

GENETIC BREEDING AND BIOTECHNOLOGY COMPONENT

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INTRODUCTION

Brazil is the major sugarcane producer worldwide with an estimated production around 571 Mt of cane (Conab-MAPA). Such leadership was possible due to the incorporation of management technologies and mainly by the utilization of improved cultivars developed by sugarcane breeding programs.

The raising demand for ethanol as a source of renewable energy, along with the need to ensure Brazilian's competitiveness, mainly about ethanol production facing international market and consequently its leadership on bioenergy, brings a series of new challenges especially for genetic breeding programs. In doing so, besides an increase in productivity, it will be necessary to develop new sugarcane cultivars with a new agroenergetic profile, aiming at biomass production for cellulosic ethanol production.

Developing a new sugarcane cultivar takes about 10 to 15 years. Thus, crossings currently performed will provide cultivars that must be able to answer to the predicted needs for sugar-alcohol sector within at least 10 years. Thus, due to the objected scenarios, it is of fundamental importance that parental genotypes to be used on further crossings have the genes of interest for breeders, in such a way that they can be incorporated on new cultivars. In turn, genetic breeding aiming to develop cultivars with a higher biomass production will demand appropriated germplasm as a source of genes that confer traits capable of contributing to an increase on biomass and fiber content, as well

as other major agronomic traits such as resistance to main diseases, pests and abiotic stresses.

Therefore, molecular technologies such as the prospection of genes of agronomic interest, genetic transformation, molecular markers, among others, will be of considerable importance and will help sugarcane genetic breeding programs.

TECHNOLOGY NEEDS AND CAPABILITIES

In this section, we intend to show the necessities and technology capabilities regarding the Genetic Breeding and Biotechnology component of *Roadmapping* of sugar-alcohol industry.

Products or technologies targeted

Genetic breeding of sugarcane plays a key role on the ethanol productive chain, since it is responsible for providing the basic raw material that drives the whole system, i.e., sugar-alcohol industry. This feedstock is essentially represented by sugarcane cultivars and must comply with a series of pre-requisites, not only the necessary for a shift from the paradigm "sugarcane for sugar and alcohol" to "sugarcane for ethanol and energy generation", but also for those related to the expansion of this crop to restrictive areas, especially drought-prone areas ("cerrado"), where water deficit is the main limiting factor.

However, genetic breeding is not an isolated subject; still, it requires the integration of other areas of knowledge such as pathology, mineral nutrition, physiology, soils and genetics. Recent

advances on genomics, genetics, proteomics and metabolomics, suggest that biotechnology must impact sugarcane breeding by making it more efficient. Molecular-assisted breeding holds the potential to speed up varietal development, while transgeny may be able to alter great paradigms in a short period of time. Though, the biggest challenge is how to overcome the technological gap resulting from the huge quantity of information obtained through genetics and genomics, where the capacity of comprehension and development of resources has exceeded the capacity to apply them in a practical manner on the genetic breeding of sugarcane.

Thus, the aimed technologies for the Genetic Breeding and Biotechnology component of sugarcane refer to a set of strategies either from conventional genetic breeding or from biotechnology, which play important roles on the development of new sugarcane cultivars, whether it be for ethanol production (1st and 2nd generation), sugar or biomass. Yet, they still have bottlenecks that need to be solved. Furthermore, these technologies

were focused on the development of cultivars to achieved two different scenarios:

Scenario 1: higher yield cultivars with higher sucrose content that can meet the demand of industries that use conventional system of ethanol production.

Scenario 2: sugarcane cultivars focused on higher energy yield, resulting from higher fiber content, whether it be from bagasse or straw (leaf sheath and lamina, including the sum of all sugars which will respond for a lower percent of this primary energy (PE) in the future.

Quantification of the PE may be calculated by the expression suggested by REGIS (personal communication, 2007): $MJ/tc = 18 \times \text{kg fiber (dry matter)} + 16 \times \text{kg sucrose} + 15.6 \times \text{kg RS (reductor sugars)}$. These new cultivars should improve the current potential either for biomass production destined to electricity production ("Energy Mills"), or to cellulosic ethanol production, depending on the market demand.

Basically, genetic breeding can be divided into two stages that involves the generation of

