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Sugarcane improvement: how far can we go?

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In recent years, efforts to improve sugarcane have focused on the development of biotechnology for this crop. It has become clear that sugarcane lacks tools for the biotechnological route of improvement and that the initial efforts in sequencing ESTs had limited impact for breeding. Until recently, the models used by breeders in statistical genetics approaches have been developed for diploid organisms, which are not ideal for a polyploid genome such as that of sugarcane. Breeding programs are dealing with decreasing yield gains. The contribution of multiple alleles to complex traits such as yield is a basic question underlining the breeding efforts that could only be addressed by the development of specific tools for this grass. However, functional genomics has progressed and gene expression profiling is leading to the definition of gene networks. The sequencing of the sugarcane genome, which is underway, will greatly contribute to numerous aspects of research on grasses. We expect that both the transgenic and the marker-assisted route for sugarcane improvement will contribute to increased sugar, stress tolerance, and higher yield and that the industry for years to come will be able to rely on sugarcane as the most productive energy crop.

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Introduction

Sugarcane is an important crop for food and energy production. Among the main traits that make it a unique crop, we note its capacity to accumulate high levels of sucrose in its stems and its characteristic high yield, making it the highest tonnage crop among cultivated plants.

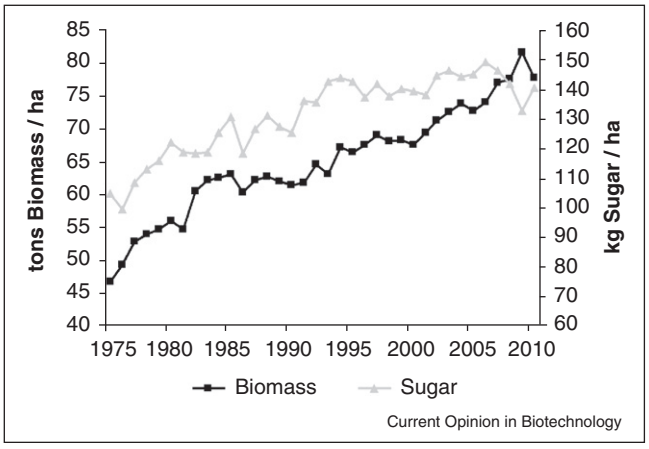
Sugar production for the 2011/12 marketing year is forecast at 168 million metric tons (MMT), in raw value, up 8 MMT over the previous year. In the 2010/11 crop, Brazil harvested about 625 million tons of sugarcane, in a cultivated area of just over eight million hectares. The average yield was 77 tons/ha [1]; higher than the corn yield in United States (9.3 tons/ha) [2] and sweet sorghum in China (60 tons/ha) [3]. Of the total of industrialized sugarcane, 53.8% (336 million tons) was destined for ethanol production, which generated a volume of 27.67 billion liters (82 l/ton of sugarcane).

The importance of sugarcane as a bioenergy feedstock has increased interest in the generation of new cultivars optimized for energy production. Breeding programs are introducing new ancestral genotypes into crosses in a quest to alter fiber content and yield. It is noteworthy that sugarcane has always been bred with the aim of improving sugar content but an evolving industry of biofuel and bio-based chemicals may require vast amounts of biomass and, therefore, higher yield. We have seen recently a desire to breed the Energy-Cane, a crop with a high yield and fiber. The world yield average is 80 tons/ha but the calculated theoretical yield potential of sugarcane has been noted to be over 380 tons/ha [4••], so there are still gains to be expected. This review will outline some of the most pressing aspects of a biotechnological route for sugarcane improvement including technological data available and the use of marker-assisted breeding, genome sequencing, transgenics, and gene discovery for traits of interest.

Classical breeding: where are we?

