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SUGARCANE:

BREEDING METHODS AND GENETIC MAPPING

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INTRODUCTION

Sugarcane is a plant species of great importance for Brazilian and world agriculture, being primarily grown in tropical and subtropical regions. Its main products are sugar and ethanol, the latter presenting high economic and environmental interest because as a renewable energy source, it can compete with petroleum derivatives (Goldemberg, 2007; FAO, 2008).

According to Daniels and Roach (1987), sugarcane is considered an allogamous species of the Poaceae family (Gramníneae), genus Saccharum, in which six species are generally recognized: *S. officinarum* L. (2n = 80), *S. robustum* Brandes and Jeswiet ex Grassl (2n = 60-205), *S. barberi* Jeswiet (2n = 81-124), *S. sinense* Roxb. (2n = 111-120), *S. spontaneum* L. (2n = 40-128), and *S. edule* Hassk. (2n = 60-80). *S. officinarum* L. stands out from the others due to its desirable industrial properties and high sucrose levels, and it is commonly called "noble cane" (Matsuoka *et al*., 1999a; Landell and Bressiani, 2008). However, the modern varieties correspond to interspecific hybrids with high genetic complexity because they present high ploidy and aneuploidy levels (Heinz and Tew, 1987; Matsuoka *et al*., 1999a; Landell and Bressiani, 2008). It is believed that, among the cultivated varieties, there is a great contribution of varying proportions of the genome of these species (Matsuoka *et al*., 1999b). In this context, sugarcane is considered to be the cultivated species with the highest genetic complexity (Manners *et al*., 2004).

Several authors have described sugarcane breeding methods and strategies (Stevenson, 1965; Blackburn, 1983; Berding and Roach, 1987; Berding and Skinner, 1987; Breaux, 1987; Heinz and Tew, 1987; Hogarth, 1987; Tew, 1987; Machado Jr. *et al*., 1987; Matsuoka and Arizono, 1987; Peixoto, 1986; Matsuoka, 1988; Bressiani, 1993, 2001; Arizono, 1994, 1999; Pires, 1993; Landell and Alvarez, 1993; Machado Jr., 1993; Matsuoka *et al*., 1999a, 1999b; Creste *et al*., 2008; Landell and Bressiani, 2008). The purpose of this chapter is to briefly present the main stages of a breeding program, as well as the concepts of genetic mapping, that is, the development of genetic maps and QTL (Quantitative Trait Loci) mapping for sugarcane. Genetic Mapping should be studied because it allows a better understanding of the genetic architecture of quantitative traits, which information could be considered in the future for breeding programs aiming to make them even more efficient through marker-assisted selection (MAS) (Mohan *et al*., 1997; Morgante and Salamini, 2003; Charcosset and Moreau, 2004; Takeda and Matsuoka, 2008).

BREEDING METHODS

Despite the genetic complexity of sugarcane, obtaining new genotypes for it is relatively easy due to mechanisms of vegetative propagation. Such programs are based on the generation of a segregating population with genetic variability, followed by several stages of selection and cloning of the superior genotypes. It is important to mention that each breeding program adopts specific

strategies according to their needs and objectives. However, the period from the crossing until the identification of the desired genotype may demand from 10 to 12 years (Matsuoka *et al*., 1999a; Landell and Bressiani, 2008).

The success of a breeding program depends on several factors, among which is the appropriate choice of parents to maximize the chance of gains with selection, experimental designed to have good accuracy, and the correct choice of the desired traits and their assessment periods. Some of the features to be considered for selection are the soluble solid content (Brix), sucrose content, diameter and number of stalks, fiber content, early maturity and resistance to pests, diseases lodging and flowering (Matsuoka *et al*., 1999a, 1999b). In most cases, these traits are controlled by various loci known as QTL.

Overall, a sugarcane breeding program include the following stages: i) generating the population with genetic variability; ii) selection and cloning in the early stages; iii) evaluation of clones in experiments using appropriate designs; and iv) trial competitions.

i) Generating the population with genetic variation

Traditionally, genetic variability in a breeding program is obtained through crosses between parents that present the desired traits. To this end, the choice of parents and crosses are planned so that the probability of finding better genotypes is maximized. One of the criteria used is the choice of parents with good performance for traits of economic interest, which is clearly the case for cultivars used commercially (Matsuoka *et al*., 1999a). It is noteworthy that this can lead to the narrowing of the genetic base and, consequently, to problems associated with inbreeding depression. With the aim of overcoming these problems, the selection of parents may be based on genealogies and/or genetic divergence obtained with molecular markers (Lima *et al*., 2002).

