BBEST Conference 2011 – Tutorials

Tutorial 2 – Sugarcane Breeding and Agricultural Practices

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An Overview of Sugarcane Production

The tutorial presents and discusses the methods used to obtain genetic improvements through the history of sugarcane breeding such as ancient selection, "traditional plant breeding" and molecular breeding techniques. Focus is placed first on crops other than sugarcane that have received much greater research effort. Parallels are drawn between advancements with those crops and what might be done for the future improvement of sugarcane. It also discusses subjects related to sugarcane production mainly those that are going through some kind of scientific or technological transition or have potential for it such as field planning, soil conditioning as well as harvesting and planting farming approaches.

1. Sugarcane Breeding: Past, Present and Future

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Past success in plant breeding made sugarcane and the three major cereals (maize, wheat, and rice) the world's most productive and most consumed crops. However, to meet the needs of a rapidly growing population and decreasing natural resources, it is critical to accelerate the rate of developing sustainably higher yields of all major crops. Accelerating increases in yields with fewer resources will require the development and use of increasingly efficient breeding methods.

From the few continuous records available, it appears that the productivity of major crops has increased more than 10-fold since they were first domesticated 8,000 – 4,000 years BCE. These dramatic increases arose from (1) accumulated knowledge about agronomic practices that manage the environment (E) to ameliorate the conditions that would otherwise limit the productivity of the crop, and (2) plant breeding practices to improve the genotype (G) of the crop so it is less impacted by "yield limiting" environmental conditions. In other words, genetically raising the crop yield potential.

The methods used to obtain genetic improvements have evolved over time and can be characterized as belonging to one of three overlapping technological periods (Eras 1, 2, and 3) based on the methodologies used to achieve these gains. Beginning from the time of earliest crop domestication (approximately 10,000 years ago) through today, breeders have used the methods of Era 1, which consist of visual selection of the best phenotype for domestication followed by selection of the best performing offspring of the domesticated lines for cultivation. Era 2, the beginning of scientific breeding, followed the discovery in 1900 of Gregor Mendel's earlier plant breeding experiments with peas. Mendel's research led to formulation of the fundamental laws of genetics, which, coupled with replicated progeny testing, contributed to understanding that an organism's traits, i.e. its phenotype (P), are the result of interactions between its genes (G) and environment (E), that is: P = G x E. Analyses of the genotype led to methods for calculating the breeding value of parental lines and greatly accelerating genetic gains. Era 3, the advanced science of molecular crop breeding, is based on a bottom-up approach using some type of proxy marker for indirect selection of the genetic determinates of the phenotype. Era 3 became possible following the discovery of the structure of DNA in 1953 and subsequent development of improved methods for DNA analyses. However, breeding applications from this information did not become practicable until the 1990s with the development of faster and cheaper technologies for producing and utilizing large numbers of markers from DNA sequences.

The history of sugarcane breeding, although not as well documented as that of other major crops, has provided improved varieties through the same three eras - - 10,000 years of domestication and visual selection, 100 years of traditional breeding, and 10 to 20 years in developing the data needed to begin molecular breeding. Yield gains made during Era 1, the era of visual selection, were based on mankind's ability to select mutants or natural occurring hybrids having higher sucrose content. Results during this era gave rise to the domesticated species, *S. officinarum*, *S. sinense*, and *S. barbari*, from the wild species *S. robustum* and *S. spontaneum*. Gradually, sugarcane industries were established based on the three ancient domesticated *Saccharum* species. With the advent of Era 2, science-based breeding, improvements were based on man's intentional hybridization and backcrossing to capture additional traits, primarily for increased pest, pathogen, and environmental stress tolerance.

Here, one should note that the scientific breeding methods of Era 2 have been more difficult to apply to sugarcane than other crops because the sugarcane genome underwent two rounds of genome wide duplication approximately 1.5-2 million years ago which created in autopolyploids with large genomes and many chromosomes. Hybrid sugarcane cultivars are aneuploids adding yet another layer of complexity to genetic and genomic analysis. This genomic complexity of sugarcane means that we do not know the number of alleles of any genes, nor their linkage to a particular chromosome. For this reason, we are unable to apply Mendelian genetics for phenotype characterization; however, we are able to use population genetics to determine the environmental and genetic components of yield and the breeding value of parental lines. Our inability to apply diploid Mendelian genetics to sugarcane makes it a massive task to directly select an improved phenotype from tens of thousands of progeny. Therefore, a common characteristic of sugarcane breeding programs around the world is their massive scale.

