FEDERAL UNIVERSITY OF SÃO CARLOS
CENTER OF AGRICULTURAL SCIENCES
POSTGRADUATE PROGRAM IN
PLANT PRODUCTION AND ASSOCIATED BIOPROCESSES

MOLECULAR DIVERSITY AND GENETIC STRUCTURE OF *Saccharum* COMPLEX ACCESSIONS AS REVEALED BY TRAP MARKERS

CAROLINA MEDEIROS DE SOUZA

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“If we long for our planet to be important, there is something we can do about it. We make our world significant by the courage of our questions and by the depth of our answers.”

Carl Sagan

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SUMMARY

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DIVERSIDADE MOLECULAR E ESTRUTURA GENÉTICA DE ACESSOS DO COMPLEXO *Saccharum* REVELADOS POR MARCADORES TRAP

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RESUMO

A cana-de-açúcar tem grande importância econômica para o Brasil e para o mundo por ser matéria-prima, principalmente, para a produção de açúcar e etanol. O melhoramento convencional é um dos responsáveis por aumentar os níveis de produtividade, no entanto, os ganhos genéticos para as características de interesse econômico têm sido cada vez menores. A utilização de marcadores moleculares para avaliar a variabilidade genética existente em bancos de germoplasma pode contribuir com o melhoramento genético na seleção de genitores para realização de cruzamentos e consequente obtenção de progênies superiores. Marcadores moleculares do tipo TRAP (*Target Region Amplification Polymorphism*) são ancorados em genes funcionais que possuem a sequência conhecida permitindo avaliar a variabilidade nesta região do genoma. Desta forma, o objetivo deste trabalho foi avaliar a diversidade e estrutura genética dos acessos do complexo *Saccharum* que compõem o Painel Brasileiro de Genótipos de Cana-de-açúcar (PBGCA), utilizando marcadores TRAP ancorados em genes dos metabolismos de sacarose e lignina. O PBGCA está instalado a campo no Centro de Ciências Agrárias (CCA) da Universidade Federal de São Carlos (UFSCar). O DNA dos acessos foi extraído a partir dos primórdios foliares e as amplificações dos marcadores TRAP para sacarose e para lignina foram realizadas. A detecção dos produtos amplificados foi realizada após eletroforese em gel de poliacrilamida 6% com coloração por nitrato de prata. Os fragmentos obtidos foram classificados como “1” para presença e “0” para ausência. A partir do conjunto total de fragmentos obtidos e utilizando o software R foram estimados: i) distância genética através do coeficiente de Jaccard; ii) PIC (*Polymorphic Information Content*) e DP (*Discriminatory Power*); iii) PCA (*Principal Components Analysis*) e; iv) dendrograma através do método Neighbor-Joining. Em adição, também foram realizados AMOVA (*analysis of molecular variance*) utilizando o software GenAIEx e análise de estrutura de população utilizando o software STRUCTURE. Os marcadores TRAP geraram um total de 595 fragmentos das quais 584 (98,15%) foram polimórficos. A distância genética média entre os acessos foi de 0,3, sendo a menor distância entre os acessos RB721012 e CB40-13 (0,10), e a maior distância entre os acessos SES205A e CAIANA FITA (0,62). O PIC variou entre 0,99 (F4+Arbi1-S) e 0,95 (SuPS+Arbi2 e SuPS+Arbi3). O DP variou entre 0,95 (F4+Arbi1-S) e 1 (StSy+Arbi3). O primeiro e o segundo componentes principais explicaram 12,4% e 5,4% da variabilidade total expressa entre os acessos, respectivamente. Para AMOVA, os acessos foram separados em três grupos: i) ancestrais (A), composto por acessos de *Saccharum* spp. e do gênero *Erianthus*; ii) melhoradas brasileiras (BB), contendo os acessos dos programas brasileiros de melhoramento; e iii) híbridos estrangeiros

(FH), composto pelos acessos oriundos de outros países. Quando a AMOVA foi realizada entre os grupos A e BB, o índice de fixação foi de 0,14 (14% da variação total entre os dois grupos), enquanto que os índices entre os grupos BB e FH e entre A e FH, foram correspondentes a 0,03 e 0,05, respectivamente. A análise de estrutura de população mostrou a formação de dois grupos: o primeiro composto pelos acessos do grupo A - e o segundo contendo os acessos dos grupos BB e FH. No dendrograma, os acessos foram divididos em 3 clados, sendo que o clado 1 foi composto majoritariamente pelos acessos do grupo A e FH, enquanto que os clados 2 e 3 pelos acessos do grupo BB. Assim, através dos marcadores TRAP associados com genes de sacarose e lignina foi possível estimar a diversidade genética do PBGCA e a estrutura de população. Os dados obtidos neste trabalho poderão ser explorados futuramente para estudos de mapeamento associativo e auxiliar programas de melhoramento na escolha de genitores e obtenção de progênies superiores.

Palavras-chave: distância genética; marcadores moleculares; cana-de-açúcar; sacarose; lignina

MOLECULAR DIVERSITY AND GENETIC STRUCTURE OF *Saccharum* COMPLEX ACCESSIONS AS REVEALED BY TRAP MARKERS

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ABSTRACT

Sugarcane has great economic importance for Brazil and for the world as a raw material, mainly for the production of sugar and ethanol. Conventional breeding is one of those responsible for raising productivity levels, however, the genetic gains for the characteristics of economic interest have been decreasing. The use of molecular markers to evaluate the genetic variability in germplasm banks can contribute to the genetic improvement in the selection of parents for crossing and consequent obtaining of superior progenies. Molecular markers of the TRAP (Target Region Amplification Polymorphism) type are anchored in functional genes that have the known sequence allowing to evaluate the variability in this region of the genome. Thus, the objective of this work was to evaluate the diversity and genetic structure of *Saccharum* complex accessions that make up the Brazilian Panel of Sugarcane Genotypes (BPSG) using TRAP markers anchored in sucrose and lignin metabolism genes. BPSG is installed in the field at the Agricultural Sciences Center (CCA) of the Federal University of São Carlos (UFSCar). The DNA of the accessions was extracted from the fresh meristem cylinder and the amplifications of the TRAP markers for sucrose and lignin were performed, respectively. The detection of amplified products was performed after electrophoresis in 6% polyacrylamide gel with silver nitrate staining. The obtained fragments were classified as "1" for presence and "0" for absence. From the total set of fragments obtained and using software R were estimated: i) genetic distance through the Jaccard coefficient; ii) PIC (Polymorphic Information Content) and DP (Discriminatory Power); iii) PCA (Principal Components Analysis) and; iv) dendrogram through the Neighbor-Joining method. In addition, AMOVA (analysis of molecular variance) was also performed using GenAlEx software and population structure analysis using STRUCTURE software. TRAP markers generated a total of 595 fragments of which 584 (98.15%) were polymorphic. The mean genetic distance between the accessions was 0.3, with the shortest distance between accessions RB721012 and CB40-13 (0.10), and the longest distance between accessions SES205A and CAIANA FITA (0.62). The PIC ranged from 0.99 (F4 + Arbi1-S) to 0.95 (SuPS + Arbi2 and SuPS + Arbi3). The DP ranged from 0.95 (F4 + Arbi1-S) to 1 (StSy + Arbi3). The first and second main components explained 12.4% and 5.4% of the total variability expressed between the accessions, respectively. For AMOVA, the accessions were separated into three groups: i) ancestral (A), composed of accessions of *Saccharum* spp. and the genus *Erianthus*; ii) Brazilian breedings (BB), containing the accessions of the Brazilian breeding programs; and iii) foreign hybrids (FH), composed of accessions originated

from other countries. When AMOVA was performed between groups A and BB, the fixation index was 0.14 (14% of the total variation between the two groups), while the indexes between the BB and FH groups and between A and FH were corresponding to 0.03 and 0.05, respectively. The analysis of population structure showed the formation of two groups: the first composed by the accessions of group A - and the second containing the accessions of groups BB and FH. In the dendrogram, the accessions were divided in 3 clades, and clade 1 was composed mainly by accessions of group A and FH, while clades 2 and 3 by accessions of group BB. Thus, through TRAP markers associated with sucrose and lignin genes, it was possible to estimate the genetic diversity of BPSG and population structure. The data obtained in this work can be explored in the future for studies of association and help breeding programs in the choice of parents and obtaining of superior progenies.

Keywords: genetic distance; molecular markers; sugarcane; sucrose; lignin

1 INTRODUCTION

Sugarcane (*Saccharum* spp.) is grown in tropical and subtropical regions of the world with a high rate of photosynthesis at high temperatures, accumulating mainly sugar (13%), fiber (12%) and water (75%) in its mature stalks (TEW and COBILL, 2008; AITKEN et al., 2018). This plant belongs to the genus *Saccharum*, in which occurs six species: two wild (*S. spontaneum* L. and *S. robustum*) and four cultivated (*S. officinarum, S. barberi, S. sinense* and *S. edule*). Currently, the commercial varieties are denominated taxonomically of *Saccharum* spp. and emerged from interspecific crosses between *S. officinarum*, the "noble cane" and the wild *S. spontaneum* (CESNIK and MIOCQUE, 2004, PARK and DA SILVA, 2016).

Sugarcane is a tropical grass and a significant component of the economy of many countries in the tropics and subtropics, playing a central role as a primary sugar-producing crop and has major potential as a bioenergy crop (SINGH et al., 2010; MOORE et al., 2013; ALI et al., 2019). It is estimated that the total production of sugarcane in the 2018/19 harvest will be 625.96 million tons, and the harvested area will be 8.61 million hectares. With the improvement in the quality of sugarcane, it is expected that there will be an increase in total ethanol production (1.4%), reaching up to 28.16 billion liters (CONAB, 2018b). It is known that sugarcane breeding programs have always aimed at improving the sucrose content. However, with increasing interest in developing biofuels and bio-based chemicals, industries

have required large amounts of biomass and consequently higher yield (DAL-BIANCO et al., 2012).

Energy cane, a type of cane with lower accumulation of sucrose in the stem and higher fiber levels, has been used for the second-generation ethanol (2GE) production (SINGH et al., 2018; LLERENA et al., 2019). Sugarcane bagasse is a lignocellulosic biomass composed of cellulose (39%), hemicellulose (25%), and lignin (23%) and has huge potential for this process. However, the presence of lignin in the bagasse radically decreases the efficiency of releasing sugars for downstream fermentation to produce 2GE (REZENDE et al., 2011; SZCZERBOWSKI et al., 2014).

The productivity gains of the current varieties are due to several years of hybridizations, crosses and selection generations. The focus of sugarcane breeding programs has been the release of cultivars that have the agronomic characteristics of interest and which satisfy the demand of the sugarcane industry (BALSALOBRE et al., 2017), which seeks to select superior varieties that contain favorable alleles for high productivity and sucrose content, acceptable fiber levels for grinding, in addition to resistance to biotic and abiotic stresses (JACKSON, 2005; LANDELL and BRESSIANI, 2008; WELHAM et al., 2010).

However, the high complexity of the genomic structure of sugarcane imposes limitations to the advances in breeding programs and studies involving its genetics, being these individuals highly heterozygous with high level of polyploidy and aneuploidy (GRIVET and ARRUDA, 2001). In addition, the genome complex associated with irregular cytogenetics of the interspecific hybrids of the *Saccharum* complex (ROACH and DANIELS, 1987; MATSUOKA et al., 2005) used in the first hybridizations makes the breeding of sugarcane a laborious practice, 10 to 15 years from the start of the program to the release of a cultivar (GAZAFFI et al., 2014).

The success of a sugarcane breeding program depends on the knowledge and understanding of the genetic diversity available in the germplasm, which usually have thousands of accessions making logistics and planning of future crosses difficult (MANECHINI et al., 2018; MBUMA et al., 2018). Although traditional methods as morphological and agronomical characterization plays an important role in the classification and organization of germplasm accessions, errors may occur since vegetative characteristics are influenced by environmental effects, showing continuous variation and a high degree of plasticity, and which many times do not

reflect the real genetic diversity of the *Saccharum* spp. accessions (LIMA et al., 2002). A complement to the morphological characterization in order to identify an access in a more reliable way is to obtain the molecular profile and to eventually use that information to facilitate the choice of parents increasing the probability of obtaining better performing varieties of the cultivated species (ALWALA et al., 2006; DOS SANTOS et al., 2012; SINGH et al., 2018). Therefore, molecular markers are useful tools to detect variations directly in the genome and are widely used in the characterization of germplasm and have been applied to investigate and measure the genetic diversity of sugarcane accessions and also to trace genetic relationship among *Saccharum* species (HAYWARD et al., 2015; MANECHINI et al., 2018).