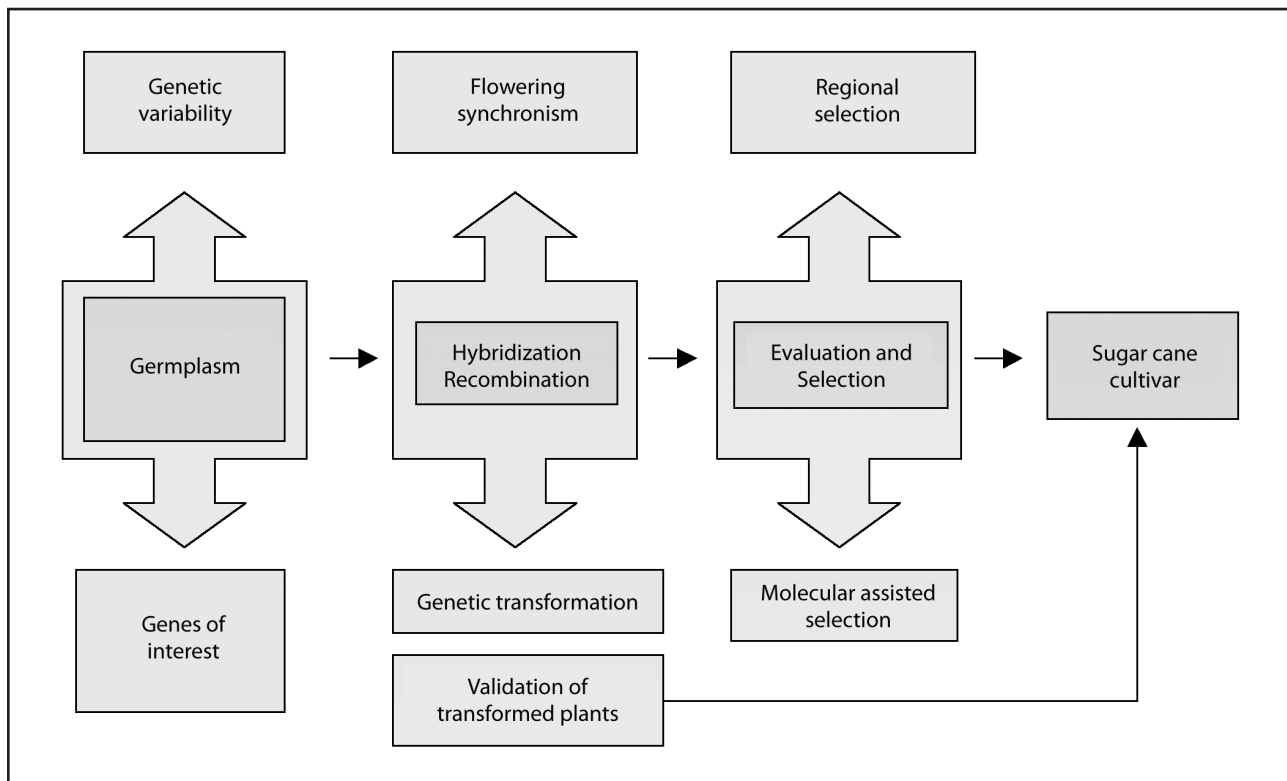


FIGURE 1 Developing process of a sugarcane cultivar and incorporation of molecular technologies.

variability through recombination (hybridization), and selection, that also includes the process of characterization of new clones. Both scenarios 1 and 2 go through these stages, although they have particularities concerning their critical requisites, since their main goals are different. Figure 1 shows a diagram of the developing process of a new cultivar, where critical steps that limit development are shown in red, as much as for conventional breeding or for biotechnology.

Critical stages for scenarios presented herein are:

1. characterization and use of germplasm;
2. regional selection strategies on expansion areas;
3. selection for drought tolerance;
4. molecular markers, genetic mapping and marker-assisted selection;
5. prospecting novel genes of interest for breeding and genetic transformation.

The identification of these stages drives the need for performing in depth basic studies in order to enable a diagnosis of the problem and identify solutions and areas of competence to solve the bottlenecks, ensuring the development of cultivars for both scenarios shown herein.

Critical system requirements – CSR

The development of sugarcane cultivars regarding to the scenarios mentioned above must meet a set of strategic goals in terms of:

- increase on biomass productivity;
- increase on sugar content (Pol%);
- increase on fiber content;
- increase of straw percent.

Sugarcane currently occupies an area of about 7.5 millions of ha. It is estimated that by 2020 the sugarcane plantation area will be at least twice as it is nowadays, and by 2025 it will probably reach an area of about 17 millions hectares. Crop expansion will occupy drought-prone areas where the main limitation is water deficit. Within this context, the development of drought tolerant cultivars, as well as their correct allocation on production environments, is imperative for increasing yield.

Current sugarcane productivity is about 81 t/ha and is predicted to reach nearly 98 t/ha in 2030 (Scenario 1). This average productivity is still much lower than the biological potential of sugarcane, and is limited mainly by severe drought, especially on fields managed for spring harvesting. Management strategies should be implemented to exploit *genotype x environment* to ensure optimize annual productivity.

