

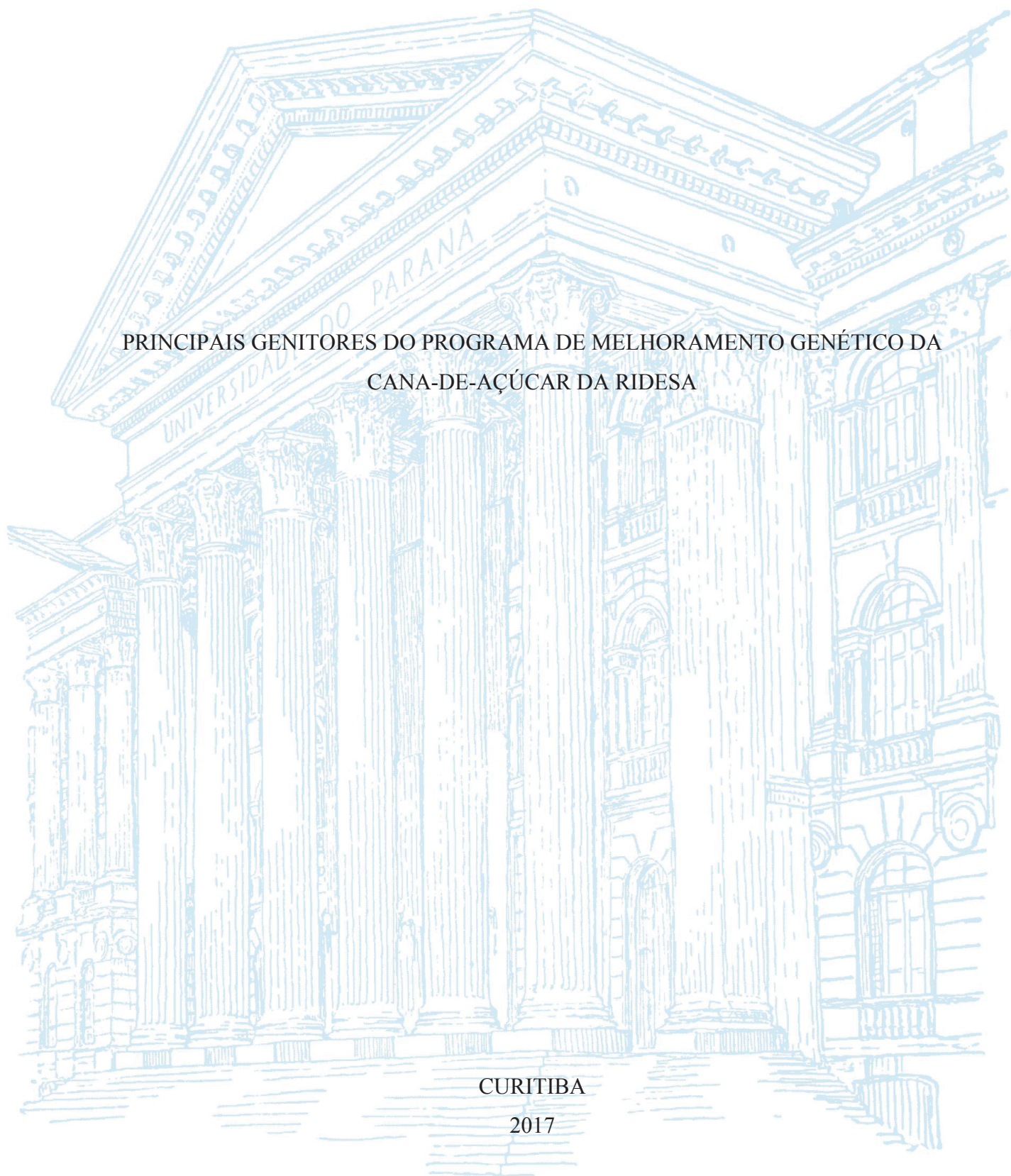
UNIVERSIDADE FEDERAL DO PARANÁ

JOÃO MARCOS TEPASSÉ MASCARENHAS

PRINCIPAIS GENITORES DO PROGRAMA DE MELHORAMENTO GENÉTICO DA
CANA-DE-AÇÚCAR DA RIDESA

CURITIBA

2017



JOÃO MARCOS TEPASSÉ MASCARENHAS

PRINCIPAIS GENITORES DO PROGRAMA DE MELHORAMENTO GENÉTICO DA
CANA-DE-AÇÚCAR DA RIDESA

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requisito parcial à obtenção do título de Mestre em
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Orientador(a): Prof(a). Dr. João Carlos Bespalhok Filho

Coorientador(a): Prof. Dr. Bruno Portela Brasileiro

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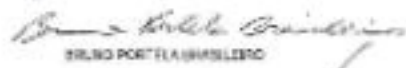
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RESUMO

A escolha de genitores e cruzamentos capazes de aumentar a probabilidade de obter variedades é a base para o sucesso dos programas de melhoramento genético da cana-de-açúcar. Diante da necessidade de avançar nos ciclos de seleção recorrente e otimizar o processo de hibridação, o objetivo desse trabalho foi identificar os principais genitores utilizados pela Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético (RIDESA) e propor a exploração de novos cruzamentos a partir das informações genealógicas. Foram utilizadas as informações do Banco Ativo de Germoplasma (BAG) da Serra do Ouro, pertencente a Universidade Federal de Alagoas, além de um arquivo de pedigree contendo as informações genealógicas de mais de 14 mil genótipos de cana-de-açúcar. Foram identificados 854 genótipos RB com pedigree, sendo 81 variedades. Esses genótipos pertencem a quatro gerações de recombinação, sendo 203 clones e 36 variedades da primeira geração RB, 445 clones e 34 variedades da segunda geração RB, 195 clones e 11 variedades da terceira geração RB e apenas 5 clones da quarta geração RB. Dos 854 genótipos RB, apenas 144 são genitores, ou seja, os clones das três últimas gerações RB descendem de 144 clones e variedades RB e de suas combinações com genitores de outros programas. Na década de 70, foram obtidos os primeiros clones e variedades RB a partir de genitores de outros programas de melhoramento genético, a exemplo dos genitores das siglas: IAC(Instituto Agrônomo de Campinas), CB(Campos Brasil), Co(Coinbatore, Índia), CP(Canal Point, EUA), NCo(África do Sul) e NA(África) . Desse período, estão presentes no BAG, 2 clones e 13 variedades, com destaque para as variedades RB72454, filha de CP53-76 e que deu origem a variedade RB867515 e RB75126 filha de Co278 e que deu origem a variedade RB92579. A RB72454 torna-se nas décadas seguintes o principal genitor da RIDESA, contribuindo com 132 genótipos para o BAG, sendo 25 variedades. Na década de 80, também são destaques os genitores: L60-14, NA56-79, TUC71-7 e SP70-1143. Nesse período foram gerados 104 clones e 30 variedades. Na década seguinte, foram introduzidos no BAG, 493 clones e 33 variedades RB, sendo que, os principais genitores desse período foram: RB72454, RB83102, RB75126, RB835486, RB855536, SP70-1143 e RB855002. Nas últimas introduções foram incorporadas 5 variedades e 179 clones gerados entre os anos de 2000 e 2010, descendentes em sua maioria dos genitores RB72454, RB72910, SP85-3877, SP80-1816, RB928064, RB931011 e RB931013. Os principais genitores RB foram divididos em três grupos heteróticos, novas combinações podem ser exploradas com a escolha correta de genitores das gerações avançadas dentro de cada grupo, visando aplicar a seleção recorrente recíproca, ou seja, obter populações segregantes em cruzamentos intrapopulacional e interpopulacional, visto que os efeitos não aditivos apresentam grande importância nos caracteres de produção da cana-de-açúcar.