Among the numerous molecular markers used for sugarcane genome analyzes, the TRAP (Target Region Amplification Polymorphism) marker is an efficient PCR-based technique that uses Expressed Sequence Tag (EST), making possible the evaluation of collections of germplasm for genes of interest. This approach uses two primers to generate the markers: a fixed primer, which is in the gene region; and an arbitrary primer, which is an AT or CG-rich sequence capable of annealing with an intron or exon, respectively (LI e QUIROS, 2001; HU e VICK, 2003; ALWALA et al., 2006)

Therefore, the use of molecular markers in plant genetic improvement can increase the efficiency in the process of obtaining more productive cultivars, since their information amplifies the comprehension of the genetic diversity between the parents, allowing the breeders a better choice of the crossings. Consequently, the incorporation of molecular markers into pre-breeding and breeding programs increases the chance of obtaining new varieties with characteristics of interest, such as varieties with higher sugar and fiber contents. The choice of parents is a crucial step in plant breeding to ensure maximum gains, particularly in sugarcane, where the generation of a new variety is performed with only a single cross.

2 OBJECTIVES

Evaluate the diversity and genetic structure of *Saccharum* complex accessions that make up the Brazilian Panel of Sugarcane Genotypes (BPSG) using TRAP markers anchored in sucrose and lignin metabolism genes.

3 LITERATURE REVIEW

3.1 Economic importance of sugarcane in Brazil

Sugarcane is a tropical grass and a significant component of the economy of many countries in the tropics and subtropics that stores sucrose in its stem and serves as an important food and bioenergy crop (MOORE et al., 2013). The high demand for clean and sustainable energy allowed the sugarcane chain to become a highlight of agribusiness, with the Brazilian sugar and ethanol sector being the largest producer of ethanol and sugar in the world.

Besides these main products, sugarcane is also used as raw material for fermentation of alcoholic beverages, biofuels and biomass production (AMORIM et al., 2011). The sugarcane bagasse (biomass), which is considered the largest residue of the Brazilian agro-industry generated from the extraction of the juice, can be used in the generation of fuel for boilers, cellulose production and cogeneration of electric energy (SINDHU et al., 2016).

Sugarcane is the most used of the sources that originate the biomass, and from the exploration of its energy potential, the production units have tried to operate with greater efficiency, helping in the reduction of costs and contributing to the sustainability of the activity (DAROS et al., 2015; SOUZA, 2015). Therefore, high investments in this sector allow a higher generation of income and employment. According to a survey carried out by the Center for Advanced Studies in Applied

Economics of the University of São Paulo (CEPEA, 2017), the sugarcane production chain handled R$ 156 billion in Agribusiness Chain.

In the 2017/18 harvest, the area harvested represented 8.73 million hectares, reaching the mark of 633.26 million tons. Sugar production was estimated at 37.87 million tons, while ethanol closed at 27.76 billion liters (Conab, 2018a). The main factor that makes ethanol so important in the sugarcane production chain and for agribusiness in Brazil is the fact that it is a clean, non-polluting and renewable source of fuel.

The production of this crop in Brazil is led by the Southeast, region where 65.9% of all sugarcane produced in this harvest (417.47 million tons) came from this region. The Midwest region remained in second place, obtaining a productivity of 133.88 million tons, followed by the Northeast region that guaranteed production of 41.14 million tons. The South and North regions, respectively, reached an estimated production of 37.52 and 3.46 million tons, respectively (Conab, 2018a).

3.2 Evolutionary and botanical aspects of sugarcane

Sugarcane are generally large, perennial, tropical or subtropical grasses that evolved under conditions of high sunlight, high temperatures, and large quantities of water (MOORE et al., 2013). This plant belongs to the *Poaceae* family, tribe *Andropogoneae*, subtribe *Saccharinae* and genus *Saccharum*, which is composed of six species: two wild (*S. spontaneum* L. and *S. robustum*) and four cultivated (*S. officinarum, S. barberi, S. sinense* and *S. edule*). The cultivated varieties are denominated taxonomically of *Saccharum* spp. and emerged from interspecific crosses between *S. officinarum*, "noble cane" and wild *S. spontaneum* (IRVINE, 1999; CESNIK and MIOCQUE, 2004; GRIVET et al., 2004).

In addition, the so-called "*Saccharum* complex", which encompasses the genera *Saccharum, Erianthus, Miscanthus, Sclerostachya* and *Narenga*, forms a very close interconnected group, being closely involved in the origin of sugarcane and it was once thought to include potential progenitors of the genus *Saccharum* (ROACH and DANIELS 1987; MATSUOKA et al., 2005; ZHANG et al., 2013; TODD et al., 2014). A study with molecular markers provided evidence that *S. officinarum* has a direct association with *S. robustum* and that *S. barberi* and *S. sinense* originate from interspecific crosses between *S. officinarum* and *S. spontaneum* (D'HONT et al. al., 2008; ZHANG et al., 2013).

There are characteristics that visually differentiate the plants of the species *S. spontaneum* and *S. officinarum*. The wild species have short and very thin stem, high fiber content, intense tillering, well developed root system, low sucrose indexes and respond well to pathogens, while the antagonistically noble cane is characterized by thick, low fibers amount and large of sucrose content. In addition, this species has a reduced and superficial root system, presenting susceptibility to various diseases. In order to unite the characteristics of agronomic interest of both species, the first crosses were made. Thus, the currently planted reeds are named *Saccharum* spp. and not only *S. officinarum* (CESNIK and MIOCQUE, 2004; CHEVEGATTI-GIANOTTO et al., 2011; MORAIS et al., 2015a).

3.3 Genomic structure of sugarcane

Sugarcane is unusual among leading crops, since it has one of the most complex genomes, with highly heterozygous individuals, presenting high level of polyploidy and occurrence of aneuploidy (ZHANG et al., 2018). The species of this culture present variation in the number of chromosomes, both among them, and in clones of the same species. The largest differences in the number of chromosomes occur in wild species, with a variation of 2n = 40-128 in *S. spontaneum* and 2n = 60-205 in *S. robustum*. In contrast, these studies of cytogenetic characterization showed that in cultivated species the variation is smaller, in which *S. officinarum* presents 2n = 70-140 chromosomes; *S. sinense* 2n = 111-120; *S. barberi* 2n = 81-124 and *S. edule* 2n = 60-80 (IRVINE, 1999; GRIVET et al., 2004).

Due to the distinct basic numbers of chromosomes manifested by *S. officinarum* and *S. spontaneum*, two divergent chromosomal organizations coexist in the current varieties of sugarcane, resulting in highly heterozygous cultivars, presence of aneuploidy and varying from 100-130 the number of chromosomes, most of which are derived from *S. officinarum*, 10–20% from *S. spontaneum*, and ~10% from interspecific recombinants (D'HONT, 2005; GARSMEUR et al., 2018).

Through the process of sugarcane nobilization, utilization of diverse clones of *S. officinarum* and *S. spontaneum* has been proposed as a way to introduce genetic diversity (YU et al., 2018). A classical cytological peculiarity of 2n chromosome transmission from *S. officinarum* in interspecific crosses with *S. spontaneum* was discovered by Bremer in 1922 (D'HONT et al., 1996). It was verified and demonstrated that this mechanism of transmission also occurs in BC1 when *S.*

officinarum is used as the female parent (BREMER, 1961). Bhat and Gill (1985) proposed the fusion of two nuclei after the second meiosis (endoduplication) to explain the peculiarity. In contrast, Roach found that n + n transmission occurs at crosses between noble cane and *S. spontaneum* with 2n = 80, but rarely occurs at crosses with 2n = 64 or 96 (ROACH, 1972).

Traditional methods that combine agronomic and morphological characteristics have been useful in identifying and describing the differences between members of the *Saccharum* genus (ARTSCHWAGER and BRANDES, 1958; SKINNER, 1971; SKINNER et al., 1987). However, these are predominantly crossed and are maintained by vegetative propagation, and because they are highly heterozygous, they present enormous plasticity in the phenotypic expression of traits. With the advent of molecular markers, it is now possible to make direct inferences about genetic diversity and interrelationships between organisms at the DNA level without the effects of variability caused by genotype x environment interactions (SRIVASTAVA and GUPTA, 2008).

3.4 Breeding of sugarcane

As a result of the new agricultural techniques and the need to meet the demands of industries and the world population, there has been a shift from old cultivars to more productive ones, with superior characteristics, but usually with a narrow genetic base. However, plant breeding is a continuous process and it is often necessary to resort to ancient varieties and primitive populations in search of specific genes, such as wild species carrying genes that confer resistance to diseases. The construction of germplasm collections was an alternative to overcome this problem, making the material available to breeders and presenting variability, as well as avoiding genetic erosion. In other words, these germplasm collections have numerous genetic components (varieties, lineages, clones), being a collection that contains the greatest genetic variability possible (BUENO et al., 2006; JANSKY et al., 2015; GULATI et al., 2018).

Nowadays, the use of these genetic resources through the collections has become of great importance, allowing access to genetic variability between the accessions of these collections and, therefore, to locate new and useful genes to transform many species of cultures. For this to be possible, a collection containing diverse, representative and limited samples is required: a core collection, which

would "represent with a minimum of repetitiveness, the genetic diversity of a crop species and its relatives." The accessions excluded from the core would be retained as the reserve collection (FRANKEL and BROWN, 1984; BROWN, 1989; LUAN et al., 2018).

In Brazil, there are three breeding programs of sugarcane: the Agronomic Institute of Campinas, whose cultivars receive the abbreviation IAC; the Sugarcane Technology Center that incorporated the SP cultivars program of Copersucar, developing cultivars with the abbreviation CTC; and the Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético (Ridesa), in which the cultivars have the abbreviation RB (MORAIS et al., 2015b).

It is known that sugarcane breeding programs have always aimed at improving the sucrose content. However, with increasing interest in developing biofuels and bio-based chemicals, industries have required large amounts of biomass and consequently higher yield (DAL-BIANCO et al., 2012). Thus, the main objectives of sugarcane breeding programs are generally productivity, sugar content, budding ability, disease resistance and acceptable fiber levels for milling (JACKSON, 2005; NARAYAN et al., 2017).

Therefore, a sugarcane breeding program is based on stages. The first stage involves the generation of segregating population with genetic variability through the crossing of parental with interesting characteristics to increase the probability of obtaining superior genotypes. The success of an improvement program is influenced by a number of factors, such as the right choice of the parents for a better chance of gaining selection, installation of experiments with experimental precision, and accurate choice of characters and epochs of evaluation (GAZAFFI et al., 2010). The second stage selects the higher genotypes obtained to propagate them which are then compared based on the experiments using appropriate statistical design to control environmental variations. Experiments in breeding programs involves simultaneous evaluation of several traits, the superior varieties must contain favorable alleles for: (i) high productivity, such as cane tonnage per hectare (TCH), tons of pol. per hectare (TPH), number of stems, sucrose content (Pol) and soluble solids content (ºBrix); (ii) disease resistance; (iii) pests; (iv) abiotic stresses; (v) among other factors (WELHAM et al., 2010). Finally, after numerous selection stages, the remaining genotypes are tested in experiments, performing stability

analysis to find genotypes that respond better to some environmental conditions and allowing them to be recommended as new cultivars (GAZAFFI et al., 2014).

The productivity gains of the current varieties are due to several years of hybridizations, crosses and selection generations. However, the irregular cytogenetics of interspecific hybrids associated with the complex genome of the *Saccharum* species used in the first hybridizations makes breeding of sugarcane a laborious practice, and a sugarcane breeding program may take from 10 to 15 years from the beginning to the launch of a cultivar (CRESTE et al., 2008; LANDELL and BRESSIANI, 2008; GAZAFFI et al., 2014). Another difficulty for obtaining superior genotypes is that the limited genetic base of modern varieties is sustained by a few initial hybrids (AITKEN and McNEIL, 2010; AITKEN et al., 2018). Evaluations of breeding programs have shown that gains in sucrose content have become smaller and smaller, and it is expected that in the future these gains will be even lower (BURNQUIST et al., 2010; DAL-BIANCO et al., 2012).

With advances in genetics and molecular biology, studies have been carried out more quickly and efficiently on the genetic makeup of the species that later will be improved by safely measuring the variability present among breeding program genotypes (CRESTE et al., 2008). In other words, biotechnology has been the main ally in the modernization of techniques that allow the identification and selection of genes that encode favorable traits facing the limitations of classical breeding (DAL-BIANCO et al., 2012).

As an example, the use of molecular markers has innovated the ability to characterize genetic variation in several organisms, since they are able to detect variability at a higher level than conventional methods (CRESTE et al., 2008; HAYWARD et al., 2015). In addition, they participate in assisted selection processes and expression of a certain gene in another organism through transgenic, a precise and efficient way to obtain new desirable traits in crops (CARRER et al., 2010; MORAIS et al., 2015b).