The purpose of today's presentation is to review methods, accomplishments, and limitations of past plant breeding efforts and then to introduce some evolving newer methods that are beginning to succeed and will likely be the basis for future gains. I will focus first on crops other than sugarcane because most of them are genetically simpler and have received much greater research and development effort than sugarcane. I will then draw parallels between advancements with those crops and what we might do for the future improvement of sugarcane.

Rates of improvement in crop yields have been extremely slow when using only the methods of Era 1. For example, over the 1,000 years between 800 and 1,800 the yield of rice in Japan increased by 1.1 tons. Although this increase represents a doubling of yield, it took so long to achieve that the annual rate of increase was a mere 0.19% per year. The same slow rates of yield increases were duplicated over centuries in other crops, including sugarcane.

The methods of Era 2 have been highly successful over the last 100+ years. They are sometimes referred to as "traditional plant breeding" to distinguish them from the ancient selection of Era 1 and the molecular breeding techniques employed in Era 3. Traditional plant breeding includes: (a) developing inbred lines that when crossed produce superior hybrids, (b) repeatedly backcrossing to introgress specific traits into improved lines, and

(c) using embryo rescue to obtain wide-cross interspecific hybrids (occasionally producing a totally new species such as the cereal triticale). During the brief 100-year period of Era 2, rice yields in Japan increased by 3.2 tons, or at an annual rate of 2.33%, which is more than 12-fold faster than the gains made during Era 1. Again, Era 2 methodologies resulted in similar rapid gains in maize, wheat, sugarcane, and other crops.

The methods of Era 2 for moving desirable traits into improved varieties are complex, imprecise, time-consuming, and labor-intensive, in part because they involve mixing whole genomes. Mixing whole genomes to obtain a particular genetic combination can require very large progeny populations (tens of thousands) tested over many years (10-20 or more), and multiple environments. Nevertheless, the increase in crop yield rates during Era 2 has been so phenomenal that it has been termed a "green revolution" that has staved off starvation in the poorer elements of the world's burgeoning population. Unfortunately, these yield gains achieved in part through increased inputs are not sustainable, may be slowing, and may not be sufficient for future needs. Therefore, alternative approaches are needed.

The earliest molecular methods used for producing improved phenotypes were the creation of mutants through radiation breeding (notable successes include Calrose rice and Ruby Red grapefruit) and the addition of unique traits through genetic engineering (notable successes include herbicide and insect resistance). These methods began with the promise of being more rapid and precise than traditional breeding because they do not involve mixing of whole genomes. However, this means that their application is limited to a very small set of traits for which we have cloned genes, none of which includes increased yield potential. In addition, barriers arose to their public acceptance including: (1) consumer and public concerns about environmental and food safety issues, (2) availability of genes, equipment, personnel, and technology, (3) intellectual property rights, (4) and costs associated with research, commercialization, and the regulatory process. These barriers are best resolved by 'Big Ag', i.e. major seed companies who hold the intellectual property rights and control the market for transgenic germplasm. Plant breeders must depend on additional molecular approaches of Era 3 to increase yield potential.

An addition to visual selection of the phenotype for discovering the underlying molecular basis is to analyze the genome to develop markers as a proxy for the desired phenotype. Molecular markers of various types (RFLPs, RAPDs/AFLPs, SSRs, SNPs, and BACs) can be applied in plant breeding and selection through genome mapping, association mapping, and marker assisted selection (MAS). Mapping with molecular markers has revolutionized the construction of high-density genetic-linkage maps that identify loci, both major genes and QTLs that are linked to traits of interest such as higher yields and increased resistance to pests, pathogens, and environmental stresses.

MAS has many potential advantages over phenotype selection: (1) it can be simpler and faster, especially for traits that involve laborious phenotype screening because the phenotype is expressed only at certain times of the year, or in plants at a specific developmental stage; (2) it can be done on seedlings and thus require less time and space than selection on mature plants; (3) it is not affected by the environment so that it is more reliable; and (4) it can discriminate between homozygotes and heterozygotes for direct selection of individual plant genotype instead of only the dominant phenotype.

MAS also has limitations: (1) in order for markers to be useful, they must be tightly linked (preferably on both sides) to the genes responsible for the trait of interest so a lot of markers are needed, and (2) most agronomic traits of interest are not due to a single, or even just a few, major genes, but are the result of interactions of a suite of genes scattered throughout the genome and expressed under environmental regulation. These two limitations can be overcome by saturating the genome of interest with a very large number of markers. However, simultaneous selection by breeders for a large number of molecular loci is infinitely more difficult and costly than selection for only one or a few. Most MAS research efforts have been directed towards identifying specific loci or genes that exert maximum effect on the desired phenotype.