Palavras-chave: *Saccharum* spp., Seleção recorrente e Melhoramento genético

ABSTRACT

Selecting capable parents and crosses to increase the probability of obtaining better varieties is the basis for the success of sugarcane breeding programs. Facing the need to advance in the recurrent selection cycles and optimize the hybridization process, the objective of this work was to identify the main parents used by the Interuniversity Network for the Development of the Sugarcane Sector (RIDESA) and propose the exploration of new crosses from genealogical information. Informations from the Genotypes Active Bank (AGB) of Serra do Ouro, belonging to the Federal University of Alagoas was used, as well as a pedigree file containing the genealogical information of more than 14,000 sugarcane genotypes. All in all, 854 pedigree RB genotypes were identified, with 81 varieties. These genotypes belong to four generations of recombination, with 203 clones and 36 varieties of the first-generation RB, 445 clones and 34 varieties of the second-generation RB, 195 clones and 11 varieties of the third-generation RB and only 5 clones of the fourth-generation RB. Only 1147 of the 854 RB genotypes are parents, in other words, the clones of the last three RB generations descend from 144 RB clones and varieties and from their combinations with parents of other programs. In the 1970s, the first clones and RB varieties were obtained from the parents of other breeding programs, such as the parents of the acronyms: IAC, CB, Co, CP, NCo and NA. From this period, 2 clones and 13 varieties are present in the AGB, with emphasis on RB72454, CP53-76 daughter's, which originated the variety RB867515 and RB75126, Co278 daughter's, which originated the variety RB92579. In the following decades, the RB72454 became the main parent of RIDESA, contributing with 132 genotypes to the AGB, with 25 varieties. In the 1980's, L60-14, NA56-79, TUC71-7 and SP70-1143 are also prominent parents. During this period, 104 clones and 30 varieties were generated. In the following decade, 493 clones and 33 RB varieties were introduced into the AGB, and the main genotypes of this period were: RB72454, RB83102, RB75126, RB835486, RB855536, SP70-1143 and RB855002. In the last introductions, 5 varieties and 179 clones were generated between the years 2000 and 2010, descended mostly from the parents RB72454, RB72910, SP85-3877, SP80-1816, RB928064, RB931011 and RB931013. The main RB parents were divided into three heterotic groups and new combinations could be explored with the correct selection of parents, from the the advanced generations within each group, in order to apply reciprocal recurrent selection, that is, to obtain segregating populations at intrapopulational and interpopulational crosses, whereas the non-additive effects show great importance in the characteristics of sugarcane production.

Keywords: *Saccharum* spp., recurrent selection, plant breeding.

TABLES

Table 1. Acronyms indicating the origin of the genotypes.....	25
Table 2. Number of clones and varieties generated by RB generation.....	26
Table 3. Origin of selected genotypes for high TCH, BRIX and resistance to main diseases (see total ratio in the appendix).....	32
Table 4. Total third-generation genotypes analyzed by origin.....	34

IMAGES

Image 1. Pedigree of cultivar RB925211 of third-generation RB.....	23
Image 2. Number of genotypes present in the Genotypes Active Bank of Serra do Ouro from multiple crosses (MP) and biparental (BP) over the decades.....	28
Image 3. Main families that originated the first generation of clones and RB varieties... ..	29
Image 4. Main biparental families (BP) that originated the second generation of clones and RB varieties.....	29
Image 5. Main biparental families (BP) that originated the third generation of clones and RB varieties.....	29
Image 6. 5 clones' parents' complete genealogy that compose the fourth-generation RB. Above 2 nd and 3 rd generation RBs.....	31
Image 7. Genotypes grouped into different heterotic groups.....	35
Image 8. Third-generation hybrids preferred for crosses with Group A.....	36
Image 9. Indicates hybrids that differ by pedigree from mentioned groups, therefore are indicated to cross with the cited groups.....	37

APPENDIX

Appendix 1. Table with genotypes analyzed by contrast in pedigree.....	45
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SUMMARY

1. GENERAL INTRODUCTION.....	12
2. INTRODUCTION.....	21
3. MATERIAL AND METHODS.....	23
4. RESULTS.....	26
5. DISCUSSION.....	32
5.1 Heterotics groups and new crosses.....	32
5.2 “Tapetinho” system x heterotic groups x number of crosses	33
5.3 Selection of elite genotypes based on pedigree	33
5.4 Crosses suggestion	36
6 Conclusion.....	38
References.....	39
Appendix.....	45

1 GENERAL INTRODUCTION

Sugarcane is cultivated in more than 100 tropical and subtropical countries and has major economic importance due to its products commercial value, especially sugar and ethanol (CONAB, 2016). In addition, the culture has gained popularity, recently, as a source of sustainable energy by the cogeneration of electricity and cellulosic ethanol through cellulose residues utilization (HOFSETZ and SILVA, 2012).

The production of sugarcane plays a fundamental role in the Brazilian economy. Its importance is increasing through time, especially with the interest in the obtainment of alternative sources of renewable energy.

Due to the increasing world demand for biofuels production, the Brazilian sugarcane production has considerably increased in the recent years, with the use of new agricultural areas, including regions with adverse edaphic climatic conditions (SILVA et al., 2012; ENDRES et al., 2010; CONAB, 2016).

In the last crop (2016/2017), it was cultivated in the country about nine million hectares, from which more than 694 million tons of sugarcane were produced (CONAB, 2016).

The average productivity in Brazilian sugarcane plantations is approximately 70 t.ha⁻¹ (CONAB, 2016) and is below what can be achieved with the cultural genetic potential, demonstrating the need of the launching of new varieties through genetic improvement and sugarcane plantations renovation to achieve higher productivity. Therefore, in order for a greater production to be achieved without major incorporations of new areas, clones that are increasingly productive and adapted to the different producing regions must be indicated by breeding programs and incorporated into crops in a shorter period of time.

Meaningful results in the sense of increasing the sugar production (Barbosa et al., 2004; Hogarth e Berding, 2006), resistance to disease (ZHOU, 2004; BAILEY, 2004) and resistance to insects (LESLIE, 2004) have been occurring through sugarcane genetic improvement.

The genetic improvement program of the Interuniversity Network for the Development of the Sugarcane Sector (RIDESA) launched 86 commercial varieties,

which occupy 15% of the cultivated areas in Brazil (DAROS et al., 2016), demonstrating the importance of the program to the energy sector.

RIDESA has annually produced about 2 million seedlings for the first test phase, named phase T1. The cane plant is cut between July-August, and the selection is made in the sugarcane ratoon. In some universities the selection is made in two periods, April and July, in order to seek, in the first period, clones that present greater precocity.

In the sugarcane genetic improvement, the process of development of new cultivars involves besides the first test phase (T1), what was conventionally agreed to call T2, T3, FE and FM, that is, first, second and third test phase, experimental phase and clonal multiplication phase, respectively (BARBOSA and SILVEIRA, 2012).

For the genotypes selection, it is considered the sprouting potencial in a lower temperature and dryness season, the sucrose content measured by soluble solids content (BRIX), the yield potential determined by the number of stalks, selecting genotypes with at least 6 stalks, the flowering, the isoporization, the tolerance to main diseases and some wanted morphological characteristics, such as the absence of deep cracks and slightly protruding gems, always taking into account experimental patterns (commercial varieties) as reference.

Usually, the launching of new varieties has occurred between 12 and 15 years, due to the long time needed to obtain and validate a promising material that can be used as a new variety and/or parent. Therefore, it is important to develop and use appropriate statistical and phytotechnical techniques to optimize the hybridization and selection process.

As the success of the development of new varieties of sugarcane is associated with the ability to properly choose the best individuals who will be the parents of the new varieties, a better understanding of the genetic diversity between the clones used as parents becomes essential for the definition of new crossings (ALWALA et al., 2006; DOS SANTOS et al., 2012; BRASILEIRO et al., 2014a). To reach this goal it is necessary to know the potential parents present in the RIDESA germplasm collection breeding program.

The process of characterizing the collection of germplasm in addition to providing basic data needed for breeding programs has a strategic application in the appreciation of genetic resources (MOHAMMADI and PRASANNA, 2003) and the advances in the areas of computational technology, numerical taxonomy and multivariate statistical methods have contributed to the conservation of genetic

resources, to the formation of nuclear collections and to the identification of possible duplicates (ORTIZ et al., 2008), as well as to provide parameters for the choice of parents that allow greater heterotic effect in the progeny, increasing the chances of obtaining transgressive genotypes in segregating generations.

The use of kinship coefficients and inbreeding has been frequent in genetic analyzes to aid in the management of germplasm banks associated with plant breeding programs (PETERNELLI et al., 2009). An inherent characteristic of the estimation of kinship coefficient and inbreeding is the dependence over pedigree information. For example, an inbreeding coefficient is not an intrinsic characteristic of the individual, but a genealogical information of each genotype (KHANG, 1989). Thus, the inbreeding and kinship coefficient estimation becomes extremely laborious when a genealogy with several generations, large numbers of individuals and overlapping of generations is available (PETERNELLI et al., 2009). These facts are presented in the Genotypes Active Bank (AGB) of the Federal University of Alagoas (UFAL) used by RIDESA, and studies of this nature have been carried out for some clones (BRASILEIRO et al., 2014a; BRASILEIRO et al., 2014b; SILVEIRA et al., 2015).