3.5 Application of molecular markers in genetic breeding of sugarcane

Molecular markers are useful tools to detect variations directly in the genome and are widely used in the characterization of germplasm banks with the purpose of identifying repeated accessions, analyzing the degree of representativity, genetic similarity and allelic richness of the collection. In addition, it aims to provide

information that allows the genetic identification of accessions (CAIXETA et al., 2006; FERREIRA et al., 2007; HAYWARD et al., 2015). The characterization of a germplasm collection provides the basic information to scale the magnitude of the genetic diversity available for use in breeding programs, allowing a better access management and consequently, germplasm conservation becomes more efficient and its use in breeding programs effective (DANTAS et al., 2012).

By definition, molecular marker is a nucleotide sequence located at a specific position in the genome, which must have sufficient variability between genotypes so that its inheritance pattern can be analyzed. When using the genome, these markers allow to reveal sites of neutral variations directly in the DNA sequence, and because they are neutral variations, these variations are not expressed in the phenotype, as with morphological markers (NADEEM et al., 2018).

Progress in plant breeding, genetics, genomic selection, and genome editing has contributed to a better understanding of molecular markers and deeper knowledge of the diversity available for crops and has contributed to breeding program strategies (NADEEM et al., 2018). Genetic breeding programs for sugarcane use the molecular markers technique mainly in studies of: genetic identification, such as fingerprinting (varietal certification); identification of the best crosses based on the genetic distance of the parents; to access the genetic diversity of the genotypes used in the programs; in paternity tests; in the identification of characteristics of agronomic interest through the construction of linkage maps (CRESTE et al., 2008; HAYWARD et al., 2015).

There are many available marker systems varying according to their complexity, reliability and information generating capacity. The molecular markers techniques most used in genetic analysis applied to sugarcane breeding are: RFLP (DA SILVA et al., 1993; D'HONT et al., 1993, 1994; LU et al., 1994; GRIVET et al., 1996; DAUGROIS et al., 1996; BESSE et al., 1997) RAPD (MUDGE et al., 1996; MADAN et al., 2000; CHEN et al., 2003), SSRs (ALI et al., 2017, 2019; CORDEIRO et al., 2000, 2003; PAN et al., 2003; PARTHIBAN et al., 2018; PINTO et al., 2004; AITKEN et al., 2005; SINGH et al., 2008; 2010; NAYAK et al., 2014; LIU et al., 2018; XU et al., 2018) and AFLP (BESSE et al. 1998; AITKEN et al. 2005, 2006, 2007, 2017; SELVI et al., 2008). However, most of these markers reflect genetic variability in non-coding or repeating regions of the genome (SINGH et al., 2017).

3.6 Target Region Amplification Polymorphism (TRAP) markers

Unlike other molecular markers, TRAP (Target region amplification polymorphism) is a PCR-based technique that uses EST (Expressed Sequence Tag) or genetic information to generate polymorphism and is capable of revealing genetic variation in the functional part of the plant genome (HU and VICK, 2003; SINGH et al., 2017). Classified as a target marker in a gene, this was developed from studies of functional genomics, which detected polymorphisms in genes that affect the variation of phenotypic characteristics. They may be functional when they are derived from sequences more likely to be involved in varying phenotypic characteristics, or not, when they target expressed regions that are not translated (POCZAI et al., 2013; MANCINI et al., 2017).

For different plant species, each PCR reaction can generate about 50 fragments ranging in size from 50-900 bp when separated on a 6.5% polyacrylamide gel. Two 18 nucleotide primers are used to generate the markers. One, called a fixed primer, is designed from ESTs or genes of interest, while the other, arbitrary primer, is a sequence with an AT or GC rich motif to pair with an intron or exon, respectively. The advantages of this technique are the high reproducibility, simplicity and access to gene-related regions and the ability to produce bands pattern equivalent to that of the AFLP technique (HU and VICK, 2003).

Molecular markers developed from expressed sequence tags (ESTs) and genomic sequences are important for potential applications in breeding programs (MANCINI et al., 2017). The TRAPs and EST-SSR markers have been extensively used in sugarcane diversity studies of the *Saccharum* Complex and commercial varieties (DA SILVA et al., 2001, 2006; ALWALA et al., 2006; QUE et al., 2009; CRESTE et al., 2010; SUMAN et al., 2012; DEVARUMATH et al., 2013; FARSANGI et al., 2018; SINGH et al., 2017, 2018), mapping and detection of QTLs (ALWALA et al., 2008; OLIVEIRA et al., 2009; ANDRU et al., 2011).

Alwala et al. (2006), using 30 genotypes of the *Saccharum* complex genera *Miscanthus* and *Erianthus*, drawn six fixed primer from EST sequences associated with sucrose metabolism and tolerance to cold, stating that the TRAP molecular marker technique is extremely useful in diversity studies genetics in sugarcane. Devarumath et al. (2013) compared the results obtained for TRAP with other markers systems by genetic similarity and groups formed in different methods unweighted pair group and concluded that TRAP can contribute substantially to evaluate genetic

diversity studies, and *Erianthus* spp. can contribute substantially to the improvement of sugarcane through the creation of *Saccharum* spp. or hybrid cultivars. Dos Santos et al. (2017), designed a set of 10 EST-SSR primer pairs and 16 fixed primers from sequences related to resistance to diseases derived from sugarcane. They observed that most TRAP primers were able to amplify and generate polymorphism in the studied genotypes.

In the present study, we characterized the genetic diversity and the population structure using TRAP markers in the sugarcane diversity panel to clarify the genetic relationship among Brazilian cultivars as ancestors and foreign genotypes. Our work focused on molecular markers associated with sucrose and lignin genomics regions.

4 MATERIAL AND METHODS

4.1 Plant material and DNA Extraction

In this study, a total of 254 accessions (Table 1) of the Brazilian Panel of Sugarcane Genotypes (BPSG) were used. This panel consisted of 81 ancestors accessions (A) (75 accessions from *Saccharum* spp. and 06 from *Erianthus* spp.), 137 hybrids from Brazilian breeding programs (BB) and 36 hybrids from Foreign breeding programs – Foreign Hybrids (FH). The BPSG accessions were chosen according to the following criteria: i) relevant Brazilian cultivars, ii) main parents for Brazilian breeding programs; iii) cultivars Genotypes of major sugarcane breeding programs; iv) parents used in mapping programs (CARDOSO-SILVA et al., 2014; BALSALOBRE et al., 2017); and v) representatives of the species from which the *Saccharum* complex originated. The genetic variability present into BPSG, for the most part, was a genetic basis for Brazilian sugarcane breeding programs.

The stalks of the accessions were collected in order to extract the total genomic DNA. The total genomic DNA was extracted from a fresh meristem cylinder according to the cetyl trimethylammonium bromide (CTAB) method for sugarcane proposed by Al-Janabi et al. (1999). DNA concentration was estimated by a Nanodrop spectrophotometer (Thermo Scientific NanoDrop One, Wilmington, DE, U.S.A) at 260 nm of UV wavelength and the DNA was stored at −20°C until further use.

Table 1. Brazilian Panel of Sugarcane Genotypes (BPSG): accessions, pedigree information, origin and predefined groups of the 254 individuals.

ID[1]	Accession	Female parent[2]	Male parent[2]	Origin[2]	Group[3]
1	28NG289	*S. robustum*	?	NA	A
2	57NG12	*S. robustum*	?	NA	A
-	75//09 ERIANTHUS	*Erianthus* spp.	?	NA	A
3	AGAUL	*S. sinense*	?	South Africa	A
4	AGOULE	*S. barberi*	?	NA	A
5	AJA X	*S. officinarum*	?	NA	A
6	AKBAR	Co270	MQ27-1124	NA	FH
7	ARCHI	*S. sinense*	?	Taiwan	A
8	AROUNDOID B	S. MUTANT	?	United States	FH
9	BADILA	S. officinarum	NG9615	New Guinea	A
10	BLACK BORNEO	*S. officinarum*	?	Indonesia	A
11	CAIANA FITA	*S. officinarum*	?	NA	A
12	CAIANA LISTRADA	*S. officinarum*	?	NA	A
13	CAIANA RISCADA	*S. officinarum*	?	NA	A
14	CAIANA VERDADEIRA	*S. officinarum*	?	NA	A
15	CANA ALHO	*S. officinarum*	?	NA	A
16	CANA BLANCA	*S. officinarum*	?	NA	A
17	CANA MANTEIGA	*S. officinarum*	?	NA	A
18	CAYANA	*S. officinarum*	?	NA	A
19	CB36-14	CO 213	?	Brazil	BB
20	CB36-24	POJ2878	?	Brazil	BB
21	CB36-25	?	?	Brazil	BB
22	CB36-68	?	?	Brazil	BB
23	CB40-13	POJ2878	Co290	Brazil	BB
24	CB40-77	POJ2878	Co290	Brazil	BB
25	CB41-76	POJ2878	?	Brazil	BB
26	CB45-155	Co413	?	Brazil	BB
27	CB45-3	Co331	Co290	Brazil	BB
28	CB46-47	POJ2878	?	Brazil	BB
29	CB47-355	POJ2878	Co413	Brazil	BB
30	CB49-260	CB44-36	?	Brazil	BB
31	CB53-98	CB46-40	?	Brazil	BB
32	CERAM RED	*S. officinarum*	?	NA	A
33	CHIN	*S. barberi*	?	India	A
34	CHINA	*S. sinense*	?	NA	A
35	CHUNNE	*S. barberi*	?	India	A
36	CINCA77-316	CP66-346	CP70-321	Bolivia	FH

Table 1. Continued.

ID[1]	Accession	Female parent[2]	Male parent[2]	Origin[2]	Group[3]
37	Co285	Green Sport	*S. spontaneum*	India	FH
38	Co290	Co221	D74	India	FH
39	Co331	Co213	Co214	India	FH
40	Co419	POJ2878	Co290	India	FH
41	Co449	Co331	POJ2878	India	FH
42	Co740	P3247	P4775	India	FH
43	Co997	Co683	P63-32	India	FH
44	CP51-22	F36-819	CP33-372	United States	FH
45	CP52-68	CP29-320	CP38-34	United States	FH
46	CP53-76	F36-819	CP36-46	United States	FH
47	CR72106	EROS	B49-119	Dominican Republic	FH
48	CREOULA	*S. officinarum*	?	NA	A
49	CRIOLLA RAYADA	*S. officinarum*	?	NA	A
50	CRISTALINA	*S. officinarum*	?	NA	A
51	D11/35	*S. officinarum*	?	United States	A
52	D152	B. cheribon	Batjan	NA	FH
53	D625	Dyer	?	NA	FH
54	EK28	POJ100	EK2	Java	FH
55	F150	PT43-52	Nco-310	Taiwan	FH
56	F31-962	Co281	CP27-108	Taiwan	FH
57	F36-819	F31-962	POJ2878	T	FH
58	F76-1762	*Miscanthus* spp.	*S. officinarum*	NA	A
59	FORMOSA	*S. officinarum*	?	Taiwan	A
60	GANDACHENI	*S. barberi*	?	India	A
61	GREEN GERMAN	*S. officinarum*	?	NA	A
-	H. KAWANDANG	*Erianthus* spp.	?	Indonesia	A
62	H53-3989	H48-3717	?	United States	FH
63	H59-1966	H50-676	H49-3646	United States	FH
64	HJ5741	H40-1184	?	United States	FH
65	IAC48-65	CP27-108	?	Brazil	BB
66	IAC49-131	CP27-108	?	Brazil	BB
67	IAC51-205	POJ2878	?	Brazil	BB
68	IAC52-150	Co419	Co285	Brazil	BB
69	IAC58-480	POJ2878	CP44-101	Brazil	BB
70	IAC64-257	Co419	IAC49-131	Brazil	BB
71	IAC68-12	Co419	IAC52-179	Brazil	BB
72	IAC82-2045	IAC65-113	IAC52-150	Brazil	BB
73	IAC82-3092	CB41-76	IAC68-12	Brazil	BB
74	IAC83-4157	IAC68-12	SP70-1143	Brazil	BB
75	IAC86-2210	CP52-58	Co798	Brazil	BB
76	IAC87-3396	Co740	SP70-1143	Brazil	BB
77	IAC91-1099	RB785148	?	Brazil	BB

Table 1. Continued.