After the choice of the parents, it is necessary to define a strategy to obtain the segregating population. For sugarcane, two widely used approaches are biparental crosses (full-sib family) and polycross (Matsuoka *et al*., 1999a; 1999b; Landell and Bressiani, 2008):

a) **Biparental crosses**: a segregating population is obtained from a cross between two parents, allowing for the identification of the male and the female. This is the type of cross most often used in practice because it takes into account both the general capacity of combination of the parents and the specific capacity of the crossing combination.

b) **Polycrosses**: certain genotypes are used as male parents and crossed with a single genotype used as female. In this case, only the one parent is known. The main advantage of this approach is the possibility of producing a larger number of seeds in relation to the two-parent cross, as well as a greater variability within each progeny (half-sib family).

Annually, a breeding program generates populations composed of thousands of seedlings, which are later submitted to selection. The number of seedlings varies according to each program and depends on technical and economical factors.

ii) Selection and cloning in the early stages

The main challenge for the breeder is to find superior genotypes in a segregating population and propagate them. However, in the earlier stages, selection cannot be intensive for traits that are highly influenced by the environment. Initially selection is done for traits with high heritability and using low intensity. Throughout the selection stages, the availability of stalks per genotypes increases gradually, allowing the implementation of experiments with high levels of accuracy and increased selection intensity. The size of plots and number of repetitions will vary depending on the availability of stems and specific traits of the breeding programs.

In contrast to some plants such as maize, sorghum, soybeans, among others, sugarcane allows the propagation of a given genotypes, which makes it possible to explore heritability in a broad sense; that is, in addition to additive variation, it is possible to explore dominance and epistatic interactions (Visscher *et al*., 2008). At the initial stages, measurements are obtained based on individual observations, and in the latter stages they

are obtained based on averages of replications. For these reasons, selection for traits of lower heritability is performed only at later stages, which can increase the gains with selection. According to the results presented by Skinner *et al*. (1987), Matsuoka *et al*. (1999a) commented that in the early stages, selection would be more effective for Brix and resistance to rust and smut. However, the results should be interpreted with caution because estimations of heritability may vary according to the population and the experimental conditions (Visscher *et al*., 2008).

Several strategies may be used in the early stages for planting and selection such as individual planting, bunch planting and families planting, because in that period it is not possible or very difficult to cultivate the plants under experiments with statistical designs.

Individual plant selection: consists of planting genotypes with a greater distance between individual plants to avoid competition. This strategy is recommended only for the selection of traits with high heritability, such as Brix, flowering, and disease resistance (Matsuoka *et al*., 1999a). Thus, this strategy can be considered in situations where it is possible to evaluate every individual genotype, which, according to Skinner *et al*. (1987) and Matsuoka *et al*. (1999a), can be accomplished in an breeding program. At this stage, many genotypes are discarded visually based only on their vigor. In other words, only the superior genotypes are measured.

Bunch planting (Mangelsdorf, 1953): consists of planting five to 10 seedlings in bunches, allowing natural selection to act by eliminating inferior genotypes. One advantage of this type of planting is the possibility of assessing several stalks simultaneously in the same area. Skinner *et al*. (1987) argued that there are successful examples of the implementation of this technique. However, Matsuoka *et al*. (1999a) indicated the impossibility of evaluating tillering as a disadvantage because this trait is positively correlated with the capacity to tillering and also productivity.

Family selection: is based on situations in which more than a two-parent cross or polycross are available, and the progeny are evaluated according to the average behavior of the individuals. In this approach, the concept of heritability is explored at means levels, which in the early stages, allows the selection of traits that would not be evaluated if this selection was considered at individual levels. When considering the progeny as a whole, it is possible to perform studies of the interactions between genotypes and environments (Hogarth and Bull, 1990; Bull *et al*., 1992; Jackson *et al*., 1995a, 1995b; Bressiani, 2002; Landell and Bressiani, 2008), identify superior crosses (Skinner *et al*., 1987), and to automate the process (Landell and Bressiani, 2008). As a result, some breeding programs prefers to apply this strategy, choosing the families that present higher phenotypic means. However, this procedure may cause the discharge of superior genotypes that are in families with low means and high variance (Matsuoka *et al*., 1999a).

iii) Evaluation of clones in experiments

The selected genotypes in (ii) are compared based on experiments using appropriate statistical design. A feature of these experiments is the large number of genotypes being evaluated and the limited number of stems available for the repetition and/or replication of the experiments in other environments. An appropriate design for this stage is the augmented blocks design (Federer, 1956).

