.68	7.81	8.12	8.06	7.55	8.06	7.68	8.00	7.55	7.14	7.62	7.28	7.75	7.94	7.42	7.00	7.94	7.75	7.81	7.87	7.68	7.35	7.48	7.48	8.00	7.68	7.75	7.14	8.12	7.81	8.19	8.00	8.12	7.75	8.19	7.28
42. I 1-05	7.42	7.68	7.81	7.75	8.06	7.42	7.94	7.42	7.75	7.14	6.40	7.48	7.00	7.75	7.81	7.00	6.71	7.68	7.48	7.55	7.48	7.55	7.35	7.62	7.21	7.48	7.55	7.48	7.00	8.12	7.42	8.06	7.48	8.12	7.62	7.94	7.00
43. I 143-01	7.07	7.35	7.75	7.81	8.00	7.07	7.87	7.35	7.55	7.35	6.78	7.42	6.63	7.42	7.48	7.07	6.63	7.35	7.55	7.35	6.86	7.07	7.00	7.14	7.00	7.28	7.07	7.14	6.78	7.94	7.35	7.48	7.14	7.94	7.14	7.75	6.93
44. I 23-05	7.21	7.75	7.87	7.81	8.12	7.21	8.25	7.48	7.81	7.48	7.07	7.14	6.78	7.28	7.87	7.07	7.07	7.87	7.42	7.62	7.81	7.62	7.42	7.55	7.81	7.94	7.35	7.81	6.93	7.94	7.48	7.87	7.55	8.19	7.81	7.75	6.63
45. I 48-05	7.48	7.62	7.87	7.68	8.12	7.21	8.12	7.48	7.55	7.21	6.78	7.14	7.21	7.55	7.48	6.78	6.32	7.75	7.28	7.62	7.14	7.21	7.42	7.42	7.55	7.94	7.35	7.42	6.78	8.06	7.62	7.87	7.81	8.06	7.68	7.87	6.63
46. Co 642	8.00	8.12	8.12	8.06	8.37	8.00	8.49	8.00	8.19	7.87	7.48	7.94	7.75	7.94	7.87	6.93	6.63	8.12	7.94	8.00	7.68	8.00	7.81	8.06	8.06	8.19	7.62	7.94	7.48	8.43	8.12	8.49	8.31	8.54	8.19	8.25	7.21
47. SC 6d	7.35	7.48	7.62	7.68	8.12	7.35	8.00	7.75	7.68	7.48	7.07	7.28	6.93	7.68	7.21	6.78	6.48	7.62	7.68	7.48	7.55	7.48	7.28	7.55	7.42	7.81	6.93	7.28	6.48	7.81	7.48	8.00	7.55	7.94	7.42	7.75	6.93
48. I 108-01	7.75	8.00	8.37	8.31	8.37	7.87	8.37	8.00	8.19	7.87	7.48	7.55	7.75	8.06	8.12	7.75	7.21	8.25	7.55	7.75	8.06	7.48	7.68	7.94	8.19	8.31	7.62	8.19	7.07	8.19	8.00	8.25	8.06	8.66	7.94	8.37	7.48
49. Isd 38	7.55	7.94	8.06	7.87	8.06	7.81	8.31	7.94	8.12	7.81	7.42	7.62	7.42	7.87	8.06	7.42	7.28	7.81	7.75	8.06	7.75	7.28	7.48	7.75	7.48	8.25	7.68	8.00	7.14	8.25	7.94	7.81	7.87	8.25	8.00	8.19	7.42
50. Isd 40	7.75	7.87	8.25	8.19	8.37	7.75	8.25	8.12	8.06	7.87	7.48	7.81	7.62	7.94	8.12	7.62	7.07	7.87	7.94	8.00	7.94	7.75	7.42	7.81	7.68	8.31	7.87	8.06	7.35	8.43	8.00	8.37	8.06	8.31	7.94	8.25	7.21
51. Isd 39	7.42	7.42	7.42	7.75	7.68	7.28	8.06	7.68	7.75	7.28	7.00	7.35	6.86	7.48	7.68	7.00	6.71	7.55	7.21	7.28	7.07	7.00	7.07	7.21	7.62	7.48	7.42	7.35	6.86	8.00	7.42	7.55	7.35	8.00	7.48	7.94	6.86

Genotype	38. I 21-00	39. SC 5b	40. I 82-98	41. I 181-03	42. I 1-05	43. I 143-01	44. I 23-05	45. I 48-05	46. Co 642	47. SC 6d	48. I 108-01	49. Isd 38	50. Isd 40	51. Isd 39
1. I 112-01														
2. I 39-04														
3. I 152-04														
4. I 111-03														
5. I 6-04														
6. I 189-04														
7. I 174-93														
8. SC 5d														
9. I 255-06														
10. CPI 96-80														
11. B 34-231														
12. I 562-85														
13. SC 2d														
14. I 326-86														
15. Bo 43														
16. I 156-97														
17. POJ 2878														
18. I 134-70														
19. CP 69-105														
20. IC 7a														
21. Co 635														
22. CL 41-229														
23. I 40-00														
24. CP 36-105														
25. CP 75-105														
26. I 14-96														
27. SC 10d														
28. I 98-98														
29. I 64-98														
30. I 17-01														
31. I 137-96														
32. I 127-96														
33. I 91-79														
34. I 33-97														
35. Saipan 17														
36. I 26-04														
37. Co 630														
38. I 21-00	0													
39. SC 5b	6.63	0												
40. I 82-98	7.07	6.63	0											
41. I 181-03	7.48	6.32	7.35	0										
42. I 1-05	7.07	6.32	7.35	6.93	0									
43. I 143-01	7.14	6.71	6.86	7.00	6.24	0								
44. I 23-05	7.42	6.56	7.00	7.55	7.28	6.93	0							
45. I 48-05	7.55	6.86	7.28	7.28	7.00	6.93	6.78	0						
46. Co 642	7.81	7.42	7.42	7.94	7.55	7.48	7.62	7.07	0					
47. SC 6d	7.42	6.86	7.42	7.42	7.28	6.78	7.21	6.93	7.07	0				
48. I 108-01	7.68	7.55	7.94	7.55	7.68	7.21	7.48	7.35	7.48	7.21	0			
49. Isd 38	7.62	7.07	7.48	7.62	7.35	7.14	7.81	7.55	7.55	7.28	7.55	0		
50. Isd 40	7.68	7.00	7.55	7.55	7.42	7.21	7.87	7.62	7.87	7.48	7.75	7.00	0	
51. Isd 39	7.07	6.63	7.21	7.21	6.93	6.56	7.14	7.14	7.68	7.00	7.42	6.93	7.42	0