ID[1]	Accession	Female parent[2]	Male parent[2]	Origin[2]	Group[3]
78	IS76-155	*S. officinarum*	?	NA	A
79	IJ76-360	*S. edule*	?	NA	A
80	IJ76-293	*S. robustum*	?	Indonesia	A
81	IJ76-313	*S. officinarum*	?	Indonesia	A
82	IJ76-314	*S. robustum*	*S. officinarum*	Indonesia	A
83	IJ76-317	*S. officinarum*	?	Indonesia	A
84	IJ76-318	*S. robustum*	?	Indonesia	A
85	IJ76-325	*S. officinarum*	?	Indonesia	A
86	IJ76-326	*S. officinarum*	?	Indonesia	A
87	IJ76-418 RED	*S. officinarum*	?	Indonesia	A
88	IJ76-560	*S. officinarum*	?	Indonesia	A
-	IM76-227	*Erianthus* spp.	?	Indonesia	A
89	IM76-228	*S. robustum*	?	Indonesia	A
90	IM76-229	*S. robustum*	?	Indonesia	A
91	IN84-103	*S. officinarum*	?	Indonesia	A
92	IN84-104	*S. robustum*	?	Indonesia	A
93	IN84-105	*S. officinarum*	?	Indonesia	A
94	IN84-106	*S. officinarum*	?	Indonesia	A
95	IN84-117	*S. robustum*	?	Indonesia	A
96	IN84-46	*S. officinarum*	?	Indonesia	A
97	IN84-58	*S. spontaneum*	?	Indonesia	A
-	IN84-73	*Erianthus* spp.	?	NA	A
-	IN84-77	*Erianthus* spp.	?	NA	A
98	IN84-82	*S. spontaneum*	?	Indonesia	A
-	IN84-83	*Erianthus* spp.	?	NA	A
99	IN84-88	*S. spontaneum*	?	Indonesia	A
100	KAVANGIRA	*S. sinense*	?	NA	A
101	KRAKATAU	*S. spontaneum*	?	NA	A
102	L60-14	CP52-1	CP48-103	United States	FH
103	LAUKONA	*S. officinarum*	?	United States	A
104	LOUSER	*S. officinarum*	?	NA	A
105	MALI	405N1133	33MQ371	NA	FH
106	MANAII	*S. officinarum*	?	NA	A
107	MANERIA	*S. sinense*	?	India	A
108	MUNTOK JAVA	*S. officinarum*	?	Indonesia	A
109	MZ-151	*S. officinarum*	?	NA	A
110	NA56-79	Co419	Co419	Argentina	FH
111	Nco-310	Co421	Co312	South Africa	FH
112	NG21-17	*S. officinarum*	?	New Guinea	A
113	NG21-21	*S. officinarum*	?	New Guinea	A
114	NG57-221	*S. officinarum*	?	New Guinea	A

Table 1. Continued.

ID[1]	Accession	Female parent[2]	Male parent[2]	Origin[2]	Group[3]
115	NG77-18	*S. officinarum*	?	New Guinea	A
116	POJ161	Black Cheribon	Chunnee	Indonesia	FH
117	POJ2878	POJ2364	EK28	Indonesia	FH
118	Q117	58N829	Q77	Australia	FH
119	Q165	Q 117	CP 33/372	Australia	FH
120	Q70	POJ2878	H31-2484	Australia	FH
121	R570	R445	H32-8560	Reunion Island	FH
122	RAGNAR	*S. officinarum*	*S. spontaneum*	United States	FH
123	RB002601	RB75126	Q107	Brazil	BB
124	RB002700	SP80-1816	?	Brazil	BB
125	RB002754	RB835205	?	Brazil	BB
126	RB721012	Co331	?	Brazil	BB
127	RB72199	NCo334	?	Brazil	BB
128	RB72454	CP53-76	?	Brazil	BB
129	RB725053	Co775	?	Brazil	BB
130	RB725828	NA56-79	?	Brazil	BB
131	RB732577	Nco-376	?	Brazil	BB
132	RB735200	Co331	?	Brazil	BB
133	RB735220	CB61-99	?	Brazil	BB
134	RB735275	IAC49-131	?	Brazil	BB
135	RB736018	M253/48	?	Brazil	BB
136	RB739359	IANE55-34	?	Brazil	BB
137	RB739735	CB52-179	?	Brazil	BB
138	RB75126	C278	?	Brazil	BB
139	RB765418	M253/48	?	Brazil	BB
140	RB785148	IAC47-31	?	Brazil	BB
141	RB785750	TUC67-11	?	Brazil	BB
142	RB805276	NA56-79	?	Brazil	BB
143	RB806043	NA56-79	?	Brazil	BB
144	RB815521	NA56-79	?	Brazil	BB
145	RB815627	IAC49-131	NA56-79	Brazil	BB
146	RB815690	IAC49-131	NA56-79	Brazil	BB
147	RB825317	L60-14	CB47-355	Brazil	BB
148	RB825336	H53-3989	?	Brazil	BB
149	RB825548	F150	?	Brazil	BB
150	RB83100	NA56-79	SP70-1143	Brazil	BB
151	RB83102	NA56-79	SP70-1143	Brazil	BB
152	RB83160	NA56-79	SP70-1143	Brazil	BB
153	RB835019	RB72454	NA56-79	Brazil	BB
154	RB835054	RB72454	NA56-79	Brazil	BB
155	RB835089	RB72454	NA56-79	Brazil	BB
156	RB835205	Co740	?	Brazil	BB

Table 1. Continued.

ID[1]	Accession	Female parent[2]	Male parent[2]	Origin[2]	Group[3]
157	RB835486	L60-14	?	Brazil	BB
158	RB845197	RB72454	SP70-1143	Brazil	BB
159	RB845210	RB72454	SP70-1143	Brazil	BB
160	RB845239	RB72454	SP70-1143	Brazil	BB
161	RB845257	RB72454	SP70-1143	Brazil	BB
162	RB845286	RB72454	SP70-1143	Brazil	BB
163	RB855002	SP70-1143	RB72454	Brazil	BB
164	RB855035	L60-14	SP70-1284	Brazil	BB
165	RB855036	RB72454	SP70-1143	Brazil	BB
166	RB855063	TUC71-7	SP70-1143	Brazil	BB
167	RB855070	SP70-1143	TUC71-7	Brazil	BB
168	RB855077	SP70-1143	TUC71-7	Brazil	BB
169	RB855113	SP70-1143	RB72454	Brazil	BB
170	RB855156	RB72454	TUC71-7	Brazil	BB
171	RB855196	RB72454	TUC71-7	Brazil	BB
172	RB855206	RB72454	TUC71-7	Brazil	BB
173	RB855350	RB72454	?	Brazil	BB
174	RB855357	RB72454	?	Brazil	BB
175	RB855453	TUC71-7	?	Brazil	BB
176	RB855463	RB72454	?	Brazil	BB
177	RB855465	RB72454	?	Brazil	BB
178	RB855511	SP71-1406	?	Brazil	BB
179	RB855533	NA56-79	?	Brazil	BB
180	RB855536	SP70-1143	RB72454	Brazil	BB
181	RB855546	SP70-1143	RB72454	Brazil	BB
182	RB855563	TUC71-7	SP70-1143	Brazil	BB
183	RB855574	SP70-1143	TUC71-7	Brazil	BB
184	RB855589	SP70-1143	TUC71-7	Brazil	BB
185	RB855595	SP70-1143	TUC71-7	Brazil	BB
186	RB855598	SP70-1143	TUC71-7	Brazil	BB
187	RB865214	RB735220	SP71-6163	Brazil	BB
188	RB867515	RB72454	?	Brazil	BB
189	RB925268	RB855511	?	Brazil	BB
190	RB925345	H59-1966	?	Brazil	BB
191	RB92579	RB75126	RB72199	Brazil	BB
192	RB935744	RB835089	RB765418	Brazil	BB
193	RB965902	RB855536	RB855453	Brazil	BB
194	RB965917	RB855453	RB855536	Brazil	BB
195	RB966928	RB855156	RB815690	Brazil	BB
196	RB975148	?	?	Brazil	BB
197	RB975157	RB855563	RB735200	Brazil	BB
198	RB975184	RB72454	SP79-1011	Brazil	BB

Table 1. Continued.

ID[1]	Accession	Female parent[2]	Male parent[2]	Origin[2]	Group[3]
199	RB975201	RB855113	?	Brazil	BB
200	RB975932	SP80-185	RB855206	Brazil	BB
201	RB975952	RB835486	RB825548	Brazil	BB
202	RB985476	H53-3989	RB855206	Brazil	BB
203	SABURA	*S. officinarum*	?	NA	A
204	SAC OFFIC 8272	*S. officinarum*	?	Malasia	A
205	SAC OFFIC 8276	*S. officinarum*	?	Malasia	A
206	SAC OFFIC 8280	*S. officinarum*	?	Malasia	A
207	SAC OFFIC 8284	*S. officinarum*	?	Malasia	A
208	SES205A	*S. spontaneum*	?	India	A
209	SP70-1005	IAC48-65	?	Brazil	BB
210	SP70-1078	IAC48-65	?	Brazil	BB
211	SP70-1143	IAC48-65	?	Brazil	BB
212	SP70-1284	CB41-76	?	Brazil	BB
213	SP70-1423	CB41-76	?	Brazil	BB
214	SP70-3370	CP53-17	?	Brazil	BB
215	SP71-1406	NA56-79	?	Brazil	BB
216	SP71-6163	NA56-79	?	Brazil	BB
217	SP71-6949	NA56-79	?	Brazil	BB
218	SP71-799	NA56-79	?	Brazil	BB
219	SP72-4928	CP52-48	?	Brazil	BB
220	SP77-5181	HJ57-41	?	Brazil	BB
221	SP79-1011	NA56-79	Co775	Brazil	BB
222	SP79-2233	H56-2954	?	Brazil	BB
223	SP79-2312	SP71-6106	?	Brazil	BB
224	SP79-2313	SP71-6106	?	Brazil	BB
225	SP79-6192	SP70-3518	?	Brazil	BB
226	SP80-1520	H48-3166	SP71-1088	Brazil	BB
227	SP80-180	B3337	?	Brazil	BB
228	SP80-1816	SP71-1088	H57-5028	Brazil	BB
229	SP80-1836	SP71-1088	H57-5028	Brazil	BB
230	SP80-1842	SP71-1088	H57-5028	Brazil	BB
231	SP80-185	BO17	?	Brazil	BB
232	SP80-3280	SP71-1088	H57-5028	Brazil	BB
233	SP81-1763	co775	NA56-79	Brazil	BB
234	SP81-3250	CP70-1547	SP71-1279	Brazil	BB
235	SP83-2847	HJ5741	SP70-1143	Brazil	BB
236	SP83-5073	SP71-1406	SP71-1088	Brazil	BB
237	SP86-155	SP78-3081	?	Brazil	BB
238	SP89-1115	CP73-1547	?	Brazil	BB
239	SP91-1049	SP80-3328	SP81-3250	Brazil	BB
240	TUC71-7	CP52-68	CP62-258	Argentina	FH

Table 1. Continued.

ID[1]	Accession	Female parent[2]	Male parent[2]	Origin[2]	Group[3]
241	UBA DEMERARA	*S. sinense*	?	NA	A
242	US57-141-5	*S. robustum*	?	NA	A
243	US60-31-3	28NG12	US57-159	United States	FH
244	US85-1008	S. spontaneum	US60-31-3	NA	A
245	WHITE MAURITIUS	*S. officinarum*	?	India	A
246	WHITE PARARIA	*S. barberi*	?	India	A
247	WHITE TRANSPARENT	*S. officinarum*	?	India	A
248	ZWART MANILA	*S. officinarum*	?	Indonesia	A

[1]Accessions number in STRUCTURE analysis (Material and Methods section 4.4.2). [2]Information from: RIDESA germoplasm bank (http://www.ridesaufscar.com.br), Miami World Collection (http://npgsweb.arsgrin.gov/gringlobal/search.aspx) and Sugarcane Variety Notes (Rossi, 2000). NA: information not available. [3]A: ancestors accessions, BB: accessions of *Saccharum* spp. hybrids from Brazilian breeding programs, FH: accessions of *Saccharum* spp. hybrids from Foreign breeding programs - Foreign Hybrids.

4.2 TRAP markers, genotyping and polymorphism analysis

To compose TRAP markers five fixed and four arbitrary primers were used (Table 2). The arbitrary primers were adapted of Li and Quiros (2001), Alwala et al., (2006) and Suman et al., (2012). In addition, three fixed primers associated with sucrose metabolism genes based on Alwala et al. (2006) (*sucrose synthase* (SuSy), *sucrose phosphate synthase* (SuPS) and *starch synthase* (StSy)) and two fixed primers associated with lignin metabolism genes based on Suman et al. (2012) (*caffeic acid O-methyltransferase* (COMT) and *ferulate-5-hydroxylase* (F5H)) were used. Thus, based on high percentage of polymorphism showed by the reference studies, we chose 08 combinations to compose the TRAP markers of this work: StSy + Arbi2, StSy + Arbi3, SuPS + Arbi2, SuPS + Arbi3, SuSy + Arbi1-A, SuSy + Arbi2 for sucrose metabolism and F2+Arbi1-S and F4+Arbi1-S for lignin metabolism.