Selection of new cultivars adapted to *cerrado* areas, is another important procedure when considering upland sugarcane cultivation, which is the most predominant type in Brazil. Sugar content (Pol%) of modern cultivars is about 15%, and it is predicted that this number may reach 16,5% in 2030, to attend demand of mills using conventional system for ethanol production (1st Scenario). So, for developing sugarcane destined for the 2nd Scenario, the main focus of genetic breeding is not sugar production, but higher content of fibers and biomass for electricity production (Energy Mills) and/or for cellulosic ethanol production. This would allow to achieve an increase of about twice of present biomass and fiber production within 20 years, starting from current 81 t/ha biomass and 12% fiber content to future 130 t/ha and 23%, respectively, or and additional 24 t/ha of trash as residues produced from cultivars with 17% or higher trash level (lamina and bundle sheath). This new situation will impact, undoubtedly, on the utilization of new sources of germplasm, different from the currently used by conventional breeding. In this case, a significant shift on the industrial profile of bioenergy cultivars will come about. Such transition period should take place during the next twenty years, as a result of current genetic breeding work.

Another interesting topic is the evolution of primary energy productivity on both scenarios. In the first one, if one projected gains of genetic breeding within the next two decades for cultivars with current non- bioenergy biotypes, it could be able to obtain gains of about 22%. On the second scenario, with the inclusion of new sources of germplasms, these gains are projected to reach up to 95.6%. Naturally, the velocity of scenario

TABLE 1 Critical System requirements (CSR) during the development of sugarcane cultivars under Scenarios 1 and 2, respectively.

CSR Scenario 1	Present (2010)	5 years (2015)	10 years (2020)	20 years (2030)
Culm biomass (t/ha)	81	83	89	98
Straw biomass (t/ha) – 14%	11.34	11.62	12.46	13.72
Total biomass (t/ha)	92.34	94.62	101.46	111.72
Sugar content (Pol%)	15	15	15.5	16.5
Fiber content	12	12	11.5	11
PE fiber (Giga Joules)	175	179.3	184.2	194
PE straw (Giga Joules)	204.1	209.2	224.3	247
PE sugar (Giga Joules)	201	205.9	228.1	267.3
Total PE (Giga Joules)	580.1	594.4	636.6	708.3
*Cost (RV)	100	96	80	69
**Energetic balance	8	9	12	14
Environmental impact	High/medium	Medium/low	Medium/low	Low

Note: estimates are based on upland cane.

* Referent to TCH (Pol and Fiber content are included on yield, since, in practice, they are associated to yield).

** Biofuel energy/invested fossil energy. PE: Primary energy.

CSR Scenario 2	Present (2010)	5 years (2015)	10 years (2020)	20 years (2030)
Culm biomass (t/ha)	81	91	111	130
Straw biomass (t/ha) – 17%	14	16	19	24
Total biomass (t/ha)	95	107	130	154
Sugar content (Pol%)	15	14	13	12
Fiber content	12	14	18	23
PE fiber (Giga Joules)	175	229.3	359.6	538.2
PE straw (Giga Joules)	252	288	342	432
PE sugar (Giga Joules)	201	210.7	238.6	257.9
PE total (Giga Joules)	627.9	728	940.2	1,228.1
*Cost	100	96	80	69
**Energetic balance	8	9	12	14
Environmental impacts	High/medium	Medium/low	Medium/low	Low

* Referent to TCH (Pol and Fiber content are included on yield, since, in practice, they are associated to yield).

** Biofuel energy/invested fossil energy. PE: Primary Energy.

two may be intensified or not, depending on the transformation dynamics of modern industry.

In relation to yield costs, we expect a decrease of about 31% within twenty years for both scenarios.

About environmental goals to be achieved by the yield component, it has to be pointed out that, generally, on qualitative terms (high, medium and low), both scenarios will change from a high/medium to a medium/low situation in twenty years. Elimination of sugarcane burning (predicted to the year of 2014) and, consequently the replacement with mechanical harvesting, will have a positive environmental impact, especially on the second scenario (production of biomass/cellulosic ethanol). In parallel, mechanical harvesting, together with the development of cultivars with better water and nutrients use efficiency will also contribute to preserve natural resources.

Major technology areas

On this section, a synthesis for each of the main technological areas from the Genetic Breeding and Biotechnology component is presented, in which scientific and technological developments are required to achieve the development of sugarcane cultivars for both scenarios.

Germplasm characterization and utilization

Germplasm is the base of all breeding programs, playing a key role in developing new cultivars. World sugarcane cultivation is based on few genotypes, which have intensively been intercrossed, originating hundreds of modern cultivars, determining a narrow genetic basis for this crop. Although some gains were achieved, the increase on productivity is each time more and more restricted, and increments that have been reached may no longer be sufficient to meet sugar and ethanol demand predicted for next decades. For this reason, it is imperative for sugarcane breeding programs to carry out strategies focusing on broadening the genetic base of sugarcane cultivars.