With the use of such an experimental design, it is possible to control some environmental variables. This allows selection for traits that are influenced by the environment, which was not addressed in the previous stage; for example, the evaluation of the capacity of regrowth. Matsuoka *et al*., (1999a) reported that it is not advisable to use a high selection intensity due to reduced experimental accuracy. Skinner *et al*. (1987) suggest that selection intensities between 10 and 30% can be considered. It is of note that these values should not be generalized because they may vary according to the characteristics of each breeding program.

At the end of each experiment, the genotype number is reduced and the availability of plant material is increased. This results in experiments with more repetitions and also replicated in differents

environments, which increases the experimental accuracy, making it possible to evaluate the traits with low heritability.

iv) Competition trials

After several selection stages, the remaining genotypes are tested in experiments with high level of accuracy, which is done also including commercial varieties as checks. In this stage, it is common to peform analysis of stability, with the goal of finding genotypes that response better to certain environmental conditions.

In Brazil, such experiments are usually implemented in randomized blocks design (Matsuoka *et al*., 1999a; Bidoia and Bidoia, 2008) that are often repeated in different environments and assessed over a number of cuts. The genotypes that are superior at this stage are recommended as new cultivars. An interesting feature of this stage is the fact breeding programs and industrial sector have an extensive involvement, which reflects at this stage. Therefore, the experiments are very close to the agronomic practices used after the cultivar is released.

GENETIC MAPPING

The importante traits considered in a breeding program are generally result of the combined action of several genes and the influence of environmental conditions. The term QTL (quantitative trait loci) has been used to specify the chromosomal regions that contain genes (or loci) that control these polygenic traits (Falconer and Mackay, 1996).

With the recent advent of molecular markers, it is now possible to study these regions more easily. Molecular markers are fast and efficient tools for genomic studies because they detect polymorphisms directly at the DNA level and do not suffer any kind of environmental influence (Souza, 2001). Based on these polymorphisms, it is possible to identify relationships between genotype (assessed based on molecular markers) and phenotype (assessed in appropriate experiments), which ultimately could increase the efficiency of breeding programs, through Marker Assisted Selection.

QTL mapping can be defined as a process of inferences across the entire genome trying to associate genotype and phenotype. These results include information on the number, position, effects, interactions of QTL alleles within loci (dominance) and among loci (epistasis), pleiotropic effects of the QTL, and interactions between QTL and the environment (Zeng, 2001). Therefore, it requires a population that presents genetic variability and high linkage disequilibrium. In this context, first a linkage map was obtained and it is the basis for future localization of regions that controls the quantitative traits. For this, sophisticated statistical methods are required, which demands strong computational support due to the complexity of analysis.

Genetic maps

The majority of the populations used for the development of genetic linkage maps are derived from inbred lines (for example, F2, Backcrosses, Recombinante Inbred Lines). The statistical methods in such cases are established and implemented in various software packages, such as MAPMAKER/EXP. However, obtaining inbred lines is impractical for sugarcane, mainly due to high inbreeding depression that exists. Thus for sugarcane, the mapping population is usually a full-sib family derived from two-parent cross between non-homozygous parents (Lin *et al*., 2003).

For sugarcane (and various other species for which there are no available inbred lines, such as passion fruit and eucalyptus), an alternative that has been widely used was the pseudo-testcross strategy (Grattapaglia and Sederoff, 1994). This approach provides two individual maps (one for each parent) through the identification of polymorphisms in a single dose marker for each parent (Grattapaglia and Sederoff, 1994; Shepherd *et al*., 2003; Porceddu *et al*., 2002; Carlier *et al*., 2004). Based on this approach, linkage maps for *S. officinarum* ('LA Purple') and *S. robustum* ('Mol 5829') were developed using RAPD, RFLP and AFLP markers in a single dose (Guimarães *et al*., 1999). Other studies were conducted with differents parents but commercial cultivars was

rarely considered in this type of study (Sobral and Honeycutt, 1993; Al-Janabi *et al*., 1993; da Silva *et al*., 1993; da Silva *et al*., 1995; Grivet *et al*., 1996; Hoarau *et al*., 2001). However, from either biological or statistical point of view, it is desirable to integrate the information contained in these individual maps into a single one. This can only be done with the presence of heterozygous markers in both parents, which are essencials in the integration process (Barreneche *et al*., 1998; Wu *et al*., 2002a; Garcia *et al*., 2006; Margarido *et al*., 2007; Oliveira *et al*., 2007).