Table 2. TRAP information: primers fixed and arbitraries, sequences 5' – 3', genbank ID and the reference studies.

Primers	Names	Sequences (5' – 3')	Genbank ID	Reference
Fixed (forward)	SuSy	GGAGGAGCTGAGTGTTTC	AF263384	Alwala et al. (2006)
	SuPs	CGACAACTGGATCAACAG	AB001338	
	StSy	GGCAAGAAGAAGTTCGAG	AF446084	
	COMT	TCGGTCATCATCACCAAGAA	AJ231133.1	Suman et al. (2012)
	F5H	ACCACCCTACGTGGACTCAG	NM_119790.2	
Arbitrary (reverse)	Arbi1-A*	GACTGCGTACGAATTAAT		Li and Quiros et al. (2001) Alwala et al. (2006) Suman et al. (2012)
	Arbi1-S*	GACTGCGTACGAATTATT		
	Arbi2	GACTGCGTACGAATTGAC		
	Arbi3	GACTGCGTACGAATTTGA		

* Arbi1-A and Arbi1-S were the arbitraries primers Arbi1 used by Alwala et al. (2006) and Suman et al. (2012), respectively.

The PCR were performed on Eppendorf Mastercycler thermocycler (Westbury, New York, USA) and according to the protocol described by Hu and Vick (2003) with some modifications. Briefly, each PCR related with sucrose and lignin metabolisms was performed in a final volume of 20 uL with 10X PCR buffer, 2.5 Mm $MgCl_2$, 0.5 mM dNTPs, 0.5 U Taq DNA polymerase (Promega, Madison, WI) and 50 to 80 ng genomic DNA. The concentrations of primers (fixed and arbitraries) and amplification conditions were different between TRAP markers for sucrose metabolism and TRAP markers for lignin metabolism. Then, for sucrose metabolism the primers concentrations were 0.5 µM and the PCR conditions were as follow: an initial denaturation at 94°C for 4 min followed by 5 cycles with denaturation at 94°C for 55 s, annealing temperature at 35°C for 55 s and extension at 72°C for 1 min, followed by 35 cycles with denaturation at 94°C for 55 s, annealing at 53°C for 55 s, extension at 72ºC for 1 min with a final extension step at 72°C for 7 min and hold at 15°C. On the other hand, for lignin metabolism the primers concentrations were 1 µM and the PCR conditions were as follow: an initial denaturation at 94°C for 4 min followed by 5 cycles with denaturation at 94°C for 1 min, annealing temperature at 35°C for 1 min and extension 72°C for 1 min, followed by 35 cycles with denaturation at 94°C for 1 min, annealing at 56°C for 1 min, extension at 72ºC for 1 min with a final elongation at 72°C for 7 min and hold at 15°C. After PCR, the amplified products were run on 6.5% (w/v) polyacrylamide denaturing gel for 4.0 h at 65 W. Silver staining procedure was employed, as described by Creste et al. (2001), to detect the fragments.

Fragments from the TRAP gel were scored as ‘‘1’’ for presence and ‘‘0’’ for absence, in all accessions. Only clearly distinguishable fragments were scored. For each TRAP marker, the presence of exclusive fragments was investigated. Through the binary matrix, the values of PIC (Polymorphism Information Content) and DP (Discriminatory Power) were calculated according to Botstein et al. (1980) and Tessier et al. (1999), respectively. PIC was used as a tool to measure the information of a given marker locus for the pool of accessions, while DP was used as a measure of marker efficiency for the purpose of identification of accession, i.e., the probability that two randomly chosen individuals have different patterns (OLIVEIRA et al., 2009).

4.3 Population differentiation

The analysis of molecular variance (AMOVA) was performed by the GenAIEx software (PEAKALL & SMOUSE, 2012) to quantify the degree of differentiation and distribution of the genetic variability between and within predefined cases: a) ancestors accessions (group A) and hybrids from Foreign breeding programs (group FH); b) ancestors accessions (group A) and hybrids from Brazilian breeding programs (group BB); and c) hybrids from Foreign breeding programs (group FH) and hybrids from Brazilian breeding programs (group BB). The BB group was subdivided according to the breeding programs CB (Estação Experimental de Campos dos Goytacazes, Campos-RJ, first incorporated by PLANALSUCAR in 1972 and after by RIDESA in 1991), IAC (Instituto Agronômico de Campinas, Campinas-SP), RB (República do Brasil, cultivars released by RIDESA - Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético) and SP (former COPERSUCAR, currently CTC, Piracicaba-SP).

4.4 Genetic structure

4.4.1 Principal component analysis

Principal Component Analysis (PCA) was performed in the R program (R Core Team, 2018) through the FactoMineR (LÊ; JOSSE; HUSSON, 2008) and factoextra (KASSAMBARA & MUNDT, 2017) packages and their respective functions PCA and fviz_pca_ind using raw data from genotyping of TRAP markers aiming to detect the presence of some clustering pattern among the evaluated accessions. The accessions of groups A, BB and FH were indicated with different colors in the graph.

4.4.2 STRUCTURE analysis

The STRUCTURE analysis was performed considering the 248 accessions of the genus *Saccharum* present in BSPG. The number of subpopulations (k) and the membership proportion (Q) were inferred using the Structure v.2.3.4 software (EVANNO; REGNAUT; GOUDET, 2005; PRITCHARD; STEPHENS; DONNELLY, 2000). The k was set from 1 to 10 (k-value), with 10 iterations at a 100,000 burning period and 200,000 Markov Chain Monte Carlo (MCMC) repeats. The Structure Harvester software was used to find the best values of k and Δk (EARL & VON-HOLDT, 2010).

4.4.3 Genetic dissimilarity and phylogenetic analysis

The pair-wise dissimilarity among the accessions of the genus *Saccharum* present in BSPG was estimated according to the Jaccard dissimilarity coefficient (Dis = 1 - Jaccard) using the vegan package and their respective function vegdist (OKSANEN et al., 2013). The genetic dissimilarity matrix was used to design a phylogenetic tree according to the Neighbor-Joining (NJ) method with 1000 bootstrapping through ape package and their respective function nj (PARADIS & SCHLIEP, 2018). To verify if the number of TRAP fragments used to estimate the genetic dissimilarities between accessions was adequate in terms of accuracy, the bootstrap resample technique (EFRON & TIBSHIRANI, 1994) was applied as in Tivang et al., (1994), Hállden et al., (1994) and Manechini et al., (2018). An exponential function was adjusted to estimate the number of markers needed to assure that the CV associated with the dissimilarity estimates were lesser or equal to 10%, considered acceptable in this research. The median of the coefficient of variation estimates were used to evaluate the accuracy of the dissimilarity values (GARCIA et al., 2004). All these procedures were performed in R software (R Core Team, 2018).

5. RESULTS

5.1 TRAP markers polymorphism

The results regarding the number of fragments, number and percentage of polymorphic fragments, PIC and PD values for each of the 08 TRAP combinations used in this study are summarized in Table 3. A total of 595 fragments were obtained of which 584 were polymorphic (98.15%). The number of fragments for each TRAP markers ranged from 88 (SuSy + Arbi1-A) to 44 (SuPS + Arbi2) with an average of 74.37 fragments per locus. The polymorphism percentage was high (> 90%), ranging from 94.64% (SuPS + Arbi3) to 100% (SuPS + Arbi2, F2 + Arbi1-S and F4 + Arbi1-S). The averages of PIC and PD values were 0.97 and 0.98, respectively. Furthermore, PIC ranged from 0.95 (SuPS + Arbi2 and SuPS + Arbi3) to 0.99 (F4 + Arbi1-S) and PD ranged from 0.95 (F4 + Arbi1-S) to (StSy + Arbi3).

Table 3. Number of fragments, number and percentage of polymorphic fragments, polymorphic information content (PIC) and discriminatory power (DP) for each TRAP combination evaluated in the Brazilian Panel of Sugarcane Genotypes (BPSG)

TRAP combination	Fragments observed	Polymorphic fragments	Percentage polymorphism	PIC	DP
SuSy + Arbi1-A	88	87	98.86	0.97	0.99
SuSy + Arbi2	84	81	96.43	0.97	0.99
StSy + Arbi2	75	72	96.00	0.97	0.99
StSy + Arbi3	87	86	98.85	0.98	1.00
SuPS + Arbi2	44	44	100.00	0.95	0.99
SuPS + Arbi3	56	53	94.64	0.95	0.96
F2 + Arbi1-S	81	81	100.00	0.98	0.99
F4 + Arbi1-S	80	80	100.00	0.99	0.95
Average	74.37	73	98.00	0.97	0.98
Total	595	584			

From the total number of polymorphic fragments, 68 (11.64%) were putative exclusive fragments. The combinations that obtained highest and lowest number of exclusive fragments were SuSy + Arbi2 (22.22%) and F4 + Arbi1-S (0%), respectively. Among the ancestor group (A), *Erianthus* spp., *S. spontaneum*, *S. robustum*, *S. officinarum* and *S. barberi* showed 49 (8.39%), 08 (1.36%), 06 (1.02%), 01 (0.17%) and 01 (0.17%) exclusive fragments, respectively. The KRAKATAU (*S. spontaneum*) access presented three exclusive fragments, one of these fragments being present in the combination StSy+Arbi2 and two fragments in the combination StSy+Arbi3. Also in the combination StSy+Arbi3, two exclusive fragments of the SES205A (*S. spontaneum*) access were also found. Among the accessions of *S. robustum*, IJ76-318 presented an exclusive fragment in the combination SuSy+Arbi1. An exclusive fragment was seen in this combination, SuSy+Arbi1, in the AGOULE (*S. barberi*) access. Furthermore, three fragments, representing 0.51% of the total fragments, were exclusive to the accessions from BB group.

Table 4. Exclusive TRAP fragments observed in genotypes in the Brazilian Panel of Sugarcane Genotypes (BPSG).

Groups*	Species	Number of exclusive fragments per TRAP markers								Total number of exclusive fragments
		SuSy+arbi1-A	SuSy+arbi2	SuPS+arbi2	SuPS+arbi3	StSy+arbi2	StSy+arbi3	F2+arbi1	F4+arbi1-S	
A	*Erianthus* spp.	8	18	3	11	8	0	1	0	49
A	*S. spontaneum*	1	0	1	0	2	4	0	0	8
A	*S. officinarum*	0	0	0	0	0	1	0	0	1
A	*S. robustum*	1	0	0	2	3	0	0	0	6
A	*S. barberi*	1	0	0	0	0	0	0	0	1
BB	*S.* spp. hybrids	2	0	0	0	1	0	0	0	3
		13	18	4	13	14	5	1	0	68

*A: Ancestors accessions (comprise representatives of the genus *Saccharum* and *Erianthus*); BB: accessions of *Saccharum* spp. hybrids from Brazilian Breeding programs; FH: accessions of *Saccharum* spp. hybrids from foreign breeding programs.

5.2 Population differentiation

Considering all predefined groups (A, BB and FH), AMOVA results revealed that the molecular variance found by TRAP markers was higher within populations than among populations (Table 5). The genetic differentiation value (Φ_{PT}) obtained between A and BB was 0.14 (Table 5), which means that 14% of the total variation found by the TRAP markers is distributed between these two groups, while 86% is within them. On the other hand, the Φ_{PT} between A and FH (Φ_{PT} = 0.05) was lower than that observed between A and BB. The smallest genetic differentiation was found when comparing BB and FH (Φ_{PT} = 0.03) accessions. In addition, Φ_{PT} values were significant for all groups ($P < 0.001$).

Table 5. Analysis of molecular variance (AMOVA) for predefined groups A (ancestors representatives of the genus *Saccharum* and *Erianthus*), BB (hybrids from Brazilian breeding programs) and FH (hybrids from foreign breeding programs).

Groups	Source of variation	d.f.	Sum of squares	Variance components	Porcentage of variation
BB and A	Between population	1	944.93	9.28	14%
	Within populations	209	12345.46	59.07	86%
	Total	210	13290.39	68.35	
			Φ_{PT}: 0.14*		
FH and A	Between population	1	242.44	3.38	5%
	Within populations	113	7943.11	70.29	95%
	Total	114	8185.55	73.68	
			Φ_{PT}: 0.05*		
BB and FH	Between population	1	138.34	1.48	3%
	Within populations	174	8761.99	50.36	97%
	Total	175	8900.33	51.83	
			Φ_{PT}: 0.03*		

d.f.: degrees of freedom.
*P<0.001.