Genetic variability for sugarcane breeding relies on species of the so-called “*Saccharum* complex OR genus”), which includes *Saccha-*

rum officinarum, *S. barberi*, *S. sinensis*, *S. spontaneum*, *S. robustum*, *S. edule* and genera *Narenga*, *Erianthus*, *Sclerostachya* and *Miscanthus*. Through recombination, it is possible to incorporate genetic variability from these genera on breeding programs, promoting a broadening of the genetic base. *Saccharum officinarum* and *S. spontaneum* are the main source of gene for breeding, whereas they were involved in sugarcane origin. *Saccharum spontaneum* represents the major species for breeding on Brazilian conditions, because it has a high biomass production potential, adaptation to several environments and tolerance to pests and diseases. Furthermore, genes can also be introduced from other species of *Saccharum* and related genera, through genic recombination (introgression) or genetic transformation. *Erianthus* shows high potential of producing biomass as a way to overcome restrictions from wild environments; therefore, it may have great potential for energy production. Recently, it has been confirmed the possibility of inter-crossings between *Erianthus* and *S. officinarum*.

A diagnosis of factors that has limited the utilization of new germplasm sources by sugarcane breeding programs identified the following bottlenecks:

- Low representativeness of species from the “*Saccharum* complex” on germplasm collections held by public institutions (IAC and RIDESA). In Brazil, collections well represented are kept by National private institutions (as is the case of CTC) and by multinational companies (i.e., MONSANTO), and therefore, have restricted access.
- Although there is a great variability inside the “*Saccharum* complex”, there is little or no possibility of immediate use of new accessions in an introgression genetic program due to a deficient characterization of materials maintained on the collections. Therefore, genetic variability present on germplasm collections is not exploited for generation of new cultivars.
- The major limitation of an introgression program is the loss on the genetic potential of the material.

- Sugarcane germplasm collections have a relatively high maintenance cost, since they are kept in field. Moreover, many accessions show problems with adaptability, and are vulnerable to a series of biotic and abiotic stresses. Thus, it has been proposed that public programs should join efforts to establish a public germplasm collection, representative of genetic variability existing on the “*Saccharum* complex”. Also, they must establish a program of wide characterization of accessions that they already have, using a robust system where it will be possible to integrate molecular, phenotypic and morphological data. *In vitro* sugarcane germplasm conservation must have special attention, ensuring the replacement of materials that may get wasted on field collections. Molecular markers also have huge potential to make viable the germplasm characterization as well as a program of genetic introgression, because both of them allow breeders to seek entire genic blocks, as well as providing faster gains. However, there is a need to invest on high performance genotyping systems (*high throughput*), substituting obsolete genotyping systems used mainly by public Brazilian sugarcane breeding programs.

Other relevant issue on the development of sugarcane cultivars refers to the behavior that the genotypes face to major diseases. There is a lack of basic studies on pathogens heritage, as well as development of diagnosis systems that may be routinely applied by breeders. Therefore, we reinforce the need for investing on the characterization and evaluation of germplasms, along with the major sugarcane diseases (brown rust, orange rust, leaf scald, ratoon stunting disease, mosaic leaf virus, sugarcane yellow leaf virus). And also secondary diseases that may end up shifting to primary diseases, due to the mechanization trend in planting and harvesting system (pineapple rot and *Fusarium*, among others). Molecular markers and prospection of resistance genes are valuable tools to elucidate these themes.

Regional selection strategies on expanding areas

Areas for expansion of sugarcane cultivation in Brazil are generally “cerrado” (savannah) areas (west and northwest of São Paulo, South of Minas Gerais, eastern Mato Grosso do Sul, north and south of Goiás, Tocantins, south of Maranhão and western Bahia). Although these areas are located in latitudes similar to each other, all of them show special particularities when compared to areas traditionally occupied by this crop. In such regions, many contrasts are observed, for instance, remarkable differences concerning vegetative growth, flowering, sucrose accumulation and responses to pests and diseases, as well as an extremely high water deficit. Sugarcane genetic breeding programs have adopted specific strategies for the obtainment of cultivars adapted to these new production environments, re-directing hybridization and selection processes, and implementing regional experimental stations for selecting more adapted cultivars. Cultivars called as eclectic or as having higher stability must be left out in benefit of specific-adapted regional cultivars, as sugarcane growers have access to new technologies.

In order to have an effective impact of such strategies on the development of new cultivars, some priorities have been pointed out, such as:

- The need for a characterization of natural variability, integrating other areas of knowledge, specially pedology, mineral nutrition, geo-statistics and climatology.
- Analysis of phenotype stability from a group of varietal competition essays. Such procedure is essential for the characterization and regional selection of new cultivars and environments.
- For traits related to yield, an important aspect on regional selection concerns the choice of yield-related traits in “cerrado”. For example, tolerance to drought can be expressed by the rationing ability, lower loss of culm’s weight and absence of flowering. These traits are considered of strategic importance under such conditions.
- For new areas, there is a need to perform a regional selection work, including the utili-

zation in the hybridization phase of germplasm more adapted to restrictive conditions. Therefore, it is necessary to develop numerous studies to investigate tolerance mechanisms present on high-performance genotypes in restrictive environments and also to identify others that may be incorporated on future materials. There is a wide genetic variability in sugarcane in relation to water deficit response. This variability is an important tool for the development of new tolerant genotypes and can be amplified with the inclusion of other species or genera on sugarcane germplasm.