The use of molecular markers has helped to identify the genetically distant genotypes, identifying constant contrast genotypes in various environments (LOPES et al., 2007). Therefore, using molecular markers corroborates with the selection thesis supported by the pedigree, since the genotypes with constant contrast were originated from different heterotic groups.

The molecular characterization of sugarcane cultivars utilizing ISSR markers allows the characterization of groups of higher or low similarity (ALMEIDA et al., 2009). From morphological characters and RAPD markers, a moderate association was found between the estimates of genetic divergence, and this is due to an unrepresentative sampling, suggesting using a larger number of genetic markers in an attempt to confirm such correlations or to obtain new approaches (SILVA, 2006).

In the study of the genetic potential of sugarcane breeders based on biparental crosses, the specific combining ability (SCA) is poorly correlated with the kinship coefficient of the parents and is not correlated with distance based on SSR markers (SANTANA, 2013).

Different distance measures are used in studies of genetic diversity, and the type of distance to be applied in the analysis is dependent on the nature of the evaluated variables. For the quantitative variables, the most commonly used distances are the

Euclidean and Generalized Mahalanobis (GONÇALVES et al., 2008). For binary variables, several coefficients of association have been proposed, with emphasis on the Sorensen-Dice coefficient and the index Jaccard complement; these methods are intensively used in the analysis of diversity from molecular data (Mohammadi and Prasanna, 2003; Amaral Júnior et al., 2010). In the case of multicategorical variables, the dissimilarity measure proposed by Cole-Rogers et al. (1997), besides being the most appropriate, is also the most used in genetic diversity studies (CRUZ et al., 2012). Gower (1971) proposed an algorithm capable of generating a measure of dissimilarity from the joint analysis of continuous, binary and multicategorical variable, being able to combine different types of variables in the obtention of a single distance matrix, making use of all information available on evaluated genotypes. Recently, this algorithm has been used in the study of sugarcane diversity (PAULA, 2013; BRASILEIRO et al., 2014).

Although the use of multivariate analysis techniques is important, morphological and/or molecular characterization requires a large amount of qualified workforce, experimental area, time and financial resources that preclude their usage throughout RIDESA's germplasm collection, because in addition to having more than 2.500 genotypes, new clones have been introduced annually and with little morphoagronomic information. Therefore, understanding the accesses origin and who were the main parents over the decades, since the 1970s, can contribute satisfactorily to defining future crosses in order to advance the cycles of recurrent selection and genetic gains.

The choice of parental combinations that will generate the segregating populations to be subjected to selection is always a major challenge for plant breeders. And for a breeding program to be efficient, it is desirable that the germplasm be organized into heterotic groups. The term heterocyclic group refers to a group of related or unrelated genotypes, from similar or different populations, but which have heterotic combining and response ability when crossed with genotypes from other genetically distinct groups (MELCHINGER and GUMBER, 1998; REIF et al., 2007).

Crossing designs show limitations mainly due to the need of obtaining large amounts of combinations (RESENDE, 2002). In the case of sugarcane, the synchronization of flowering has also been a problem for the achievement of the desired combinations. An alternative is the use of the disconnected factorial (BURDON and BUIJTENEN, 1990), as it offers the breeder flexibility in the number of crosses to be performed by the parent. Other advantages of this crossing scheme are: i) the possibility

of identifying superior crosses, ii) the efficiency of clone selection in the hybrid population, and iii) the ability to evaluate a large number of parents (RESENDE and BARBOSA, 2005).

In the disconnected factorial it is recommended the formation of small groups of crosses, usually 3x3 or 4x4, maximizing the number of parents to be evaluated in a single experiment (BURDON and BUIJTENEN, 1990). Unbalanced data are common in these cases, thus to obtain reliable estimatives of components of variance and genetic and genotypic values, the methodology of restricted maximum likelihood (REML) and best linear unbiased prediction (BLUP) are considered the best statistical methods (RESENDE, 2002; PHIEPO et al., 2008). Piepho et al. (2008) suggests the application of BLUP in plant breeding, including pedigree information to explore genetic correlations between relatives and to obtain more accurate estimates.

Nature knowledge and magnitude of the variability present in the breeding populations is of great importance. Parameters such as phenotypic variance, genotypic variance and their components divided into additive and dominant, restrict and broad heritability are relevant, since they allow selecting the crosses, predicting genetic gains and defining the best strategies for conducting the segregating population (HOGARTH et al., 1981; FALCONER and MACKAY, 1996).

In sugarcane, although the potential use of RRS was recognized, with the objective of exploring both non-additive and additive effects of the characters of economic value (BARBOSA et al., 2012), few studies were carried out aiming at the obtention of heterotic groups and at the choice of parents for the realization of future crossings (BRASILEIRO et al., 2012a; BRASILEIRO et al., 2014b; PAULA, 2013). The general combining ability (GCA) is more important than the specific combining ability (SCA) for most of the economic value characters, with emphasis on sugar and fiber contents, as well as on resistance to diseases such as coal and rust. The main exception is the sugarcane ton per hectare, where both variances have presented similar values (WU et al., 1980; HOGARTH et al., 1981; HOGARTH et al., 1991; BASTOS et al., 2003; BARBOSA et al., 2004; BARBOSA et al., 2005).

Different selection strategies can be proposed according to the inheritance of the characteristics. The presented evidences suggest that the additive gene effects are as important as the non-additives gene effects in the expression of the economic importance characters of sugarcane. Therefore, exploiting the specific combining ability (SCA) becomes as important as the general combining ability (GCA). In this sense,

sugarcane breeding programs should seek to exploit both fractions of total genetic variation, and for this, the use of the Reciprocal Recurrent Selection (RRS) as represented by Resende and Barbosa (2005) is an interesting strategy for the sugarcane genetic improvement program.

Reciprocal recurrent selection (RRS) is the main method for improving the average interpopulational crossing. It must be used in the breeding of species in which heterosis is relevant in characters of economic importance (RESENDE, 2002).

As described by Resende and Barbosa (2005), RRS can be applied at two levels: (i) populational, involving the crossing of several individuals of a population with individuals from the reciprocal population; (ii) individual (IRRS), involving only one individual from each population, which produce excellent crossings, with high genotypic value and also high SCA. In the first case, there is limitation for its use in sugarcane breeding, since the recombination step would be impaired by the lack of individuals blossoming synchronicity to be recombined. As for the second case (ii), it is suggested that such individuals originated from upper crossings be self-fertilized to produce inbred populations (S1), in which higher individuals would be selected to integrate an individual reciprocal recurrent selection program.

One of the most important steps in the sugarcane improvement is the initial phase (T1), when the first selections of plants or families are carried out. After phase T1, new materials are no longer introduced, which makes the selection performed at this stage crucial to the success of the program. It is in this phase that data about the family level can be collected, aiming at the estimation of genetic parameters as well as SCA and GCA of the parents.

Resende and Duarte (2007) suggest that family assessments should be approached from a genetic and statistical point of view. In addition, Resende (2002) proposes the use of selective accuracy due to its property of reporting on the efficacy of inference about genotypic value. However, there are difficulties in applying the selection of families in sugarcane breeding at the field level, especially due to the increase in activities as a result of operational problems related to the obtaining of plant-level data, and/or the need of weighing all the plots in the experiments, which often restricts the number of families to be evaluated at one time. These difficulties have been blocking the use of family selection in breeding programs, currently promoting little impact on the development of new sugarcane cultivars.

As a way to overcome the difficulties involved in evaluating families, it must be considered that each University that composes RIDESA takes annually to the field millions of seedlings and hundreds of families.

Therefore, a better understanding of the clones present in the AGB is essential in face of the need to advance in the recurrent selection cycles and to optimize the RIDESA hybridization process, since the success of the development of new sugarcane varieties is associated to the capacity to properly choose the parents, in this sense, new strategies must be evaluated and in case they are shown to be efficient they must be incorporated to the genetic improvement program routine.