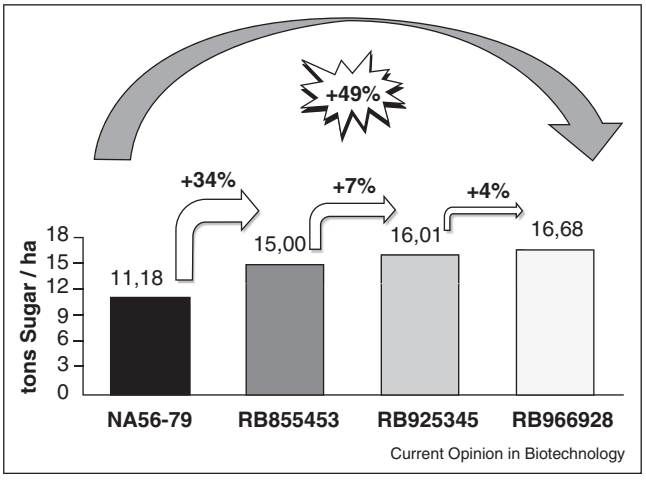
Brazilian sugarcane productivity increased 66% in tons of sugarcane per hectare and increased 34% in sugar content per tons of sugarcane from 1975 to 2010 (Figure 1) [1,5]. This increase in yield was due to breeding and better agronomical practices [6••]. The introduction of a new variety does not imply large changes in the production system and is always a hope in the search of productivity gains. The selection of the superior genotypes within a population obtained by crossing two individuals is a long duration work, which lasts no less than ten years to

Figure 1



Evolution of sugarcane productivity and sugar content from 1975 to 2010 in Brazil. The productivity and sugar content increased 1.89% and 0.98% (average per year), respectively. If we consider only the last 10 years, the increase was 1.2% and 0.2% (average per year), respectively. *Source*: [1,5].

Figure 2



Decreasing productivity gains of varieties. Sugar yield of three cultivars released in 1985 (RB855453), 1992 (RB925345) and 1996 (RB966928) compared to the most important cultivars at the time. The data are from São Carlos Federal University's Breeding Program in Brazil.

generate results. On average, one variety can be obtained for each 250 thousand seedlings evaluated in the first stage of the breeding program [6••].

A recent evaluation of breeding programs indicates that increases in sugar yield are becoming less pronounced (Figure 2) [7]. The productivity gains of sugarcane crop have been in the order of 1.0–1.5% a year in recent years [4••]. In the future, it is believed that the productivity increases will be even lower. We have observed an increasing number of varieties in use in Brazil, probably due to the exploration of new environments by breeders (Figure 3). In 1995, five cultivars occupied 70% of the cultivated areas. In 2010, this number has doubled. The number of cultivars in use is larger and their genetic similarity has been decreasing over the years (evaluated by the coefficient of parentage [8]). Breeding programs still need though to broaden the genetic basis of sugarcane, since many common ancestors are present in their pedigrees. The lack of diversification in the genotypes may be the underlying difficulty in increasing sugar content. Biotechnology may become crucial to face the limitations of classical breeding.