- It is possible to obtain selection gains through better characterization of parents adapted to specific regions, and regional allocation of populations generated from these planned crosses until their final selection and characterization as a regional cultivar. Generally, cultivar allocation has been performed in an inefficient way, due to an incorrect utilization by growers of the characterizations generated by breeding programs.
- One area of future effective contribution will be the development of molecular markers to allow a better selection of superior genotypes for traits that show important interaction, allowing the allocation of genotypes in a specific environment.

Selection for drought tolerance

Tolerance to drought is defined as the ability to minimize losses on production in a water-deficient soil. In plants, mechanisms that lead to tolerance to drought may be divided in two main groups: Escape and tolerance mechanisms. Escape (or avoiding) mechanisms allow plants to avoid losing water on tissues during drought conditions through the maintenance of cell pressure and volume, whether it is by absorbing water using a more abundant root system, or by a reduction on non-stomata's transpiration rates such as leaf cuticle. Tolerance mechanisms enable plants to keep their metabolism even under the same low

soil water potential, especially due to an osmotic adjustment and an antioxidant capacity. Drought escaping mechanisms involve complex alterations on development that require temporal and spatial regulation due to drastic changes that they may cause on plant's metabolism. Therefore, drought tolerance mechanisms are more desirable to plant genetic breeding than escape mechanisms. Although all the advances obtained through conventional breeding, the lack of genetic, molecular and physiologic information on drought tolerance mechanisms as well as their heritage, are all limiting factors for a greater success in generating better cultivars.

Thus, strategies for selecting sugarcane genotypes tolerant to drought have been proposed, such as:

- Promoting the selection of new genotypes in environmental drought-prone areas, characterized by having a serious drought period during the year (that is, to promote selection where this problem occurs).
- Intensifying experimentation (e.g. increase number of repetitions and plot size; use subdivided plots to minimize local effect), when essays are designed for selecting drought-tolerant materials, whereas environment variations under drought condition (mainly from soils), tend to impact negatively on heritability and repeatability from each cycle.
- Intensifying molecular characterization, setting associations between genotypes and phenotypes, so that the possibility of predicting phenotypic performance from genetic information can be exploited during selection. Establishing genetic mapping programs associated to conventional breeding may also lead to the identification and manipulation of genes involved in responses to drought. In addition, prospection of genes of interest to sugarcane breeding (tolerance to drought, sucrose accumulation, lignin biosynthesis etc), must be intensified.
- Exploring the genetic information existing in Sorghum (*Sorghum bicolor*), consider-

ing that it is a model plant, with a complete sequenced diploid genome, and within all the grasses, it is the closest relative to sugarcane (84% homology between genomes). Moreover, it is a drought-tolerant plant, and may be used as a link to many interest traits of genetic breeding.

Molecular markers, genetic mapping and molecular marker assisted selection

The utilization of molecular markers, especially functional markers, may enable the utilization of a fast and efficient technology that would permit a previous selection of seedlings before taking them to the field, known as marker assisted selection – MAS. MAS represents the main contribution of molecular markers on genetic breeding, considering that they promote an increase on selection efficiency as well as reducing costs in a breeding program. As a pre-requisite for MAS it is necessary the existence of an intensive genetic mapping program. However, MAS is not a reality in sugarcane yet, although there are many articles on the identification of molecular markers associated to qualitative and quantitative traits. Major bottlenecks concerning the use of MAS are:

- Speed of analysis (genotyping) used by current systems is too slow.
- There is a need to identify not only one marker, but also the individual haplotype, which will verify the presence of traits of interest in genotyped individuals. Individuals selected by having the marker could then be taken for field evaluations through assisted selection. However, this strategy must involve fast genotyping of thousands of individuals, and would be crucial the use of high-throughput sequencing technology, capable of generating huge quantity of information.
- Sugarcane mapping usually makes use of F1 populations (derived from crossings between two parents) where marker's linkage phase is not known. In comparison to other species, on sugarcane genetic mapping just a part of the data is used for

analysis (genome sampling) where only single-dose markers are used and fitted in actual theoretical models.

- Ligation tests between markers are impaired by the enlargement of the distance between marks, what causes the loss of many existing ligations, which are not statistically detected.
- ESTs-based markers must be prioritized, because even that map position cannot be correctly estimated, these markers show higher probability of being linked to an interest QTL.
- To perform assisted selection it is necessary to know the linkage phases among markers and especially among QTLs. It is also needed to study the interaction between *QTL x environment* and the correlation among traits, and also how to verify if the correlation is due to ligation or pleiotropy and how to predict breeding values.
- On current sugarcane scenario, there is little development of theoretical models for the identification of QTLs, and it is such a complex issue considering the need for estimating linkage phases between markers and QTLs. Generally, models are adapted to accommodate them in more ordinary software.
- Association mapping in sugarcane seems to be a very interesting alternative, although it answers to different questions than those raised when using mapping populations.
- The development of sugarcane's genetic mappings anchored on sorghum's genetic or physic maps will allow for a direct utilization of information about genes derived from sorghum's genome complete sequencing on sugarcane breeding.
- Sequencing not only of the expressed sequences, but getting a draft of the complete sugarcane genome through high-throughput sequencing will provide a better comprehension of the complex polyploidy nature of this crop and will also help on the development of biotechnological systems capable of optimizing genetic breeding.