However, to do this one needs (1) a low-cost method for developing and analyzing the markers and (2) extensive, replicated evaluations of the trait of interest to link as many markers as possible to the phenotype expressed under a wide range of environmental conditions. These two limitations are so great that most research papers published on MAS are really just progress reports on developing the technology rather than reports of successful implementation of MAS for the production of an improved plant variety. A notable exception includes the development of soybean cyst nematode resistant lines. This success had not been previously possible because of the difficulty in conducting repeatable and reliable nematode-effect yield trials. The soybean nematode resistance success required many years and hundreds of thousands of dollars with work in several laboratories, even though the mechanism for resistance is a major gene which makes this the simplest case possible. More recently there has been success in developing higher yielding and improved quality tomatoes and maize by combining a large number of QTLs into select lines. In these cases the effort required has been at least an order of magnitude higher than with soybean. The lesson here is that at this time, a great deal of effort is required to successfully implement MAS on even the most highly characterized crops. Nevertheless, the potential for MAS is so great that we can expect to see large research efforts directed towards implementing molecular breeding.

MAS is projected to be involved in producing our next wave of improved crops.

An alternative to MAS is to apply molecular markers through association mapping that identifies genetic loci associated with phenotypic trait variation. Association mapping shares much in common with QTL mapping, but is based on unstructured populations (genealogy) instead of the structured populations of QTL analyses. The use of unstructured populations in association mapping means that they represent many more recombination events and are often many generations from a common ancestor, providing the potential of a greater resolution for a set population size. Advances in genome sequencing technology for the production of large quantities of molecular marker genotyping data favors association mapping over traditional QTL mapping and is thus likely to become more common.

In sugarcane currently, considerable resources are being allocated towards developing data (molecular markers, linkage maps, and haplotype sequence analyses) needed to facilitate MAS and association mapping. Genetic maps, although all incomplete, have been published from ten segregating sugarcane populations. Follow up mapping of QTLs has been done on some of these populations for sugar content, sugar yield, disease resistance, and physiological traits contributing to yield. A large collection of expressed sequence tags (ESTs) has been generated and applied for SNP mining, gene expression profiling, and gene discovery. Sequence haplotypes represented by sugarcane BACs have demonstrated a high level of gene retention and gene structure through sequence conservation. Although these advances contribute significantly to our plant science knowledge about sugarcane, we have thus far been unable to use them to improve our crop. At this stage it is impossible to know which of these approaches will progress sufficiently to assist sugarcane breeders. To this plant physiologist, the task of applying molecular breeding to sugarcane improvement looks daunting, but with the generation of ever faster and cheaper sequence data analyzed by a new population of bright young scientists devoted to sugarcane, I am confident that these efforts will succeed. However, I must add a cautionary warning noting that "success can be achieved only through increased collaboration between plant breeders and plant scientists, with each group having a better understanding of the other's expertise." – I hope this will be achieved within my lifetime.

2. Sugarcane Agricultural Practices

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2.1 - Field planning

Sugar industries are a complex integrated system involving the planting, harvesting, transportation, milling and marketing sectors (Higgins and Muchow, 2003). Agricultural planning is an important tool to increase profitability. Two important factors having impact on profitability are the optimized management of the harvest and transport operations taking into consideration cane quality through the production season. Even though it is not possible to harvest all fields at maximum sugar content it is possible to maximize total sugar from the production area. The planting schedule should distribute varieties and production environments taking into consideration ripening performance and transport distance to the mill of each environment. Varieties having early, mean and late ripening performance should be included in the field planning program. Early ripening varieties placed at longer distances from the mill and late varieties planted close to the mill help to compensate the lower capacity of the field operations under unfavorable weather conditions.

Working capacity of transport and harvesting equipments depend on three main efficiencies related to field layout, logistics and maintenance. Logistics is usually the one requiring most attention. Maintenance planning for transport and harvesting infrastructure are also important in order to keep the performance estimates used for field planning. The available transport capacity must be allocated to short, medium and long distant harvesting areas in order to be able to process all fields through the dry and wet periods of the season. Multiple harvesting areas being harvested simultaneously are required to manage weather and cane quality conditions. Not less important are the industry constrains related mainly to the quantity and quality of raw material to be delivered considering that in the early season canes have lower sucrose content so higher tonnages are crushed. The milling capacity determines the length of the milling season and also interacts with the performance of the transport and harvest infrastructure considering that reduced capacity will push the field operations to the initial and final periods of the season where weather conditions are less favorable.