The development of integrated genetic map using molecular markers with different segregation patterns has major advantages because it increases the saturation of the map and also can represent better the polymorphism variation across the genome. Specifically, for polyploid species such as sugarcane, co-dominant markers can be useful to gather linkage groups into their respective homology groups (da Silva *et al*., 1993; Grivet *et al*., 1996). Moreover, statistical power for QTL mapping can be increased when integrated maps are used (Maliepaard *et al*., 1997). However, in a full sib family each segregating locus may differs at the number of alleles, as well as at their linkage phases, making it difficult to detect recombination events (Maliepaard *et al*., 1997; Wu *et al*., 2002a).

Wu *et al*. (2002a) proposed a statistical method based on maximum likelihood, which allows for the estimation of both the recombination fraction and the linkage phases between loci in full-sib families. This method allows the development of an integrated genetic map that is a result of the combination of several types of information generated from different types of molecular markers with variable information content. This approach was used by Garcia *et al*. (2006) and Oliveira *et al*. (2007), who developed integrated genetic maps consisting of 357 markers distributed over 131 groups of co-segregation from a cross between two pre-commercial cultivars of sugarcane (SP80-180 x SP80-4966). These results were superior to those obtained when the same data were analyzed using the JoinMap program, which indicates, in this case, a greater efficiency in the estimation of linkages and linkage phases of the method proposed by Wu *et al*. (2002a). Initially, a software called OneMap (Margarido *et al*., 2007) was developed, which allows for the development of genetic maps in full-sib family, considering the approach proposed by Wu *et al*. (2002a). However, in its new version, the methodology was adapted for a multipoint approach based on Hidden Markov Models (Lander and Green, 1987; Jiang and Zeng, 1997; Butcher *et al*., 2002; Wu *et al*., 2002b). It is important to note that this software has also been used for other outcrossing species such as citrus, pinus, eucalyptus and passion fruit.

Despite the great superiority of these new approaches, it is still only possible to use markers that show 1:1 and 3:1 fashion, which are known to be less informative than other types such as markers that segregate in 1:2:1 or 1:1:1:1 fashion, in the case of diploid species (Wu *et al*., 2002a). For sugarcane, the presence of autopolyploids is also complicated because there are few studies focusing on the development of genetic maps with markers with different doses (da Silva, 1993; da Silva *et al*., 1995; Ripol *et al*., 1999). Overall, these factors make it difficult to obtain linkage groups and the ordering of markers within these groups, resulting in less saturated maps with less coverage of the genome. Furthermore, the integration of maps from parents is not always possible.

QTL mapping

Similar to linkage maps, QTL mapping also has several statistical methods available that were mainly developed for inbred lines, such as single markers analysis (t-test, analysis of variance, simple linear regression and likelihood ratio test (Weller, 1986, Edwards *et al*., 1987; Stuber *et al*., 1987, Lynch and Walsh, 1998), interval mapping (IM) (Lander and Botstein 1989), composite interval mapping (CIM) (Zeng 1993, 1994; Jansen and Stam 1994), and multiple interval mapping (MIM) (Kao and Zeng, 1997; Kao *et al*., 1999). These methods are implemented in software platforms such as the QTL Cartographer (Basten *et al*., 1999). From a theoretical point of view, there are several advantages of the MIM models over the others (Zeng *et al*., 1999). They provide useful

results for breeding programs, for example, for assessing breeding values of each individual that can be used in marker-assisted selection.

For sugarcane, several QTL mapping studies were developed using the analysis of individual makers, or interval mapping (for example, Grivet *et al.*, 1996, Guimarães, 1999; Hoarau *et al.*, 2001; Ming *et al.*, 2001, 2002; Kido, 2003), generally considering the pseudo testcross strategy. Among the results, it could be point out the identification of RFLP probe that is associated with resistance to rust that showed a segregation pattern 3 (resistant):1 (susceptible) in the progeny (Grivet *et al.*, 1996). Guimarães (1999) detected an RFLP probe associated with sugarcane flowering. Ming *et al.* (2001, 2002) detected 36 QTL regions for sugar production, stem weight, number of stalks, and fiber and ash content. Kido (2003) mapped QTL regions for several agronomic traits in crosses of two Brazilian species of sugarcane detecting 11 QTLs for Brix, 9 for stem diameter, 7 for tiller number, 21 for stalk height, 6 for fiber content, 2 for percentage of sugar, and 3 for productivity. It becomes clear that advances in sugarcane breeding can be achieved with QTL mapping.