Therefore, it is necessary to establish multi-disciplinary teams for these topics, having in mind the complexity of such themes and the existing volume of information. There is also a need for investment on genetic mapping programs as well as on the development of software more appropriately designed to analyze sugarcane data.

In conclusion, it is strategically very important for Brazil to be a world reference country not only about sugarcane production, but also on biotechnology research; and for this the country needs the support from universities and public research institutes. Although having a leading position in sugarcane genomics, this could be threatened by the great interest that foreign biotechnology companies have on bioenergy and in the establishment of many bioenergy centers on biomass research, including sugarcane such as EBI in the United States.

Genetic transformation and prospective interest on breeding genes

Significant gains on sugarcane yield were achieved through conventional genetic breeding. However, increases on sugar levels did not occur as the same proportion, indicating that the genetic base used for crossings is not broad enough for this trait. A similar scenario would make viable the development of a genetically-modified plant that overpasses the limits of gains obtained through conventional breeding.

Innovative processes, such as biotechnology, would allow much greater gains, never reached through crossings before as the barrier of sexual compatibility is broken by this kind of technology; and virtually any kind of living organism is capable of supplying interest genes for breeding.

There is a well-established and very efficient sugarcane transformation process that is based on biobalistics. Nevertheless, this system has some limitations, e.g. the insertion of high number of copies of a gene of interest. It becomes necessary to improve the transformation system, as well as implementing new technologies as the *Agrobacterium*-mediated transformation technique, which offers some advantages as a lesser number of copies and a more precise insertion of the transgene.

Add to the development of methodologies for obtaining sugarcane transgenic plants, it is important to seek promoters that show stability of expression, since gene silencing is very frequent on this plant. Basic studies and development of strategies to avoid transgene silencing are of great relevance for successfully applying biotechnology techniques in sugarcane.

The development of commercial transgenic sugarcane is impaired because each transformation event obtained must be individually deregulated. In genetically-simpler crops (soybean and maize, for instance) sexual propagation, trait (or transgene) is transferred to the interest cultivar through crossings, not being necessary a new deregulation of this cultivar. For genetically-complex species, as sugarcane, transference of interest traits through crossings is unviable, since the probability of recovering an initial genotype is nearly zero. Thus, it is necessary to come up with an especial deregulation process that reflects individual features of this important crop. A second commercial sugarcane variety with the same interest feature of another already deregulated, should be submitted to a simplified regulatory process analysis that contemplate only the genetic characterization of GMOs.

Thus, factors that interfere on genetic transformation of sugarcane were discussed and the major aspects pointed out were:

- The use of genetic engineered cultivars presents a great challenge for breeding programs, even though they still have a great opportunity for the obtainment of more efficient and sustainable agriculture.
- The need to improve sugarcane transformation techniques (regeneration, evaluation of event number etc.) as well as the promoter cloning process and the execution of an evaluation of the risks.
- Transformation through biobalistics usually shows high efficiency, however, it needs to be improved to minimize copy number insertion.
- In parallel, it becomes necessary to develop an *Agrobacterium*-mediated transformation protocol in order to increase available

options for obtaining sugarcane transgenic plants.

- Perform basic research on sugarcane gene silencing in order to get a better understanding of the process and try to establish strategies to avoid this problem.
- Invest on GMO development, making advantageous use of public-domain genes as well as using strategies to unravel new genes.
- Make efforts to enact a specific legislation for sugarcane regarding to GMOs, since it is a vegetative-propagated species and deserves special treatment to make economically viable the release of different cultivars with the same interest gene.
- To invest in studies focusing on prospection of genes of interest for genetic breeding, based on high-throughput technologies, capable of generating large quantity of data.
- To establish multi-disciplinary teams, integrated into breeding programs.

TECHNOLOGY DRIVERS

Critical requirements of the system presented on section Critical system requirements – CSR were transferred to technological conductors for each of the main technological areas (characterization and use of germplasm; strategies for regional selection in expansion areas; selection for drought tolerance; molecular markers, genetic mapping and marker-assisted selection; prospection of interest genes for breeding and genetic transformation), identified on section Major technology areas. Goals were presented for each of the technological conductors, and they were referenced to critical requirements of the system that the product or technology must have (see Tables 2 – 4).