5.3 Genetic structure

5.3.1 Principal component analysis

Principal component analysis (PCA) was firstly performed based on 595 TRAP fragments with all 254 accessions of BPSG, which includes accessions of predefined A, BB and FH groups (Figure 1A). The A group was composed by representatives of the genus *Saccharum* and *Erianthus*. On the other hand, the BB and FH groups include accessions of *Saccharum* spp. hybrids, being the first one from Brazilian breeding programs and the second from foreign breeding programs. Considering that

the panel under study presents accessions of two genera (*Saccharum* and *Erianthus*), a second PCA was performed without accessions of the genus *Erianthus* and using 546 TRAP fragments (Figure 1B).

So, in the first PCA the first two principal components, PC1 and PC2, explained 17.8% of the total variability expressed among accessions (Figure 1A). According to PC1 it is possible to note that *Erianthus* accessions (75//09 Erianthus, H. KAWANDANG, IM76-227, IN84-73, IN84-77 and IN84-83) were grouped in a cluster isolated from the others accessions. In addition, the accessions of *S. spontaneum* were positioned together (IN84-58, IN84-82, IN84-88, KRAKATAU and SES205A). In contrast, accessions of FH group were spread in non-clustered way, being some closer to accessions of A group (for example, CR72/106 and US60-31-3), while others closer to accessions of BB group (for example, NCo-310 and EK28). The accessions of BB group showed a tendency of clustering greater than A and FH groups, and it is possible to note two formation of genotypes within the group.

Already in the second PCA, PC1 and PC2 explained 12.7% of the total variability expressed among accessions (Figure 1B). The accessions of A group were spread over PC1, being some accessions of *S. officinarum* (for example, WHITE TRANSPARENT, CAIANA RISCADA, SAC OFFIC 8272, NG21-21, NG57-221, CAYANA, WHITE MAURITIUS and AJAX) closely positioned with accessions originated from breeding programs. In addition, accessions representatives of *S. barberi* (GANDACHENI and WHITE PARARIA) and *S. sinense* (MANERIA) also were nearby of improved accessions. The accessions of the FH group were spread almost equally along PC1 and PC2, as can be observed by the blue ellipse with center near the 0-0 coordinate and also by the presence of FH accessions in the four quadrants of the graph. The BB group apparently showed the division of their accessions into two clusters, one with most accessions in the second quadrant and another in the fourth quadrant of the graph. In general, this division agrees with pedigree information. For example, the accessions RB965917 and RB965902 are full-sibs originated from the cross between RB855453 and RB855536 and all were positioned into the cluster at second quadrant, while RB845197, RB845210, RB845257, TB855036, RB855002 and RB855113, which are full-sibs originated from the cross between RB72454 and SP70-1143, all positioned into the cluster at the fourth quadrant. The presence of half-sibs should also contribute to this division. For example, RB806043, RB815521, RB83102, RB855533, SP71-6163, SP716949,

SP81-1763, RB815627, RB815690 and RB835054, all sharing the parent Na56-79 and were all positioned into a cluster at the second quadrant, while IAC87-3396, SP83-2847, RB845197, RB845210, RB845257, RB855036, RB855002, RB855070, RB855113, RB855595 and RB855598 sharing the parent SP70-1143, were all positioned into a cluster at the fourth quadrant.

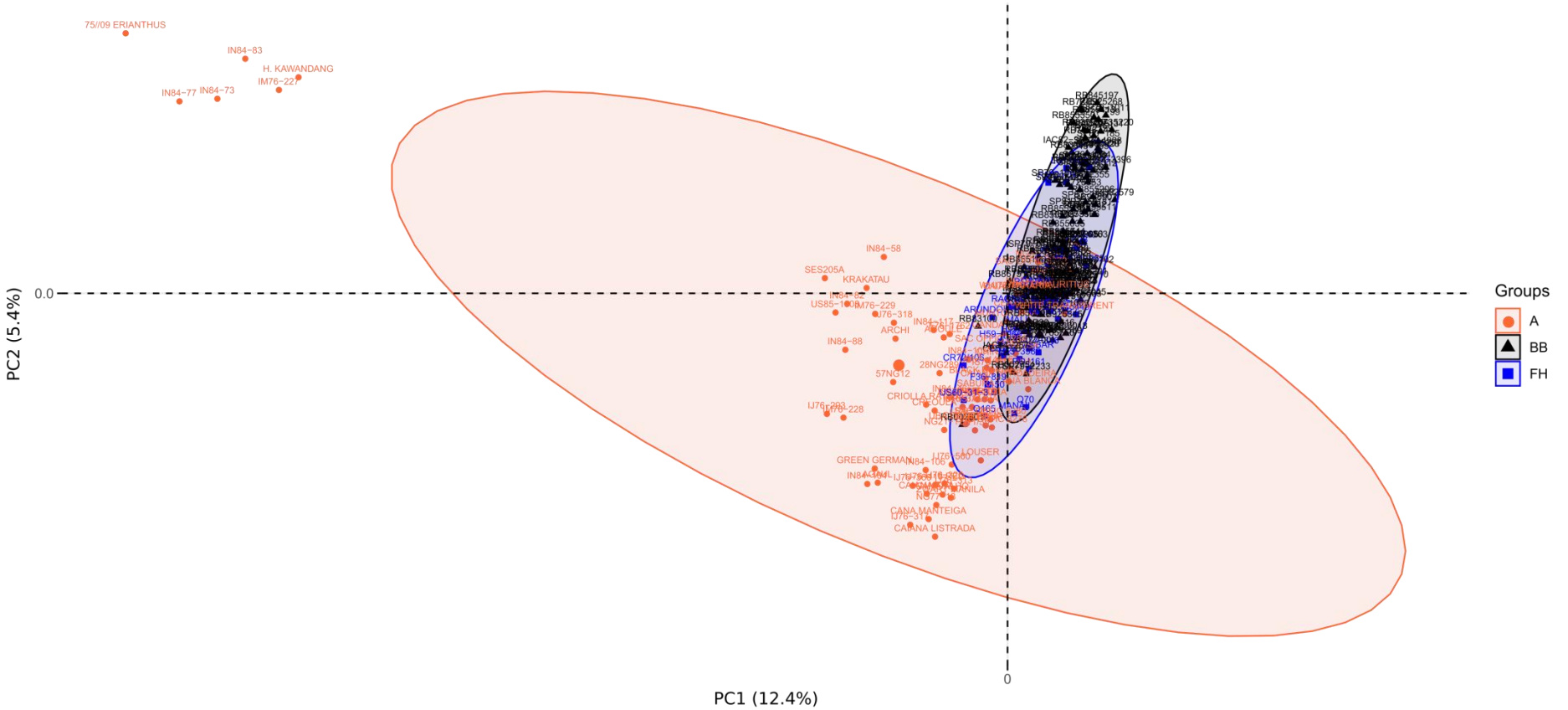


Figure 1. Principal Components Analysis (PCA) using accessions of the Brazilian Panel of Sugarcane Genotypes (BPSG) based on TRAP markers **Figure 1A.** Analyzes carried out with all 254 accessions of BPSG. **Figure 1B.** Analyzes carried out without 06 accessions of *Erianthus* spp.. A: group composes by ancestors representatives of the genus *Saccharum* and *Erianthus* in Figure 1A and only genus *Saccharum* in Figure 1B. BB: hybrids from Brazilian breeding programs; FH: hybrids from foreign breeding programs.

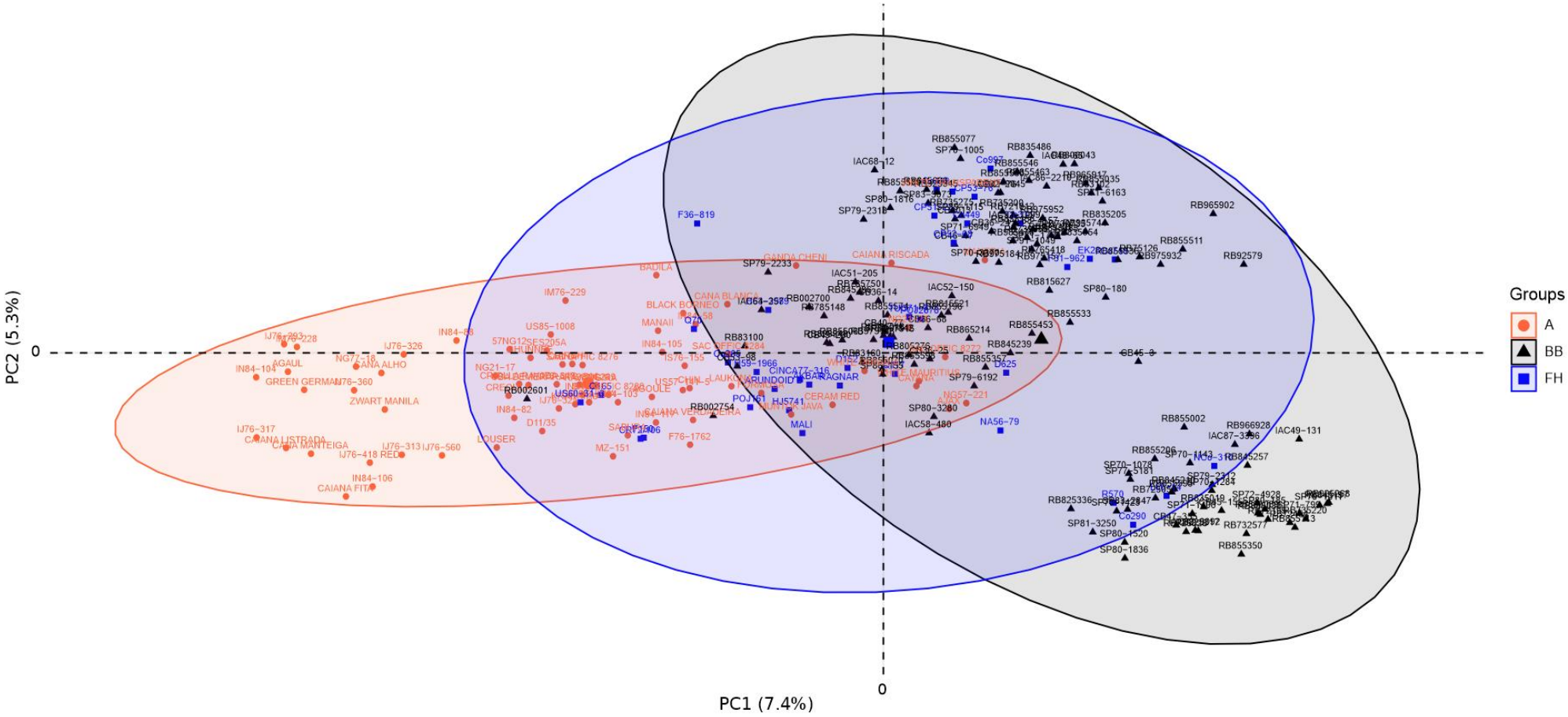
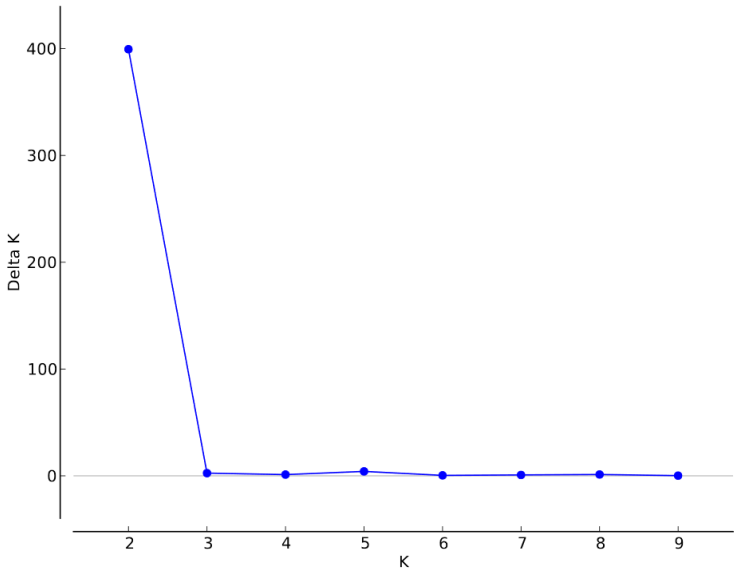