In populations derived from a full sib family, as in the case of sugarcane, there is an additional complication: the linkage phases between different loci and QTL are not known and should be estimated. This complicates linkage analysis and QTL mapping in these cases. Lin *et al.* (2003) presented a statistical method based on interval mapping that estimates linkage and linkage phases between marker loci and QTL simultaneously, considering all types of markers (informative and partially informative). However, this model considers only the information between a pair of flanking markers (probabilities are not obtained via multipoint analysis). To avoid the problem of the lack of knowledge of linkage phases between QTL regions and markers, they are considered as parameters in the mixtures model, which makes the likelihood complex, in addition to presenting computational difficulties when obtaining estimates of linkage phase between QTL regions and markers. The proposal of Lin *et al.* (2003) was important because, to our knowledge, it was the first mapping model that used an integrated genetic map using markers that show different segregation.

To avoid some of these limitations, Gazaffi (2009), Gazaffi *et al.* (under development) and Pastina (2010) developed approaches for QTL mapping in this case, expanding the approach by Lin *et al.* (2003) in the context of composite interval mapping (Zeng, 1993, 1994) and using multipoint probabilities (Jiang and Zeng, 1997). However, there are no statistical models available for multiple features in both the context of CIM and MIM, which would allow for the study of the genetic basis of the correlation between traits and the interaction of genotypes and environments, in a full sib family. In addition, it is expected that more sophisticated approaches will be available in a near future.

FINAL CONSIDERATIONS

Currently, sugarcane breeding programs benefit from methods developed for the estimation of genetic parameters. Additionally, the development and dissemination of molecular markers has enabled the unraveling of the architecture of quantitative traits through QTL mapping.

It is important to mention that, for sugarcane (an autopolyploid species), proposals have been made for genetic mapping that consider polyploidy (da Silva, 1993; da Silva *et al.*, 1995; da Silva and Sorrells, 1996; Ripol *et al.*, 1999; Doerge and Craig, 2000; Wu *et al.*, 2001; Luo *et al.*, 2001; Luo *et al.*, 2004; Wu *et al.*, 2004; Cao *et al.*, 2005), although the approach used in most practical situations corresponds to the application of mapping similarly to what occurs in the diploid species. This approach is based on the use of markers that segregate at 1:1 and 3:1 ratios. However, several different patterns of segregation can occur (Grivet *et al.*, 1996; Edmé *et al.*, 2006), and only part of the genetic polymorphism is considered in this approach, which impedes the complete study of the genetic architecture of quantitative traits in polyploids. We hope that, in the future, these species can be studied in details, by developing models that consider this genetic polimorphism.

A common criticism made in QTL mapping concerns the results obtained for a particular

population are not necessarily repeated in other crosses because only part of the variability of the species is sampled (Flint and Mott, 2001). One possibility to alleviate this problem would be to conduct QTL mapping in several full sib families, followed by the search for corresponding polymorphisms in others genotypes via associative mapping (Salvi and Tuberosa, 2005). This type of study can only be conducted with the presence of a high rate of polymorphism. For this reason, SNP markers are used in such studies (Kruglyak, 2008). It is believed that by reducing the cost of this type of technology results for sugarcane will be available in the near future.

QTL mapping studies also aim to find regions that control quantitative traits. However, there is interest in finding polymorphism at the gene and individual nucleotide levels (Mackay, 2001). For this purpose, one option would be to conduct fine mapping studies (Mott and Flint, 2001). However, such studies are expensive and burdensome. The development of DNA microarray technology allows for eQTL (expressed QTL) mapping (Jansen and Nap, 2001; Schadt *et al*., 2003) because the gene expression of individuals of a certain population can be used as a phenotype to obtain regions that control this expression. The main advantage of this approach is that a single DNA chip allows for a simultaneous genetic expression profile of hundreds or thousands of genes for a single individual. The phenotyping of a segregating population would allow the observation of which genes present QTL regions that are coincident with the QTL regions associated with agronomic traits. Thus, it is possible to infer which genes are responsible for the control of the phenotypes under study. The use of eQTL also allows the study of epistasis from a new perspective, for example, through the reconstruction of gene networks (Jansen and Nap, 2001; Zhu *et al*., 2004). Although it still presents few practical results, this approach would allow for studies of the genetic architecture of traits of economic importance in great detail.

We believe that genetic mapping studies have allowed for a better understanding of the genetic control of traits of economic interest in sugarcane. However, such knowledge can still be expanded if we consider other approaches that require the development of statistical models and new biotechnology tools. The main contribution of these studies is the possibility of incorporating this information into breeding programs, either by selecting regions that control traits of interest or by guiding new strategies.

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