The development of more efficient conventional breeding methods, will propitiate an increase on yield and sucrose content. Efficient technologies of inoculation and evaluation of response to important diseases, such as smut, mosaic leaf virus, leaf scald, brow rust, orange rust and sugarcane yellow leaf virus as well as exotic diseases

(Fiji, Sereh etc.) are fundamental to ensure yield. In the same way, it is also needed a better comprehension of genetic heritage of these diseases in order to define selection strategies.

For the development of cultivars focused on a greater fiber content (scenario 2), studies on the characterization of cell wall components, evaluation of fiber quality and allied to the quantification of genetic variability of germplasm available for such traits, are of major importance. Such studies could identify the most suitable type of biomass for 2nd generation ethanol.

The adoption of a quick analysis system for fiber content through NIR (*Near infra-red*) technologies will allow for a correct and efficient selection of seedlings.

The development of genetically modified cultivars that contain genes promoting enzymatic hydrolysis would permit a faster and lower-cost fiber processing. Biotechnology must contribute to fiber quality (composition) which may be altered through the manipulation cell wall composition genes (celluloses, hemi-celluloses etc.).

Conventional genetic breeding may have its efficiency increased through the incorporation of molecular markers (marker assisted selection). Moreover, it is imperative to reiterate that both scenarios will become reality only if investment on R&D is given priority.

GAPS AND BARRIERS

This section identifies current and future gaps, and barriers on technological conductors of the section Technology drivers. Thus, we present herein abilities and knowledge that, both breeding and biotechnology programs must be able to develop and implement, to develop new cultivars. Thus, one can indicate strategic decisions that should be taken by the government concerning generation of new knowledge:

- There is a deficiency in raw material (germplasm) used for genetic breeding. Thus, it is necessary to incentive the introduction and characterization of germplasm, as well as promoting the evaluation of natural resources available to traits that are of

TABLE 2 Technology drivers of CSR yield (To simplify, Pol requisite and fiber content were incorporated on yield).

CSR	Yield (TCH) Scenario 1	Present	5 years	10 years	20 years (Vision)
	Biomass production	79	83	89	98
	Content sugar (Pol%)	15	15	15.5	16.5
	Content fiber	12	12	11.5	11
Technology area	Characterization and use of germplasm				
Indicator	<i>Use level</i>	Low	Medium	Medium/ high	High
Technology area	Strategies for regional selection in expansion areas				
Indicator	<i>Gains in terms of productivity (%)</i>	0	5	12	20
Technology area	Selection for drought tolerance				
Indicator	<i>Gains in terms of productivity (%)</i>	0	8	18	25
Technology area	Prospection of interest genes for breeding and genetic transformation				
Indicator	<i>Cultivated area with transgenic cultivars (%)</i>	0	3	10	40

CSR	Productivity (TCH) Scenario 2	Present	5 years	10 years	20 years (Vision)
	Biomass production	79	91.3	111.25	130.34
	Content sugar (Pol%)	15	14	13	12
	Content fiber	12	14	18	23
Technology area	Characterization and use of germplasm				
Indicator	Fiber quality evaluation	Low	Medium	Medium/ high	High
Technology area	Strategies for regional selection in expansion areas				
Indicator	<i>Gains in terms of productivity (%)</i>	0	5	10	15
Technology area	Selection for drought tolerance				
Indicator	<i>Gains in terms of productivity (%)</i>	0	8	15	40
Technology area	Prospection of interest genes for breeding and genetic transformation				
Indicator	<i>Cultivated area with transgenic cultivars (%)</i>	0	1	5	20

Obs.: actually, it is expected that the development of indicators for common areas are similar. In the case of fiber cane, the use of wild germplasm will possibly confer to the new clones a higher yield potential in drought-prone lands.

greatest interest for traditional breeding (Scenario 1) or for biomass (Scenario 2), ending up with an evaluation of responses against major diseases.

- There is a lack of interest among breeding groups to share genetic material. Also, public programs are in a disadvantageous situ-

ation concerning the availability of genetic resources. Thus, it would be interesting to establish a public use germplasm collection, as well as sharing germplasm in non-competitive phases among breeding programs.

- There is also a lack of training of human resource on the different approached areas.

TABLE 3 Technology drivers of CSR “cost”.

CSR	Cost Scenario 1	Present	5 years	10 years	20 years (Vision)
Technology area	Characterization and use of germplasm				
Indicator	Cost (reference = 1)	1	1	0.85	0.75
Technology area	Strategies for regional selection in expansion areas				
Indicator	Cost (reference = 1)	1	0.95	0.85	0.75
Technology area	Selection for drought tolerance				
Indicator	Cost (reference = 1)	1	0.90	0.80	0.65
Technology area	Prospection of interest genes for breeding and genetic transformation				
Indicator	Cost (reference = 1)	1	0.8	0.50	0.10