Hence, the aim of this study was to understand and identify the main parents used in the last four decades by the Interuniversity Network for the Development of the Sugarcane Sector (RIDESA), to separate the clones developed in heterotic groups and in different generations of recombination. By these means, to propose the exploration of new crosses from pedigree information and the selection rates applied in the first test phase over the last years, aiming at contributing to obtain new varieties and genetic gains.

CHAPTER I

MAIN PARENTS OF THE RIDESA SUGARCANE GENETIC IMPROVEMENT PROGRAM

RESUMO

A escolha de genitores e cruzamentos capazes de aumentar a probabilidade de obter melhores variedades é a base para o sucesso dos programas de melhoramento genético da cana-de-açúcar. Diante da necessidade de avançar nos ciclos de seleção recorrente e otimizar o processo de hibridação, o objetivo desse trabalho foi identificar os principais genitores utilizados pela Rede Interuniversitária para o Desenvolvimento do Setor Sucroenergético (RIDESA) e propor a exploração de novos cruzamentos a partir das informações genealógicas. Foram utilizadas as informações do Banco Ativo de Germoplasma (BAG) da Serra do Ouro, pertencente a Universidade Federal de Alagoas, além de um arquivo de pedigree contendo as informações genealógicas de mais de 14 mil genótipos de cana-de-açúcar. Foram identificados 854 genótipos RB com pedigree, sendo 81 variedades. Esses genótipos pertencem a quatro gerações de recombinação, sendo 167 clones e 36 variedades da primeira geração RB, 411 clones e 34 variedades da segunda geração RB, 184 clones e 11 variedades da terceira geração RB e apenas 5 clones da quarta geração RB. Dos 854 genótipos RB, apenas 87 são genitores, ou seja, os clones das três últimas gerações RB descendem de 87 clones e variedades RB e de suas combinações com genitores de outros programas. Na década de 70, foram obtidos os primeiros clones e variedades RB a partir de genitores de outros programas de melhoramento genético, a exemplo dos genitores das siglas: IAC, CB, Co, CP, NCo e NA. Desse período, estão presentes no BAG, 2 clones e 13 variedades, com destaque para as variedades RB72454, filha de CP53-76 e que deu origem a variedade RB867515 e RB75126 filha de Co278 e que deu origem a variedade RB92579. A RB72454 torna-se nas décadas seguintes o principal genitor da RIDESA, contribuindo com 132 genótipos para o BAG, sendo 25 variedades. Na década de 80, também são destaques os genitores: RB72454, L60-14, NA56-79, TUC71-7 e SP70-1143. Nesse período foram gerados 104 clones e 30 variedades. Na década seguinte, foram introduzidos no BAG, 493 clones e 33 variedades RB, sendo que, os principais genitores desse período foram: RB72454, RB83102, RB75126, RB835486, RB855536, SP70-1143 e RB855002. Nas últimas introduções foram incorporadas 5 variedades e 179 clones gerados entre os anos de 2000 e 2010, descendentes em sua maioria dos genitores RB72454, RB855536, RB855156, RB855511, SP85-3877, SP80-1816, SP83-2847, SP77-5181. Os principais genitores RB foram divididos em três grupos heteróticos, novas combinações podem ser exploradas com a escolha correta de genitores das gerações avançadas dentro de cada grupo, visando aplicar a seleção recorrente recíproca, ou seja, obter populações segregantes em cruzamentos intrapopulacional e interpopulacional, visto que os efeitos não aditivos apresentam grande importância nos caracteres de produção da cana-de-açúcar.

Palavras-chave: *Saccharum* spp., Seleção recorrente e Melhoramento genético

ABSTRACT

Selecting capable parents and crosses to increase the probability to obtain better varieties is the basis for the success of sugarcane breeding programs. Faced in the need to advance in the recurrent selection cycles and to optimize the hybridization process, the objective of this work was to identify the main parents used by the Interuniversity Network for the Development of the Sugarcane Sector (RIDESA) and propose the exploration of new crosses from the information Genealogical. Were used informations from the Genotypes Active Bank (AGB) of Serra do Ouro, belonging to the Federal University of Alagoas, was used, as well as a pedigree file containing the genealogical information of more than 14,000 sugarcane genotypes. All in all were identified 854 pedigree RB genotypes, with 81 varieties. These genotypes belong to four generations of recombination, with 167 clones and 36 varieties of the first-generation RB, 411 clones and 34 varieties of the second-generation RB, 184 clones and 11 varieties of the third-generation RB and only 5 clones of the fourth-generation RB. Only 87 of the 854 RB genotypes, are parents, in other words, the clones of the last three RB generations descend from 87 RB clones and varieties and from their combinations with parents of other programs. In the 1970s, the first clones and RB varieties were obtained from the parents of other breeding programs, such as the parents of the acronyms: IAC, CB, Co, CP, NCo and NA. From this period, 2 clones and 13 varieties are present in the AGB, with emphasis on RB72454, one CP53-76 daughter's, and that gave origin to the variety RB867515 and RB75126, one Co278 daughter's, that gave origin to variety RB92579. In the following decades, the RB72454 became the main parent of RIDESA, contributing with 132 genotypes to the AGB, with 25 varieties. In the 1980's, also they are prominences the parents: RB72454, L60-14, NA56-79, TUC71-7 and SP70-1143. During this period, 104 clones and 30 varieties were generated. In the following decade, 493 clones and 33 RB varieties were introduced into the AGB, and the main genotypes of this period were: RB72454, RB83102, RB75126, RB835486, RB855536, SP70-1143 and RB855002. In the last introductions, 5 varieties and 179 clones were generated between the years 2000 and 2010, descended mostly from the parents RB72454, RB855536, RB855156, RB855511, SP85-3877, SP80-1816, SP83-2847, SP77-5181. The main RB parents were divided into three heterotic groups and new combinations could be explored with the correct selection of parents, from the the advanced generations within each group, in order to apply reciprocal recurrent selection, that is, to obtain segregating populations at intrapopulational and interpopulational crosses, in view of that the non-additive effects show importance in the characteristics of sugarcane production.

Keywords: *Saccharum* spp, recurrent reciprocal selection, breeding.

2. INTRODUCTION

Sugarcane (*Saccharum* spp.) is one of the most efficient agricultural crops in the conversion of solar energy into chemical energy, because it is a C4 specie with high biomass production (TEW and COBILL, 2008). In Brazil, it is one of the main options for biomass production, being economically competitive in relation to other crops, mainly due to the fact that it has a well-functioning and fully-equipped industrial park with mastery over cultivation and processing techniques, in addition to the considerable number of plants distributed through the country (MATSUOKA et al., 2014).

The average productivity in the Brazilian sugarcane plantations is approximately 70 t.ha⁻¹ (CONAB, 2016) and is far below what can be achieved with the genetic potential of the crop when there is no water restriction, reaching up to 160 tons per hectare in the first and second cutting (SILVA et al., 2014). This demonstrates the need to launch new varieties through genetic improvement and sugarcane plantations renewal in order to achieve higher productivity. Therefore, for greater production to be achieved without major incorporation of new areas, clones that are increasingly productive and adapted to different producing regions must be indicated by the breeding programs and incorporated into crops in a shorter period of time.

Significant results in the sense of increasing sugar production (BARBOSA et al., 2004; HOGARTH and BERDING, 2006), resistance to diseases (ZHOU, 2004; BAILEY, 2004) and resistance to insects (LESLIE, 2004) have been occurring through sugarcane genetic improvement program.

Nature knowledge and the magnitude of variability present in the breeding populations are of great importance. Parameters such as phenotypic variance, genotypic variance and their components divided into additive and dominant, restrict and broad heritability, are relevant, since they allow selecting the crosses, predicting genetic gains and defining the best strategies to conduct the segregating population (HOGARTH et al., 1981; FALCONER and MACKAY, 1996).

The choice of paternal combinations that will generate the segregating populations to be subjected to selection is always a great challenge to plant breeders. And for a breeding program to be efficient, it is desirable that the germplasm be organized in heterotic groups. The term heterotic group refers to a group of genotypes related or not, from similar or different populations, but that present combining capacity and heterotic response when crossed with genotypes from other genetically distinct groups (MELCHINGER and GUMBER, 1998; REIF et al., 2007).