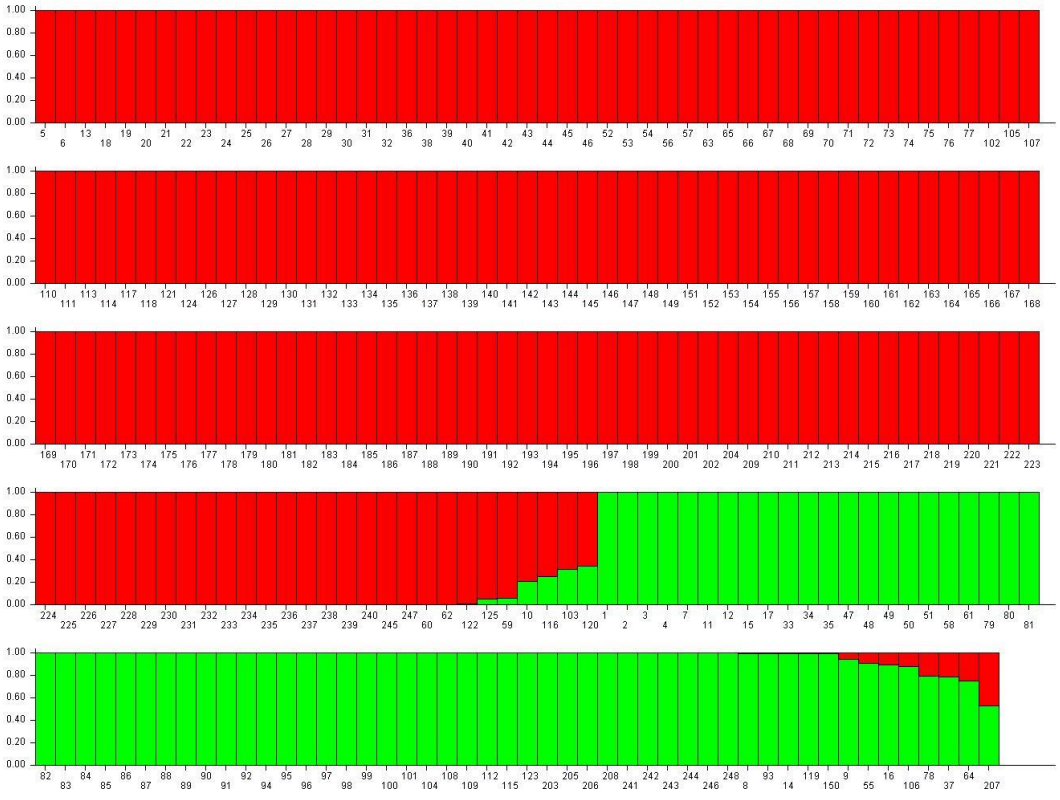
Figure 1. Continued.

5.3.2 STRUCTURE analysis

According to the STRUCTURE analysis (without accessions of genus *Erianthus*), the best k value was 02 (ΔK = 399.43, Figure 2A), suggesting that the 248 accessions of genus *Saccharum* can be divided into two subpopulations (Figure 2B), P1 and P2 containing 178 and 70 accessions, respectively (Figure 2B and Figure 4). P1 had 164 accessions belonging to BB and FH groups and only 14 accessions belonging to A group. The ancestors accessions into P1 were representatives of *S. officinarum* (AJAX, BLACK BORNEO, CAIANA RISCADA, CAYANA, CERAM RED, FORMOSA, LAUKONA, NG21-21, NG57-221, SAC OFFIC 8272, WHITE MAURITIUS and WHITE TRANSPARENT), *S. barberi* (GANDACHENI) and *S. sinense* (MANERIA), in accordance with the evolutionary and breeding history of sugarcane. In contrast, P2 had 61 accessions belonging to A group and only 09 accessions were improved accessions (AROUNDOID B, CR72/106, Q165, RB83100, RB002601 and US60-31-3, Co285, F150, HJ5741). Therefore, P1 had most of the accessions of BB and FH groups while P2 had most of accessions of A group. Furthermore, 14 accessions showed probabilities to be part of both subpopulations (Figure 2B). Among these, 06 accessions were more likely to be allocated in P1 (BLACK BORNEO, FORMOSA, LAUKONA, POJ161, Q70 and RB002754) and the other 08 accessions were more likely to be included in P2 (BADILA, CANA BLANCA, Co285, F150, HJ5741, IS76-155, MANAII and SAC OFFIC 8284).



A



B

Figure 2. STRUCTURE analysis using 248 accessions of the Brazilian Panel of Sugarcane Genotypes (BPSG). **Figure 2A**. Best k analysis showing k values from 2 to 9 (10 suppressed). **Figure 2B.** Membership proportions (Q) for each accession for k = 2. Red and green colors indicate the membership proportions of each accession to subpopulations P1 and P2, respectively. The names of the accessions can be consulted in Table 1 with their correspondent number (ID) in Material and methods section.

5.3.3 Genetic dissimilarity and phylogenetic analysis

The number of TRAP fragments used in this study was sufficient to estimate the pair-wise genetic dissimilarity with an acceptable level of accuracy. Considering the 546 fragments used in this analysis the CV was 8.64% (Figure 3), under the threshold previously established of 10%. An amount around 400 fragments would be sufficient to obtain a CV average estimate around 10%.

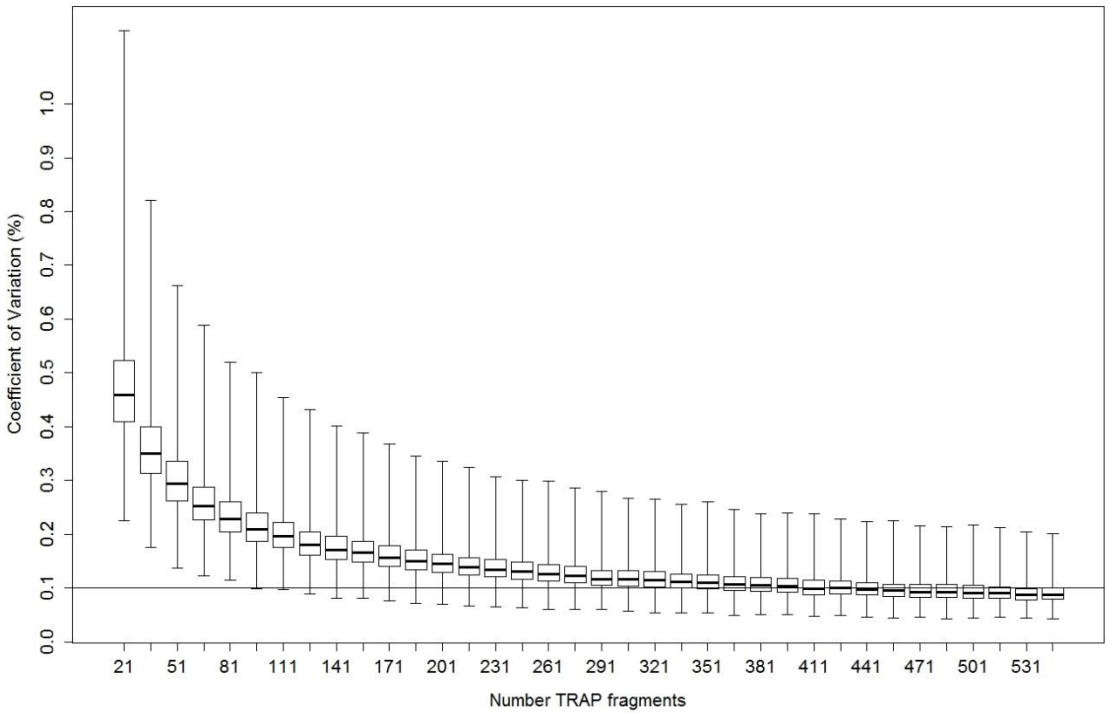


Figure 3. Boxplots of the coefficients of variation (CV%), associated with the estimates of genetic dissimilarities, by bootstrap analysis for subsamples with different numbers of TRAP fragments.

The highest dissimilarity value was between SES205A (*S. spontaneum*) and CAIANA FITA (*S. officinarum*) accessions (0.62), and the lowest dissimilarity value was observed between CB40-13 (BB) and RB721012 (BB) accessions (0.10). The average dissimilarity values within the A, BB and FH groups were 0.36, 0.34 and 0.29, respectively. Considering a subdivision of BB group according to different breeding programs the average dissimilarities were 0.23, 0.24, 0.26 and 0.26 to CB, IAC, RB and IAC subgroups, respectively (Table 6). The highest average dissimilarities were found when the A group was compared with the FH group and BB subgroups (average of 0.34). In contrast, smaller average dissimilarities occurred between and within the FH group and BB subgroups (CB, IAC, RB and SP), ranged

from 0.23 (within CB subgroup) to 0.29 (within FH group and between FH group and SP subgroup).

Table 6. Average dissimilarities between predefined groups A (ancestors representatives of the genus *Saccharum*), BB (hybrids from Brazilian breeding programs CB, IAC, RB and SP) and FH (hybrids from foreign breeding programs) according to Jaccard coefficient.

Groups		**A**	**BB**				**FH**
			CB	IAC	RB	SP	
A		0.36					
BB	CB	0.32	0.23				
	IAC	0.34	0.24	0.24			
	RB	0.34	0.25	0.26	0.26		
	SP	0.35	0.25	0.26	0.27	0.26	
FH		0.34	0.27	0.27	0.28	0.29	0.29

The phylogenetic tree carried out with accessions of the genus *Saccharum* revealed the prevalence of 3 major clades (Figure 4). The clade C1 was composed mainly by accessions of the A group (68 accessions), followed by 18 FH accessions (AKBAR, CINCA77-316, Co285, Co997, Co449, CP51-22, CP52-68, CR72/106, D625, F150, H59-1966, HJ5741, MALI, POJ161, Q70, Q165, RAGNAR and US60-31-3) and 31 BB accessions (CB36-24, CB36-25, CB4013, CB41-76, CB46-47, IAC48-65, IAC82-2045, RB002601, RB002700, RB721012, RB735275, RB736018, RB785750, RB805276, RB806043, RB815690, RB83102, RB845286, RB855035, RB855070, RB855463, RB865214, SP70-1005, SP70-3370, SP79-2233, SP79-2313, SP80-1816, SP81-1763, SP83-5073, SP86-155 and SP89-1115). On the other hand, the clades C2 and C3 were composed largely of BB accessions. Clade C2 had 74 BB accessions, 8 FH accessions (Co290, Co419, Co740, EK28, F31-962, L60-14, Nco-310 and R570) and 3 from the A group (CERAM RED, NG57-221 and WHITE MAURITIUS). Finally, clade C3 was composed by 32 BB accessions, 10 FH accessions (AROUNDOID B, Co331, CP53-76, D152, F36-819, H53-3989, Na56-79, POJ2878, Q117 and TUC71-7) and 4 A accessions (CAIANA RISCADA, MANERIA, NG21-21 and SAC OFFIC 8284).

In addition, the clustering of the phylogenetic tree was similar to the arrangement of accessions in the PCA (Figure 1B) and, in general, the clades were also in agreement with pedigree information. Evidence of this is that some

accessions that were widely used as parents by Brazilian breeding programs were grouped at the same clade with their progenies of full-sibs or half-sibs. In clade C2, for example, the full-sibs RB845197, RB845210, RB845239, RB845257, RB855036, RB855002, RB855113 and RB855536 were grouped together with their parents SP70-1143 and RB72454. In clade C3, there are three interesting cases: a) F36-819, IAC58-480 and IAC51-205 are half-sibs with the parent POJ2878 in common; b) RB835054, RB83100, RB855533 and SP716949 are half-sibs with the parent NA56-79 in common; and c) RB855156, RB855196, RB855070, RB855077, RB855574, RB855589 and RB855453 are half-sibs with the parent TUC71-7 in common. On the other hand, the half-sibs CB36-24, CB40-13, CB46-47, CB41-76, Q70 and Co449, that have POJ2878 as common parent, were grouped in clade C1, but the other parents of these accessions were not included in the clade.