CSR	Cost (US\$/t) Scenario 2	Present	5 years	10 years	20 years (Vision)
Technology area	Characterization and use of germplasm				
Indicator	Cost (reference = 1)	1	1	0.85	0.75
Technology area	Strategies for regional selection in expansion areas				
Indicator	Cost (reference = 1)	1	0.95	0.85	0.75
Technology area	Selection for drought tolerance				
Indicator	Cost (reference = 1)	1	0.90	0.70	0.50
Technology area	Prospection of interest genes for breeding and genetic transformation				
Indicator	Cost (reference = 1)	1	0.8	0.50	0.10

- Although a large number of labs carry out research in sugarcane biotechnology, there is a lack of cooperation among groups and also a connection among basic and applied research (genetic breeding).
- Although breeding programs have incorporated molecular markers for numerous purposes, it is necessary to use more efficient genotyping systems, so that Brazil can effectively implement breeding through marker assisted selection.
- There is also a shortage of human resources and infrastructure in research labs and experimental stations in public programs.
- There is a lack of suitable software for genetic analysis in polyploids. Models cur-

rently utilized in sugarcane were developed for diploid species, and show little efficiency in sugarcane. Investment on the development of specific software for polyploids should bring major advances on the comprehension of sugarcane genetics.

FINAL CONSIDERATIONS

Brazilian sugarcane breeding programs play a key role on the establishment of a new energy matrix derived from sugarcane, considering that they are responsible for the development of cultivars, which consists in the major technological input to energy generation. This way, it is necessary to re-orientate processes involved in breeding,

TABLE 4 Technology drivers of CSR "environment".

CSR	Environmental impacts Scenario 1	Present	5 years	10 years	20 years (Vision)
Technology area	Strategies for regional selection in expansion areas				
Indicator	<i>Reduction of inputs (%)</i>	1	0.90	0.85	0.80
Technology area	Selection for drought tolerance				
Indicator	<i>Water use efficiency (%)</i>	1	0.87	0.77	0.68
Technology area	Prospection of interest genes for breeding and genetic transformation				
Indicator	<i>Reduction of inputs (1)</i>	1	1	0.90	0.60

RCS	Environmental impacts Scenario 2	Present	5 years	10 years	20 years (Vision)
Technology area	Strategies for regional selection in expansion areas				
Indicator	<i>Reduction of inputs (%)</i>	1	0.90	0.80	0.70
Technology area	Selection for drought tolerance				
Indicator	<i>Water use efficiency (%)</i>	1	0.95	0.68	0.50
Technology area	Prospection of interest genes for breeding and genetic transformation				
Indicator	<i>Reduction of inputs (1)</i>	1	1	0.90	0.60

as well as the implementation of adequate and efficient tools.

The use of new germplasm sources constitute one of the major bottlenecks on the development of biomass cultivars, emphasized by the low representativeness of species with high potential for bioenergy in germplasm collections from public programs, as well as by the precarious characterization of accesses maintained on germplasm collection from different Brazilian programs. Also, for traditional breeding, where main focuses are sugar-rich cultivars, a narrow genetic base derived from the low number of clones used for breeding, has been a limiting factor for the increase on sugar content in modern cultivars. The implementation of a parallel genetic introgression program, making use of new sources of germplasm for sugar, fiber, biomass, biotic and abiotic stresses, will definitively bring a major contribution to increases yield, ensuring a more sustainable cultivation of sugarcane in Brazil. In conventional breeding, we must seek for the development of new selection

methods and strategies that explore genetic variability on genetic breeding programs.

Resistance to diseases is the chosen method to control most of sugarcane pathogens, and is considered as an elemental trait on the development of new varieties, but has been superficially broached by Brazilian programs. Thus, it is imperative to emphasize the development of methodologies more efficient to inoculate and evaluate responses against important diseases such as smut, mosaic, leaf scald, rust and yellow leaf as well as other exotic diseases (Fiji, Sereh).

Biotechnology offers important tools for breeding programs, whereas molecular markers have been usefully utilized to the determination of the genetic distance between genitors, germplasm characterization (identifying accesses of interest for breeding), besides enabling a speed up on time for developing new cultivars and allowing the selection of genes of interest for a genetic introgression progress. However, systems currently used by Brazilian breeding programs,

mainly public, are obsolete and little productive requiring a lot of work and workforce. There is a necessity to incorporate more efficient genotyping systems, capable of generating large amounts of data in a shorter period of time. In addition, statistical methods for analyzing molecular data also show bottlenecks that need to be overcome, since they were developed for diploid species. Therefore, investment on software development for molecular data analyses are required together with the training of human resources.

Transgeny provides a promising strategy either to broaden genetic variability of sugarcane,

or to introduce traits of interest. However, transformation techniques have some limitations that need to be overcome. Thus, investment is necessary on the development of a efficient system for sugarcane genetic transformation, in order to make transgenic breeding feasible. It is also needed to reduce technological dependence that is controlled multinational companies (e.g. through patents and royalties). For all of these reasons, we must prioritize the unraveling of new genes, as well as making good use of those from the public domain. Finally, it is necessary to introduce specific legislation for genetically modified sugarcane.