The reciprocal recurrent selection (RRS) is the main method to improve the interpopulational cross average. It must be used in the breeding of species which

heterosis is relevant in characters of economic importance (RESENDE, 2002). In sugarcane, although the recognized potencial use of RRS, with the objective of exploring both additive and non-additive effects for the characters of economic value (BARBOSA et al., 2012; PAULA et al., 2001), few studies were carried out aiming at obtaining heterotic groups and at choosing the parents for future crosses realization (PAULA, 2013; BRASILEIRO et al., 2014a; BRASILEIRO et al., 2014b).

The best families in mass (TCH) and sugar (TBH) productivity and with high specific combining ability (SCA) come from crosses between divergent parents (OLIVEIRA, 2007).

Therefore, a better understanding of the clones present in the Genotypes Active Bank becomes essential in view of the need to advance in the recurrent selection cycles, and to optimize the RIDESA hybridization process, since the development of new sugarcane varieties success is associated with the ability to choose the parents correctly. In this sense, new strategies must be evaluated and if they show efficiency, they should be incorporated into genetic improvement program routine.

That said, the objective of this work was to identify the main parents used by the Interuniversity Network for the Development of the Sugarcane Sector (RIDESA) and to propose the exploration of new crosses from the pedigree information and the selection rates applied in the first test phase over the last few years aiming at contributing to the achievement of new varieties and genetic gains.

3. MATERIAL AND METHODS

It was used information from 2017 of the Genotypes Active Bank (AGB) of Serra do Ouro, property of the Federal University of Alagoas, along with a pedigree file containing genealogical information of more than 14,000 sugarcane genotypes.

For decades, it was made a separation of the families, also discriminating three different generations of RB genotypes. By first generation, it is understood every genotype synthesized within the program with parents from outside the program, in other words, they are not RB. Second generation genotypes originate from crosses with at least one parent, whether male or female, from the first RB generation. The third generation is the product of a parental second-generation RB and some other, being RB or not. The fourth generation is the product of a crossing between a third-parental RB generation and another parental that may or may not be RB, but in several cases both parents happen to be already RB and with RB ancestry.

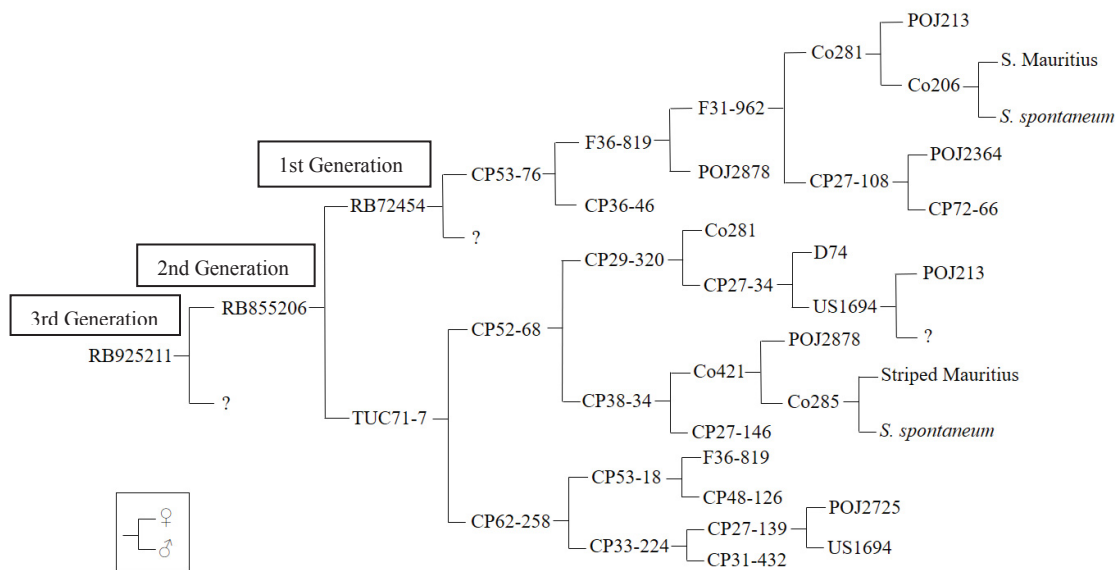


Image 1. Pedigree of cultivar RB925211 of third-generation RB.

As for the separation of genotypes into groups of distinct heterotic patterns, the criteria was the genotypes origin and also the groups' kinship degree, if they coincide, then they were considered of the same group with similar heterotic pattern and apt to be used in intrafamilial crosses.

It was defined as GROUP A, acronyms of origin Co, IAC, NA and NCo. As GROUP B: CP, TUC and L, and as GROUP C: SP, ROC, H, Q, F.

These acronyms correspond to the following locations:

Co – Coimbatore, India

IAC – Agronomical Institute of Campinas

NCo – Coimbatore, Índia

NA – Natal – Africa

CP – Canal Point, U.S.A

TUC – Argentina

L – Louisiana

SP – Coopersucar, Brazil

H – Hawaii

Q – Australia

F – Formosa

From the parents genealogical information present in the AGB and from the selection rates performed in the families during the first test phase (T1), the parents were separated into different groups and new hybrid combinations were proposed to explore the intra and interpopulational selection and to advance in recombination cycles.

GROUP A	GROUP B	GROUP C
Co	CP	SP
IAC	TUC	ROC
Nco	L	H
NA	RB72454	Q
		F

Table 1. Acronyms indicating the origin of the genotypes

The pedigrees of 164 third-generation genotypes for the formation of heterotic groups and intra and intergroup crossing schemes were analyzed.

4. RESULTS

In the year of 2017, it was identified in the AGB database 956 RB genotypes, 854 of which have pedigree and from these 81 are varieties. These genotypes belong to four recombined generations, being 167 clones and 36 first-generation RB varieties, 411 clones and 34 second-generation varieties, 184 clones and 11 third-generation varieties and only 5 fourth-generation RB clones.

The 854 RB genotypes are descendant from a combination of 275 parents from different breeding programs, 300 of which are genotypes descendant from multiple crosses and the remaining ones from biparental crossings. Among the 854 clones, only 87 are parents, in other words, the clones of the last three RB generations descent from 87 RB clones and varieties and from their combinations with parents from other programs.

AGB 2017	
TOTAL RB GENOTYPES	956
WITH PEDIGREE	854
VARIETIES	81

FIRST GENERATION	
CLONES	167
VARIETIES	36

SECOND GENERATION	
CLONES	411
VARIETIES	34

THIRD GENERATION	
CLONES	184
VARIETIES	11

FOURTH GENERATION	
CLONES	5
VARIETIES	0

Table 2. Number of clones and varieties generated by RB generation.

In the 1970s, the first clones and RB varieties from other breeding program parents were obtained, such as the breeders IAC, F, M, TUC, CB, Co, CP, NCo and NA. From that period, 2 clones and 13 varieties are present in AGB, although 22 varieties were returned in 1970, all of which come from multiple crosses. From this period, the highlights are the varieties RB72454, the only descendant from CP53-76 and that originated the variety RB867515, besides the variety RB75126, Co278's daughter and that originated the variety RB92579. In the following decades, RB72454 became the main parent of RIDESA, contributing with 132 genotypes to the AGB, being 25 varieties.

In 1980s, the main parents were: RB72454, L60-14, NA56-79, TUC71-7 and SP70-1143. From that period, 104 clones and 30 varieties of the 31 developed in that decade are present in AGB, the only missing variety of that period in the AGB is RB8495. The varieties RB83102, RB835486, RB855536, RB855206 and RB855002 developed in that decade stood out as important genitors in the following decade.

In the last introductions, 5 varieties and 179 clones generated between the years of 2000 and 2010, descent mostly from the parents RB72454, RB855536, RB855156, RB855511, SP85-3877, SP80-1816, SP83-2847, SP77-5181, were incorporated.

Although the L60-14 had contributed with only 12 genotypes in the AGB, she is an important parent, because she is the RB835486 variety's mother, which has 71 descendants in the AGB, being 55 as female parent.

Out of the 27 descendants of the cross between RB72454 and SP70-1143, the most prominent as parent is the RB855536 variety, because it is not only an excellent variety but also a parent of 51 clones and 2 varieties (RB965902 and RB965917) present in the AGB. Another important parent of the SP70-1143 × RB72454 combination is RB855002, which became one of the main parents of the 1990s.