Biotechnological tools for the improvement of sugarcane

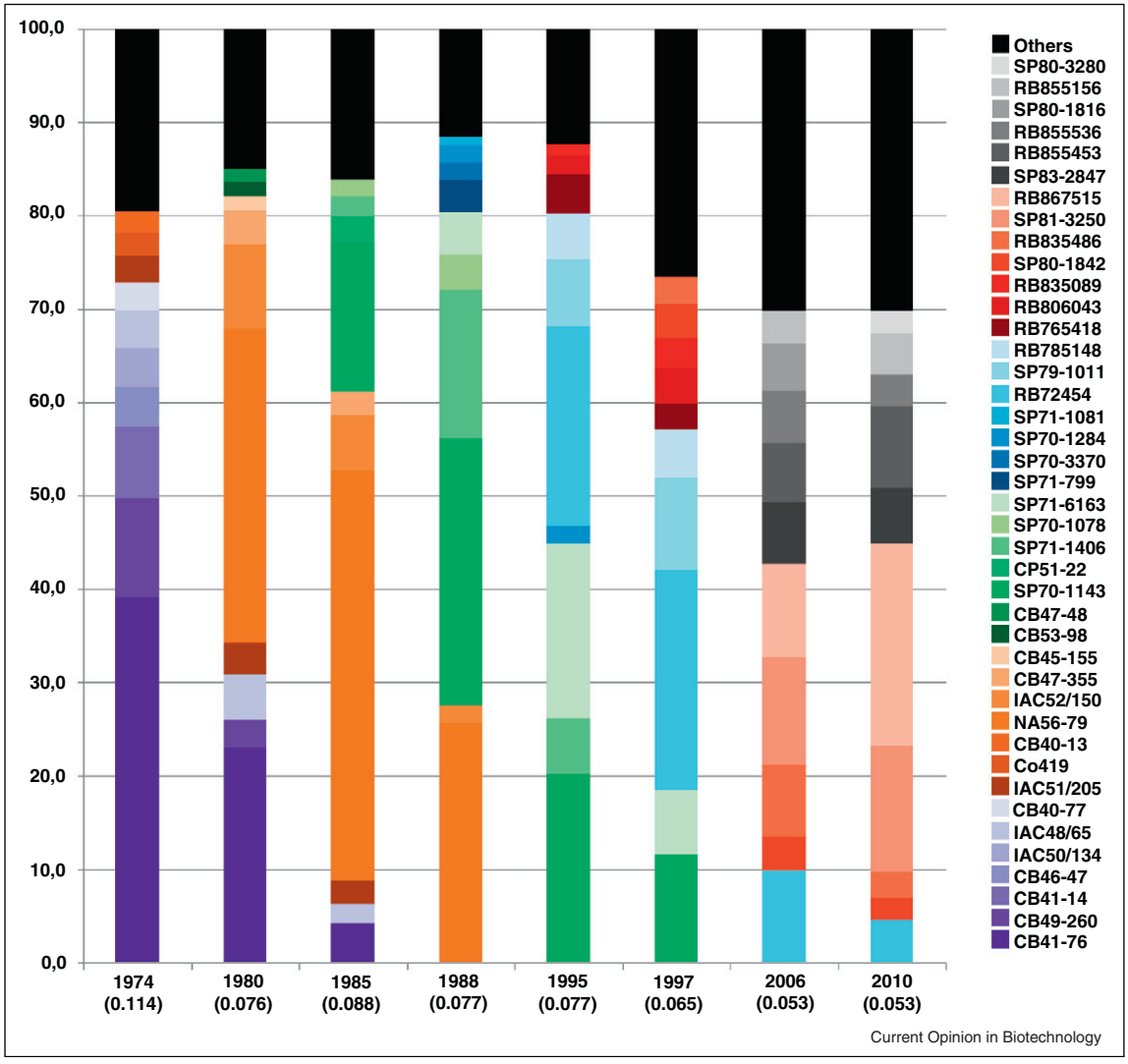
Sequencing the sugarcane genome

A sugarcane modern cultivar is a hybrid of *Saccharum officinarum* and *Saccharum spontaneum*. Sequencing the sugarcane genome poses new challenges due to its highly polyploid and aneuploid structure with a complete set of homeologous genes predicted to range from 10 to 12 copies (alleles). The monoploid genome is estimated to be around 1 Gb but the high level of polymorphism requires new assembly algorithms that can take into account allelic variation and a high content of repetitive regions. Obtaining a reference assembled monoploid genome for this crop is one of the greatest challenges in genomics at this time. There are 1585 nucleotide sequences (including 491 mRNA sequences), 283 158 ESTs and 10 728 genome survey sequences (GSSs) of *Saccharum* species at NCBI [9•]. Efforts underway include BAC-by-BAC and whole genome shot-gun sequencing (WGS) [9•]. The most comprehensive effort so far is devoted to sequencing BACs corresponding to regions of interest of the cultivar R570. A BAC library of 103 296 clones representing 14× the monoploid genome and 1.3× the total genome and 3D-pools of BAC clones are available. Moreover, a total of 6021 overgo probes were analyzed on the library to provide links with sorghum and there is ongoing effort to obtain R570 BAC-end sequences [9•]. Sequencing of R570 using the BAC library is being pursued by groups in Australia, France, South Africa, USA, and Brazil (http://sugarcanegenome.org). It is also worth mentioning that BAC and WGS sequencing are underway for SP80-3280, the Brazilian cultivar that most contributed to the available ESTs, and *S. officinarum* and *S. spontaneum* genotypes (LA Purple and SES208) (G. Souza, Ray Ming; personal communication).