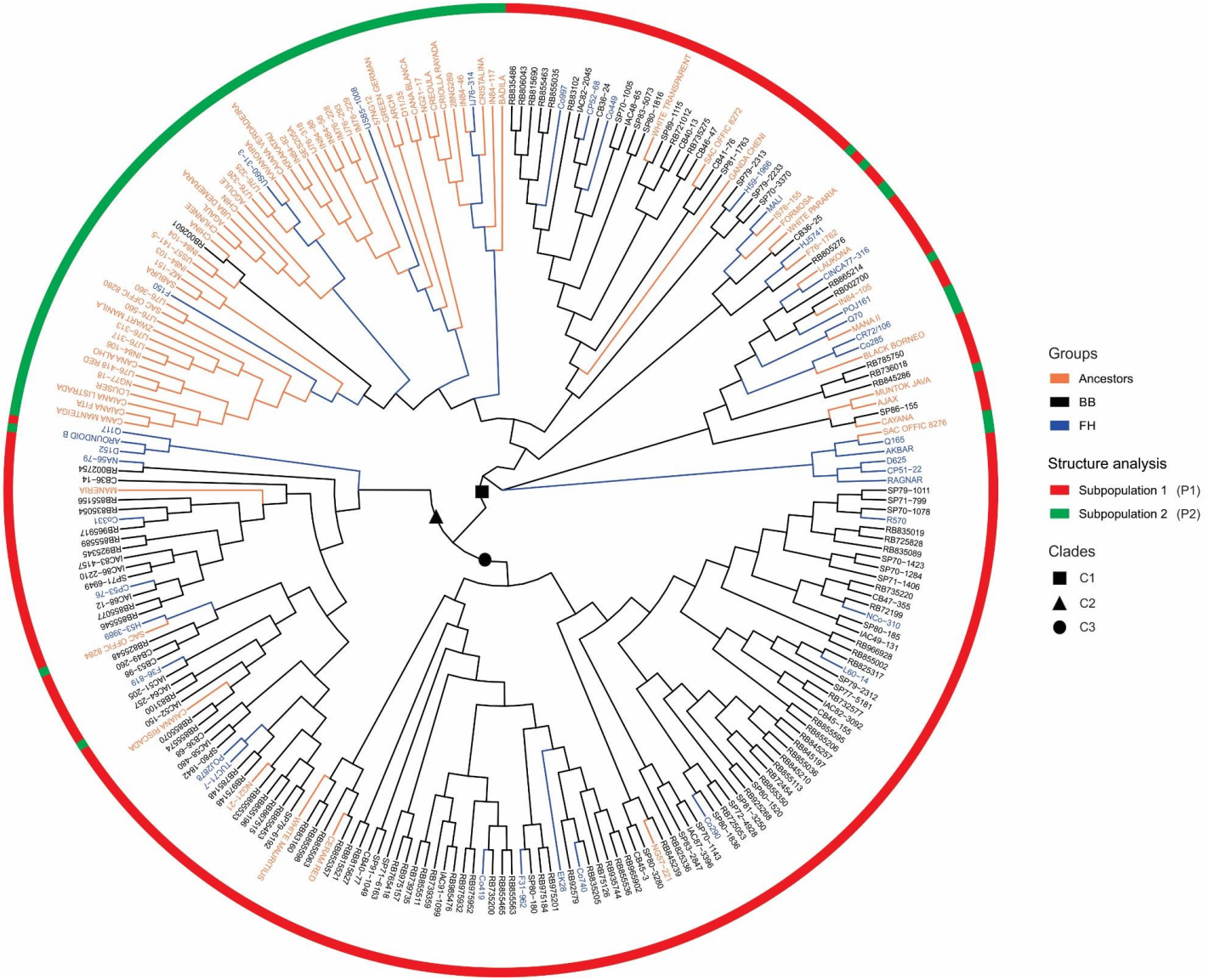


Figure 4. Phylogenetic tree estimated through Neighbor-Joining method for 248 accessions of the Brazilian Panel of Sugarcane Genotypes (BPSG). The circumference around the phylogenetic tree represents the two subpopulations estimated by the STRUCTURE analysis (red and green colors indicate accessions of the P1 and P2 subpopulations, respectively).

6. DISCUSSION

TRAP markers polymorphism

TRAP markers have been widely used in assessment of sugarcane genetic diversity, as well as other plants with complex genomes (ALWALA et al., 2006; QUE et al., 2009; CRESTE et al., 2010; SUMAN et al., 2012; DEVARUMATH et al., 2013; Da SILVA et al., 2016; SINGH et al., 2017; FARSANGI et al., 2018). The BSPG was composed of accessions representatives of different species of the *Saccharum* complex and also by different hybrids from Brazilian and foreign breeding programs, which constitutes a broad genetic background and allelic pool to be explored. These features may have been responsible for a high number of polymorphic fragments of each TRAP marker. The high polymorphism rate (98%) and high PIC (average of 0.97) and DP (average of 0.98) values showed the efficiency of TRAP markers by revealing a large amount of genetic information for a panel containing highly diverse accessions (SUMAN et al., 2008; DEVARUMATH et al., 2013; SINGH et al., 2017; FARSANGI et al., 2018).

The number of polymorphic fragments was higher when compared to other studies in sugarcane, even using a smaller set of TRAP combinations. The average of polymorphic fragments with the six TRAP markers associated with sucrose metabolism was 70.50, higher than reported by Alwala et al. (2006) (29.38 polymorphic fragments) and Singh et al. (2017) (14.66 polymorphic fragments), being that both used the same set of 18 TRAP markers. Alwala et al. (2006) evaluated

TRAP markers in a panel containing 30 accessions of the *Saccharum* complex (*Saccharum* species and other genera as *Miscanthus* and *Erianthus*), while Singh et al. (2017) used a panel composed of 25 accessions, being 18 commercial cultivars and seven accessions of the *Saccharum* complex (three *S. officinarum*, two *S. spontaneum* and two *S. barberi*). Likewise, similar results were found for TRAP markers related to lignin metabolism. In the present study the average of polymorphic fragments for these two TRAP markers was 80.50, while Suman et al. (2012), working with 16 TRAP markers and 64 accessions (*Saccharum* complex accessions and commercial cultivars), showed an average of 25.10 polymorphic fragments. Furthermore, the PIC and DP values found in the present study exceed the values showed by other works. Creste et al. (2010) showed an average PIC value of 0.30 using a panel of 82 Brazilian sugarcane cultivars. Alwala et al. (2006) and Devarumath et al. (2013) obtained an average PIC value of 0.24, and Suman et al. (2012) and Farsangi et al. (2018) achieved an average PIC value of 0.28.

The ancestors group was responsible for 65 exclusive fragments (10.9%), and among the genus *Saccharum*, the species that presented the highest number of exclusive fragments (eight fragments) was *S. spontaneum*, which is considered the most diverse species of the genus *Saccharum* due to its great ecogeographic distribution (LIU et al., 2016) and, therefore, can explain the occurrence of these exclusive fragments observed. Although a large number of polymorphic fragments were identified for the whole panel, only seven exclusive fragments (1.2%) were observed among the BB group suggesting that ancestor accessions did not encompass the whole genetic pool used in prior breeding programs, or that these new alleles observed in breeding accessions may have emerged over time, as a result of mutations (MANECHINI et al., 2018). Furthermore, the absence of exclusive fragments in these groups separated may be due to the fact that genotypes have common ancestors that are associated to strong selection pressure imposed to specific traits, such as sucrose content (CRESTE et al. 2009).

Population differentiation

The results of AMOVA, based on Φ_{PT} values, indicated that the molecular variance found by TRAP markers was higher within populations than between populations, a result that may reflect the high degree of variation among sugarcane accessions. The high values of within population genetic differentiation found in the

current assignment are in accordance with other studies in sugarcane (GLYNN et al., 2009; MANECHINI et al., 2018). According to Wright (1978), Φ_{PT} values between 0 and 0.05 signal small population differentiation, while values in the 0.05–0.15 and 0.15–0.25 ranges, respectively, indicate moderate and high genetic differentiation. The genetic differentiation value between the A and BB groups was higher (14%) than that observed between the A and FH groups (5%) and, in turn, these values reflect a moderate genetic differentiation between the breeding accessions and the ancestor accessions. A similar result was found by Manechini et al. (2018) using a panel of 81 Brazilian cultivars and 56 accessions of the genus *Saccharum*, in which the genetic differentiation between cultivars and *Saccharum* species corresponded to 17%. On the other hand, the genetic differentiation between BB and FH was slightly lower (3%) showing small population differentiation.

The Φ_{PT} values suggest a substantial genetic differentiation between the improved accessions (BB and FH groups) and ancestors accessions (A group). In the first interspecific crosses to develop sugarcane commercial cultivars were used extensively a limited number of accessions of *S. officinarum* and *S. spontaneum*, resulting in the narrow genetic base of current commercial cultivars (RAMDOYAL and BADALOO, 2001; PAN et al., 2005). This, associated with the genetic drift effect, reflects the moderate genetic differentiation values. Besides that, there is a large number of parents shared among the Brazilian accessions, which allows increasing the genetic differentiation between these accessions and the ancestors (MANECHINI et al., 2018).

Genetic structure

The genetic structure of the BSPG was evaluated by different methods: i) PCA; ii) STRUCTURE analysis; and iii) genetic dissimilarity and phylogenetic analysis. In general, all methods were able to relate evolutionary history and breeding orientations of modern sugarcane. However, the first and third methods showed better capacity of clustering and differentiation among the accessions.

In the PCA method, two PCAs were performed being the first with all 254 accessions of BPSG and the second, with 248 accessions of the BPSG (without *Erianthus* accessions). In the first PCA the *Erianthus* accessions were highly divergent from the *Saccharum* accessions, supporting the taxonomic evidence which assigned each of them to a separate genus (DANIELS et al., 1975). Our result

agrees with other studies using rDNA spacers (AL-JANABI et al., 1994), RFLP (BURNQUIST et al., 1992), TRAP (ALWALA e t al., 2006; SUMAN et al., 2012; DEVARUMATH et al., 2013), and SRAP (SUMAN et al., 2008) markers. Already in the second PCA, the dispersion of accessions was better visualized in the graph and some clustering could be analyzed. For these reasons, *Erianthus* accessions were also removed from all subsequent analyzes.

Considering both PCAs, the close position between breeding accessions and some representatives of *S. officinarum* (for example, AJAX, CAIANA RISCADA, CAYANA, NG21-21, NG57-221, SAC OFFIC 8272 and WHITE MAURITIUS) could be explained by the fact that this species was one of the main progenitors of modern sugarcane cultivars, which carry 80-85% of the *S. officinarum* genetic base (D'HONT et al., 1996). Furthermore, *S. barberi* accessions (AGOULE, CHIN, CHUNNE, GANDA CHENI and WHITE PARARIA) were grouped together with *S. officinarum* (CAIANA RISCADA, CAIANA VERDADEIRA, CANA BLANCA, IN84-103, NG21-17, SAC OFFIC 8272, SAC OFFIC 8276, SAC OFFIC 8280 and WHITE MAURITIUS) and *S. spontaneum* (KRAKATAU and SES205A), possibly due to the fact that *S. barberi* originated from the hybridization of *S. officinarum* and *S. spontaneum* (LU et al., 1994; SINGH et al., 2018). Moreover, in the second PCA, some FH accessions (NA56-79, POJ2878, TUC71-7, Co290, Co331, Co413 and Co419), used as parents in crosses to obtain Brazilian cultivars, were close positioned of BB accessions (for example, the parent TUC71-7 was close to their progenies RB855453, RB855574 and RB855196). It is interesting to note the central position of NA56-79, which could be explained by the fact that it was used as parent of several accessions that were located into BB subgroups in the second quadrant (RB806043, RB815521, RB83102, RB855533, SP71-6163, SP71-6949, SP81-1763, RB815627, RB815690 and RB835054) and in the fourth quadrant (RB725828, RB805276, SP71-1406, SP71-799, SP79-1011, RB835019 and RB835089) of the graph. On the other hand, some FH accessions (CR72/106, POJ161, Q165, Q70 and US60-31-3) were also found close to accessions of the A group, which could be explained by the few generations of these foreign hybrids.

The STRUCTURE software was able to separate the ancestors from the Brazilian breedings accessions. STRUCTURE is based on the assumption that, within each population, all loci are considered to be in Hardy-Weinberg equilibrium, without any linkage disequilibrium among loci, if they are not closely linked. These

assumptions are violated in sugarcane, which comes from generations of selection. The robustness of this method is not clear in studies with polyploids, so one faces difficulties when using structure with sugarcane data (HENRY and KOLE, 2010; WEI et al., 2006, 2010; RACEDO et al., 2016).

This separation between groups A and BB can also be seen in the dendrogram, in which most of the ancestors accessions were grouped within a cluster. The two wild species of sugarcane, *S. spontaneum* and *S. robustum,* were closely related, which agrees with the first PCA. These results are in accordance with the taxonomical classification proposed for the *Saccharum* complex (DANIELS et al., 1975). As expected, most of the accessions of Brazilian breeding programs were grouped in two clusters by sharing common parents and were bred aiming to increase sugar levels and ethanol production. *S. officinarum*, also known as "noble cane", has thick stalks, high sugar and low fiber content, characteristics that have promoted utilization for breeding purposes (MANECHINI et al., 2018).

7 CONCLUSION

The TRAP markers associated with sucrose and lignin genes used in this work were able to estimate the genetic diversity of the BPSG and to identify population structure. The data obtained in this work can be explored in the future for studies of association and incorporate this information into breeding programs for the choice of parents and obtention of superior progenies.

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