Another important cross involving the SP70-1143 was with the NA56-79. Among several clones and varieties originated from this combination, the variety RB83102 stood out, one of the main parents from 1990s, with 45 clones and 2 varieties present in the AGB, with the majority of these genotypes coming from their combination with RB72454, such as varieties RB9879350 and RB988082.

The combination RB72454 × TUC71-7 also originated a major RIDESA parent, the variety RB855156, which when combined with RB815690 gave rise to RB966928, currently the most planted variety in the south-central region of Brazil.

When evaluating the types of crosses that gave rise to AGB parents, it is possible to observe an increase in the number of individuals originated from biparental crossing through time, while in 1970s all genotypes descent from multiple crossings, in the following decades a predominance of biparental crossing descendants' genotypes occurred, as can be observed in Image 2.

Number of genotypes generated from BP and MP crosses

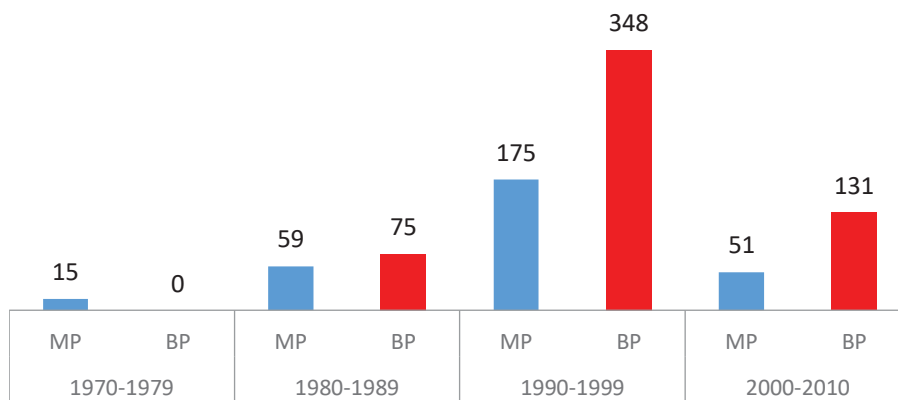


Image 2. Number of genotypes present in the Genotypes Active Bank of Serra do Outro from multiple crossings (MP) and biparental (BP) over the decades

When the information is analyzed by generation of recombination, it is observed that first-generation RB clones are predominantly descendant from multiple crosses, mainly involving the parents NA56-79, TUC71-7, F147, SP70-1143, SP80-1816, SP83-2847, SP80-3280 and SP71-1406. From the first-generation RB, 146 genotypes originating from MP crosses and 57 genotypes from BP crosses are present in the AGB, most of these genotypes descent from combinations involving the parents mentioned above, especially the crosses SP70-1143 × TUC71-7, NA56-79 × SP70-1143 and their reciprocal (Image 3).

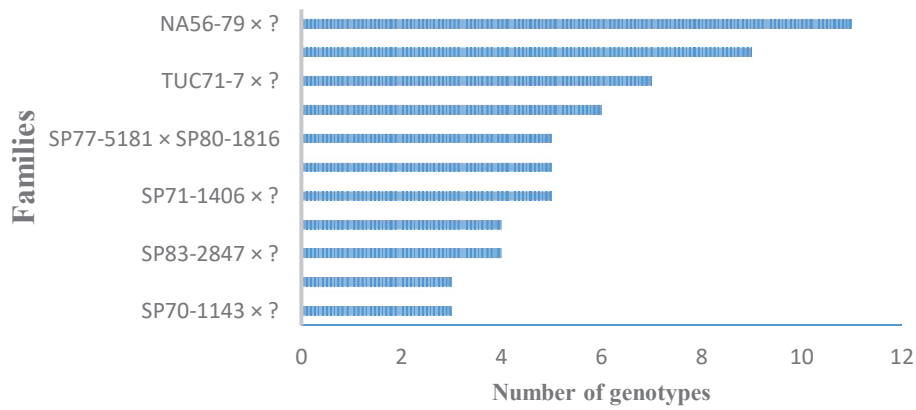


Image 3. Main families that originated the first generation of clones and RB varieties.

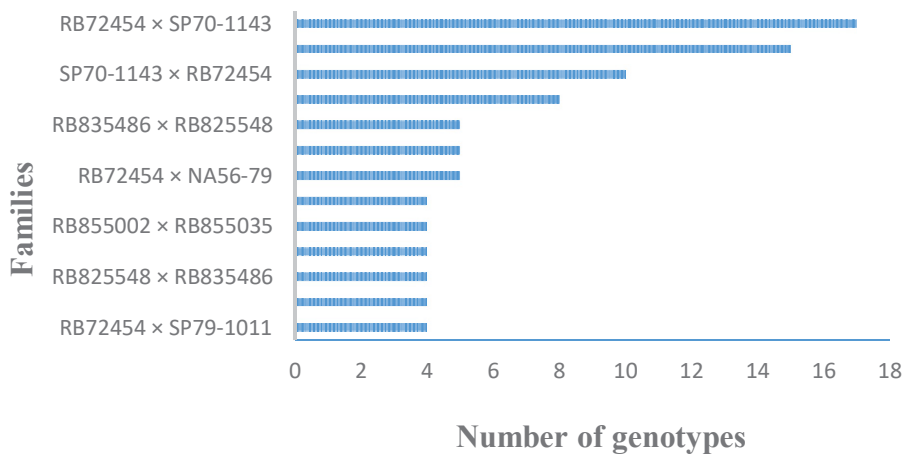


Image 4. Main biparental families (BP) that originated the second generation of clones and RB varieties.

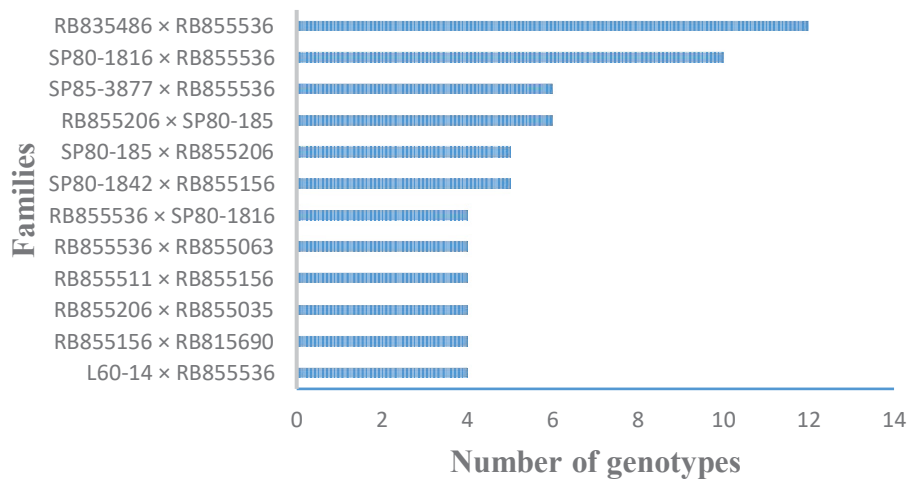


Image 5. Main biparental families (BP) that originated the third generation of clones and RB varieties.

In the second-generation RB, 120 genotypes come from MP crosses. Of these genotypes, 30% have as mother the parents RB72454, RB735220 or RB835486. Another 325 genotypes of the second-generation RB descent from BP crosses. The main families that originated these individuals are shown in Image 3, which shows the importance of the variety RB72454 as the parent of the RIDESA breeding program.

In the third-generation RB of clones and varieties, 164 individuals descent from BP crosses, against 31 from MP crosses, being RB855206 clone the mother of 8 genotypes, one of them being the variety RB925211. The main families that originated the third-generation RB are presented in Image 4, which shows the importance of the variety RB855536 as parent of the third-generation RB of clones and varieties, such as RB965902 e RB965917 varieties.

The fourth-generation RB of clones is composed by only 5 clones: RB981805, RB981812, RB981819, RB036110 and RB036122, the first three are children of a third-generation clone; RB931566 and the latter two are children of the variety RB925211. Both parents have as grandmother the RB72454 variety (Image 5).