The transgenic route

No commercial transgenic sugarcane cultivar exists, even though field trials are being conducted in several countries [6••,10]. The first sugarcane transgenics were transformed with traditional agronomical traits [10] but alternative approaches seek to change source and sink

Figure 3



Increasing number of sugarcane varieties in use in Brazil. Numbers in parenthesis are the average of the coefficient of parentage among the top 10 varieties of a given year [8].

relations [11] or to use sugarcane to synthesize value-adding products such as polyhydroxyalkanoates (PHAs) [12]. The incorporation of new metabolic pathways through systems biology and synthetic biology may allow sugarcane to be a source of new carbon compounds to replace petrochemistry. However, the potential of sugarcane as a biofactory has not been fully explored.

Sugarcane transformation is hindered by low transformation efficiency, transgene inactivation, somaclonal variation and difficulties in backcrossing [10]. Transformation methods must be optimized. Transgene expression must be better controlled and stability must be achieved. The difficulties of sugarcane transformation reduce the speed in which candidate genes can be tested.

Candidate genes for sugarcane improvement have been selected using the large number of gene expression data accumulated for this crop [4••,13•,14•]. Transcriptome analysis of culm maturation of sugarcane cultivars contrasting for sucrose content showed differential expression of genes related to cell wall metabolism, which suggests that accumulation of sucrose leads to alterations in cell wall synthesis [14•,15]. Downregulation of enzymes in lignin synthesis, such as COMT, or monolignol changes in lignin could improve ethanol production by increasing fermentable sugar release from lignocellulose [16].

Sugarcane's tolerance to drought is another important trait to be incorporated as cultivation is expanding

into water-limited regions [17••]. Transcriptome studies of sugarcane submitted to drought or treated with stress-related phytohormones identified genes associated with stress [13•,18,19]. These genes must be tested to determine whether they can confer sugarcane with enhanced stress tolerance. One solution is to use systems biology to target regulatory networks. An alternative is to use different model systems, such as *Brachypodium distachyon* [20], *Setaria italica* and *Setaria viridis* [21], as they have shorter life cycles and simpler genomes.

The long time required to transform a new transgenic sugarcane cultivar also makes yield lag a potential problem [10]. A transgenic cultivar has to be re-introduced in a breeding program and re-evaluated for traits of the original variety. Trials are expensive and regulatory aspects slow down its commercial release. When the new transgenic cultivar is eventually released, its benefits may have been overcome by a cultivar developed using classical methods. Thus, the added traits will have to outweigh considerably the yield of the original cultivar. In contrast, when sugarcane reaches its yield plateau, further increases in productivity may have to rely on the transgenic strategy. In this scenario, yield lag ceases to be a problem, and the importance of candidate genes will increase.

Marker-assisted breeding and statistical genetics for polyploids

Given the complexity of the genome of modern sugarcane varieties, information from molecular markers is crucial for genetic studies. Reliable linkage maps based on molecular markers are required to increase sequence assembly precision [22•] and to find genomic regions associated with variation on quantitative traits, or QTL [23••]. There are 19 linkage maps constructed from 13 mapping populations [23••] based on 1500–2000 markers. There are no saturated genetic maps covering all sugarcane chromosomes [24].