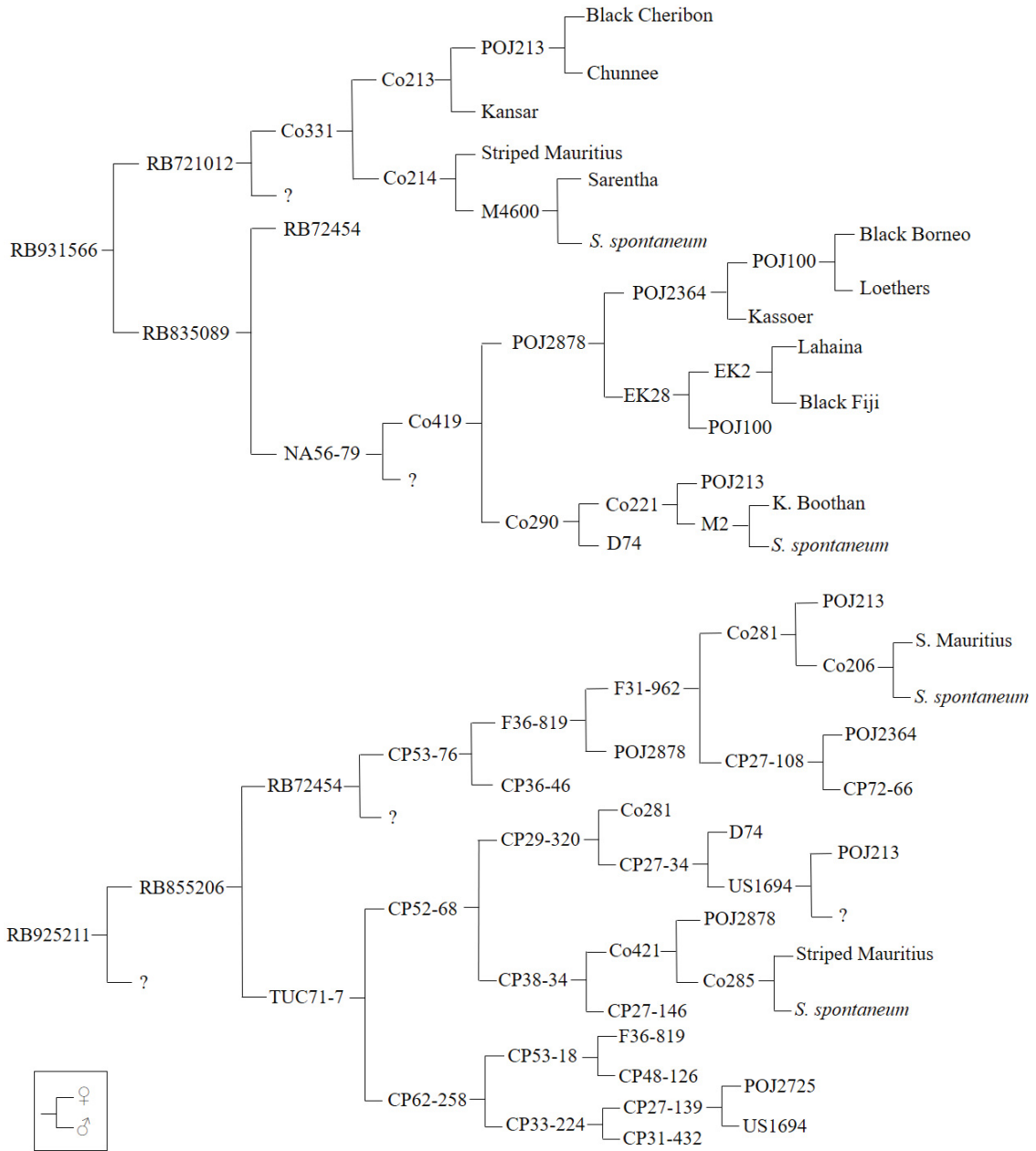


Image 6. 5 clones' parents' complete genealogy that compose the fourth-generation RB. Above 2nd and 3rd generation RBs .

Figure 6 explains the RB925211 genotype as a third generation and RB931566 as being of second RB genotypes generation.

5 DISCUSSION

5.1 Heterotics groups and new crosses

Analyzing the genealogy of the new varieties and promising clones inserted in the AGB, that is, of higher productivity of stalks per hectare (TCH), BRIX and resistance to the disease complex analyzed, we observed those originated from parents from different origins. This fact supports the thesis that the separation of a germplasm bank by groups of different heterotic pattern based on its origin generates a different organization in the conduction of new crosses and a new optic for the sugarcane genetic improvement program.

In Table 3, some examples of RB products originated from biparental crossings contrasting in their origin were observed. Note that RB72454, acting as female parent, in crosses with NA56-79 and SP70-1143 as male parents, originated new clones and demonstrated that they have good combining ability. NA56-79, as well as SP70-1143, NCo310, F141 and B337, possesses different genetic from RB72454, all of them producing good new genotypes, demonstrating that biparental crosses between contrasting genotypes are an efficient technique to originate superior genotypes.

GENOTYPE	FEMALE PARENT	MALE PARENT
F160	NCo310	F141
RB83102	NA56-79	SP70-1143
RB83594	RB72454	B3337
RB835054	RB72454	NA56-79
RB835089	RB72454	NA56-79
RB845065	SP70-1143	NA56-79
RB845197	RB72454	SP70-1143
RB845210	RB72454	SP70-1143
RB845239	RB72454	SP70-1143
RB845257	RB72454	SP70-1143

Table 3. Origin of selected genotypes for high TCH, BRIX and resistance to main diseases (see total ratio in the appendix)

The parents' origin control and subsequent evaluation of the new clones' performance is fundamental to guiding the future of a breeding program. This control and knowledge do not pretend to go against the multiparental cross in which the male parent is unknown, but it reduces the action of chance and places parameters and measurements on known individuals, allowing greater effective selection and genetic gains per generation.

Other crops of commercial interest such as corn work with hybridizations between higher individuals or with the hybrid vigor exploitation (SHULL, 1948; SURESH and KHASMA, 1975; FALCONER and MACKAY, 1996; HALLAUER et al., 1988). Naturally, sugarcane's genetic nature differs from the crops cited, but the exploration of new crosses between recognized clones and varieties is fully justified.

This approach opens up wide possibilities for self-fertilization within heterotic groups, selecting more homozygous individuals for future crosses and exploration of hybrid vigor. It also provides many possibilities for the generation of new populations and heterozygous individuals, but with a concentration of alleles really favorable to the characteristics sought with the most significant genetic advance for the sector.

5.2 “Tapetinho” system x heterotic groups x number of crosses.

It is important to mention that the orientation of new crosses based on origin and heterotic groups allow a greater number of crosses with known parents. It is possible to use an expressive number of self-fertilizations within the populations, from biparental crosses and also from crosses with many female parents with some known and of proven good combining ability parents.

This way of working gladly complements with the system of T1 conduction through “Tapetinho” system (RIDESA, 2013), since this requires much less experimental area and allows the number of crossings to increase significantly.

5.3 Selection of elite genotypes based on pedigree

The criteria for selecting clones or varieties to form a heterotic group or to be grouped can be varied, broadened or reduced, based on TCH, resistance to disease, tillering or other characteristics, but inclusion within a group should always obey the origin of this genotype. With this, the success and the orientation of the breeding program will depend on the objectives and parameters of each breeder.

Table 4 shows the total of 164 third-generation parents analyzed based on the criteria of selection by pedigree or origin.

RB985476	RB935875	RB037186	RB011715	RB975949	RB925367	RB976328	RB005917
RB985840	RB935925	RB985580	RB037048	RB965635	RB946905	RB975053	RB005983
RB985841	RB965578	RB955312	RB991530	RB971755	RB971765	RB976930	RB006610
RB985820	RB965580	RB955980	RB011518	RB975013	RB975375	RB965602	RB006613
RB985829	RB965586	RB956911	RB086000	RB971108	RB935581	RB965604	RB006981
RB985834	RB965904	RB966200	RB97341	RB971703	RB935903	RB965731	RB975101
RB985843	RB965906	RB975031	RB006655	RB975007	RB965692	RB965741	RB975932
RB931565	RB966220	RB975032	RB985696	RB961517	RB995118	RB965519	RB975933
RB931566	RB966229	RB975033	RB015861	RB026968	RB028008	RB965902	RB975934
RB931569	RB966925	RB975086	RB015895	RB015836	RB965739	RB006607	RB976934
RB931003	RB966926	RB975241	RB005014	RB016746	RB935788	RB006609	RB036129
RB0451	RB966927	RB975948	RB005040	RB935744	RB945040	RB006970	RB98710
RB0459	RB975081	RB965917	RB005498	RB936109	RB965922	RB006971	RB028005
RB0442	RB975930	RB966923	RB005632	RB036142	RB966249	RB018198	RB036152
RB0449	RB961	RB037081	RB005695	RB935608	RB966920	RB106819	RB027042
RB04803	RB9441	RB965908	RB005935	RB935621	RB966928	RB026971	RB027046
RB945203	RB981802	RB965909	RB006995	RB936095	RB965699	RB026970	RB027050
RB991532	RB98703	RB026965	RB006984	RB975942	RB015898	RB106802	RB027052
RB036075	RB955970	RB995198	RB006629	RB945276	RB015934	RB036059	RB027059
RB006988	RB975939	RB965518	RB006638	RB945955	RB015935	RB018117	RB027060
			165	RB945957	RB005928	RB00509	RB027040

Table 4. Total third-generation genotypes analyzed by origin.

Analyzing the related genotypes and their respective parents, we observe that some of them have some parent from divergent origin, but usually the genetic origin division is composed by a percentage greater than or equal to 50% of origin similarity so they can be considered from the same heterotic pattern.

Thus, we can divide them into three groups of distinct heterotic patterns as shown in Image 7.

GRUPO A	GRUPO B		GRUPO C	
RB931565	RB985840	RB9441	RB995118	RB036129
RB931566	RB985841	RB955970	RB028008	RB028005
RB931569	RB985820	RB975939	RB006607	RB027042
RB981802	RB985829	RB955312	RB006609	RB027046
RB98703	RB985834	RB955980	RB006970	RB027050
5	RB985843	RB956911	RB006971	RB027052
	RB931003	RB966200	RB00509	RB027059
	RB975942	RB965904	RB006655	RB027060
	RB945276	RB965906	RB985696	RB005695
	RB945955	RB966220	RB005014	RB005935
	RB945957	RB966229	RB005040	RB006995
	RB935875	RB966925	RB005498	RB006984
	RB935925	RB966926	RB005632	RB006629
	RB965578	RB966927		RB006638
	RB965580	RB975081		27
	RB965586	RB975930		

32

Image 7. Genotypes grouped into different heterotic groups ¹.

With this division, one can work with recurrent crosses within families, to concentrate favorable alleles characteristic of these families. Broad possibilities for self-fertilization and biparental crosses within each family can happen, increasing the homozygousness degree and the need for selection.

The crosses between distinct genetic groups do not cease, so new varieties can be generated.

The use of clones or varieties from unknown parents, or that have question marks in their pedigrees, is not excluded or prohibited, but clearly becomes incompatible, because knowing the origin and the genetic characteristics of the parents at future crosses is a key point of this type of work.

¹ In order: Group A, Group B and Group C.

5.4 Crosses suggestion.

Based on the divisions between heterotic groups, all crosses between groups A, B and C above are recommended.

3rd-GENERATION HYBRID CLONES			
FOR PREFERENTIAL CROSSINGS WITH GROUP A			
RB985476	RB976930	RB005928	RB015861
RB975949	RB965602	RB037186	RB015895
RB925367	RB965604	RB975031	RB005917
RB946905	RB965731	RB975032	RB005983
RB975375	RB965741	RB975033	RB006610
RB935581	RB965519	RB975086	RB006613
RB935903	RB965902	RB975241	RB006981
RB965692	RB018198	RB975948	RB975101
RB965739	RB026971	RB965917	RB975932
RB945040	RB026970	RB037081	RB975933
RB965699	RB036059	RB965908	RB975934
RB015898	RB018117	RB965909	RB976934
RB015934	RB011715	RB026965	RB98710
RB015935	RB991530	RB975053	RB036152
			RB027040

Figure 8. Third-generation hybrids preferred for crosses with Group A

3rd GENERATION HYBRID CLONES

FOR PREFERENTIAL CROSSINGS WITH GROUP B

RB945203
RB991532
RB971108
RB971703
RB975007
RB026968
RB015836
RB016746
RB935608
RB935621
RB936095
RB106819

3rd-GENERATION HYBRID CLONES

**FOR PREFERENTIAL
CROSSINGS WITH GROUP B**

RB971755
RB975013
RB971765
RB011518

WITH GROUP A and C

RB965635
RB037048

WITH GROUPS A,B and C

RB995198
RB965518
RB976328
RB106802
RB00509
RB97341

WITH GROUP C

RB036075
RB006988
RB961517
RB935744
RB936109
RB036142
RB961
RB935788
RB965922
RB966249
RB966920
RB966928
RB985580
RB966923
RB086000

Image 9. Indicates hybrids that differ by pedigree from mentioned groups, therefore are indicated to crosses with the cited groups

6. CONCLUSION

Biparental crosses have effectively contributed to finding superior individuals and cultivars in advanced generations, with known parents and at more promising crossings.

It was possible to discriminate 3 contrasting heterotic groups based on the origin, 5 genotypes from the group called A, 32 from group B and 27 from group C. All intergroup crossings are recommended.

The following crossings are also recommended:

- 57 hybrid genotypes, similar to group B and C and therefore recommended for crosses with group A
- 12 hybrids similar to group C, recommended for crosses with group A and B
- 4 hybrids for crosses with group B
- 2 hybrids for crosses with group A and C
- 6 hybrids for crosses with group A, B and C
- 15 hybrids for crosses with group C

The genotypes were divided into four generations:

- First generation: 167 clones and 36 varieties
- Second generation: 411 clones and 34 varieties
- Third generation: 184 clones and 11 varieties
- Fourth generation: only 5 clones: RB981805, RB981812, RB981819, RB036112, RB036122.

Due to the low proportional number of 4th generation genotypes, the need for advances in generations and new crosses within the 3rd generation is evident.

It bases the possibility of discarding clones without parents by exclusion or use of testers. Also, the use of self-fecundation and intrafamiliar reciprocal recurrent selection for fixation of the heterotic pattern.

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APPENDIX

GENOTYPE	FEMALE PARENT	MALE PARENT
F160	NCo310	F141
RB83102	NA56-79	SP70-1143
RB83594	RB72454	B3337
RB835054	RB72454	NA56-79
RB835089	RB72454	NA56-79
RB845065	SP70-1143	NA56-79
RB845197	RB72454	SP70-1143
RB845210	RB72454	SP70-1143
RB845239	RB72454	SP70-1143
RB845257	RB72454	SP70-1143
RB855113	SP70-1143	RB72454
RB855536	SP70-1143	RB72454
RB855546	SP70-1143	RB72454
RB855595	SP70-1143	TUC71-7
RB858927	NA56-79	RB739735
RB862821	H39-3633	L60-14
RB872552	RB754665	RB773720
RB872601	NA56-79	B42231
RB925367	RB845239	RB855035
RB9350	Q107	RB72199
RB931569	RB721012	RB835089
RB935621	RB835089	SP70-1143
RB935788	RB855113	RB835089
RB941019	RB72454	SP70-1143
RB942549	RB75126	RB765418
RB943161	NCo376	CB45-3
RB943365	ROC3	RB83100
RB945276	RB835486	RB845239
RB9571	RB72454	RB83102
RB95549	RB72454	NCo310
RB951539	RB83102	RB751194
RB951541	RB72454	SP79-1011
RB951551	RB80457	RB72454
RB9622	RB75126	SP79-2313
RB965902	RB855536	RB855453
RB965906	RB835486	RB855536
RB965917	RB855453	RB855536
RB965918	RB855511	RB855002
RB965920	RB855574	RB845210
RB97319	RB72454	RB83102
RB97711	SP80-1520	RB83102
RB971508	RB83102	RB72454
RB971534	RB83102	RB855453
RB971551	RB83102	RB855453
RB975157	RB855563	RB735200
RB975932	SP80-185	RB855206
RB975944	RB855563	RB735200
RB975952	RB835486	RB825548
RB98709	SP81-3250	RB825336
RB99386	NA69-12	RB835205
RB005983	SP80-1842	RB855156
RB005989	RB835486	TUC71-7
RB011509	BJ7504	RB72454
RB011518	RB9367	RB91539
RB015895	SP80-1816	RB855156
UFPR1	RB855536	SP80-1816
UFPR2	SP81-3250	RB835486
UFPR3	SP81-3250	RB835486
UFPR4	SP80-1520	RB855536
UFPR5	RB855536	NA84-3920