Several types of molecular markers have been used to construct genetic maps in sugarcane, for example RFLPs, AFLPs, TRAP [25,26], EST-SSRs [27,28], and DARTs [29]. Most sugarcane maps are based on dominant marker loci that have only one copy in a given parent (single dose loci), segregating in a 1:1 ratio for presence or absence of bands on the F1 progeny of a biparental cross [30•]. Mapping uses statistical methods and software already available for diploids, but there are reports using statistical methods to simultaneously estimate recombinant fraction and linkage phases for a number of different segregation ratios [31,32•]. These methods were successfully used to estimate integrated linkage maps on sugarcane, using 1:1 and 3:1 markers [25,27,33]. However, this is only an approximation, since sugarcane is polyploid.

Although there is some evidence that single dose loci correspond to about 70% of the detectable polymorphic loci [30•], more saturated maps will only be available if the whole genome is analyzed and included on the maps.

The abundance of Single Nucleotide Polymorphisms (SNPs) in plant genomes has prompted interest to develop panels of SNP markers to expand resolution of maps [34]. SNPs behave like codominant markers for polyploids and allow dose estimation. Statistical methods for automated genotype calling for biallelic markers were recently proposed for autotetraploids [35], but these are not necessarily suitable for sugarcane.

In contrast to the widely used linkage analysis, association mapping identifies QTLs by examining the marker–trait associations that can be attributed to the strength of linkage disequilibrium between markers and functional polymorphisms across a set of diverse germplasm [36,37]. The breeding history of sugarcane, consisting of a strong foundation bottleneck, followed by a small number of cycles of intercrossing and vegetative propagation suggests that linkage disequilibrium should be extensive [38,39]. Nevertheless, in sugarcane, due to low-density markers and nonrefined statistical methods, the association studies are only just beginning.

The use of Marker Assisted Selection (MAS) in sugarcane breeding programs is a challenging task. Most important traits, such as yield, are explained by multiple quantitative trait loci, each only contributing a small proportion of the overall phenotypic effect [40•,41]. Sugarcane QTL mapping is mostly based on single marker analysis or (composite) interval mapping [23••]. In order to provide useful results for genetic studies and breeding purposes, new models need to be developed, taking into consideration QTL versus environment interaction and epistasis. Although, no MAS has been reported in sugarcane, the *Bru-1* and *Bru-2* haplotypes have potential use in the identification of durable rust resistance gene in sugarcane germplasm [42•].

SUCEST-FUN, an integrated sugarcane database

The development of biotechnological tools for sugarcane requires an effort to manage the increasing amount of data related to sugarcane genomics and functional genomics. In this regard, the SUCEST-FUN database is an important resource to manage sugarcane genome data and to provide tools for geneticists and breeders. The SUCEST-FUN database integrates the Sugarcane EST Project (SUCEST) [43], the Sugarcane Gene Index (SGI), gene expression data [4••,13•,14•,44], the GRASSIUS database [45] and records of the agronomic, physiological, and biochemical characteristics of sugarcane cultivars (http://sucest-fun.org). The database contains 237 954 ESTs clustered into 43 141 assembled transcripts, 32 848 predicted proteins and 68 383 differential

gene expression data points [46]. The database is being modeled to include proteomics and metabolomics data as well as molecular markers and genomic sequences and promoters.

Conclusions

Sugarcane biotechnology has been receiving considerable attention over the last few years. New breeding programs and germplasm collections are being established and we expect to see an increasing arsenal of tools to improve this crop. Commercial transgenic plants may yet take years to come to commercialization and will probably be targeted at insect and drought resistance. The assembly of a reference genome sequence for this crop is paramount to aid both the development of transgenics and the marker-assisted improvement of this crop. A reference sequence will be important to define gene promoter sequences that may allow gene networks to be defined as well as speed up gene discovery projects and the development of tools for transgenic plant generation. SNP discovery and QTL determination will also profit from a reference genome. With the aid of statistical genetics for polyploids and the introduction of new genotypes, we expect breeding to progress much further toward achieving higher levels of productivity.

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