This complex arrangement of variables can benefit from the use of operations research to optimize plantation management instead of the more frequently used empirical management procedures. Reliable yield and cane quality predicting models are required to optimize harvest scheduling (Scarpari and Beauclair, 2004). Digital planning tools contribute to reduce the need of long experienced field managers, especially when significant areas of sugarcane expansion are under way. One major challenge of agricultural planning is related to scheduling of transport and allocation of the fields to be harvested so as to keep continuous flow of cane to the mill using a fully occupied infrastructure for harvesting and transport. Monitoring tools such as GPS are an alternative to simulation to help in determining the best route in real time.

2.2 Land preparation

Proper soil conditioning is essential for good establishment and growth of the crop. Traditionally soils have been harrowed or ploughed to a depth of 0.25 m to control weeds, pests and improve physical condition. Several additional tillage operations are frequently used to crush the soil clumps so as to make it soft and friable. As environmental legislation enforces green cane harvesting in Brazil mechanical harvesting grows rapidly and trash blanketing becomes a farming approach not well compatible with conventional tillage. The adoption of trash blanketing returns organic matter to the soil, improves structure and nutrient status, as well as reduces the cultivation and erosion losses. It may also contribute for rationalization of herbicide usage, and efficiency of water use. However, many of the soil improvements arising from trash blanketing may take several crop cycles to show up. Even though trash blanketing has positive effects on sugarcane agriculture it brings along mechanical harvesting and heavy traffic over the soil surface in such a way that tillage is needed at every planting cycle. Sub-surface hard pans or compaction is a frequent phenomenon in heavy mechanization. It increases bulk density which in turn reduces porosity, water infiltration rates and the soil storage capacity as well as increases impedance to root proliferation. Shallow root system makes the plant susceptible to droughts, which is a major factor for productivity. The conflicting factors just described point in the direction of the need for a land preparation approach, specific for sugarcane, that will be able to take advantage of the benefits of trash blanketing reducing the negative impacts.

2.3 - Traffic reduction for sugarcane mechanization

The positive impacts expected from trash blanketing and no-till farming bring up the challenge of reducing equipment traffic to much lower levels that presently practiced. Some partial solutions are been tested such as minimum tillage and controlled traffic, but they are not yet fully compatible with the no-till farming concept. No-till farming has been very successful in grain agriculture over the last 30 years and seems to be a natural trend for sugarcane if adequate solutions are found to maintain physical conditioning and pest control of the soil. Sugarcane root development and vehicle wheels require widely opposite soil physical conditions for optimum performance. Soil compaction induced by wheels is a form of physical degradation in which biological activity and agricultural productivity of the soil are reduced.

The use of wide span axles for mechanization vehicles combined with controlled traffic is a practical way of separating the wheeled areas from the cropped (zero traffic) areas in a field. A vehicle specially designed for sugarcane production is being developed by the Brazilian Bioethanol Science and Technology Laboratory aimed at a trafficked area reduction from the present level of 60%, attainable with conventional farm tractors and harvesters, to near 10 % of the planted area. Several 5 to 12 m span vehicles (gantries) have already been used throughout the world for research on soil compaction, Chamen et al.,1994, but little effort has been focused specifically on sugarcane.

2.4 Planting

As technological changes take place in sugarcane agriculture, such as the planting and harvesting processes that are shifting from manual to mechanical solutions a careful review of the planting process is required to reduce negative impacts of the new coming technologies. Plant spacing and seed quality are among those factors that require special attention. Under favorable soil fertility and climate conditions sugarcane can reach higher productivities when planted in narrower row spacing. However commercial fields are planted in rows spaced near 1.5 m just to fit available tractors and harvesters that have a wheel track larger than 1 m.

Sugarcane is planted from vegetative stalks. They are cut into seed pieces called "setts" from which sugarcane propagates. Setts contain two or three buds that sprout under favorable soil, water and temperature conditions and give rise to primary stalks. Bud germination lasts around 30 days and starts 7 to 10 days after planting. Tillering takes place from about 40 days to almost 120 days after planting. Though 6 to 8 tillers are produced from a bud, only 1 to 2 tillers remain to form millable stalks. Even though the early formed tillers are likely to result in heavier stalks the competition process will not necessarily give better surviving chances to the older ones. This tiller establishment process based on competition is associated with two negative factors: time is lost for developing of millable stalks and plant spacing becomes randomly distributed far from an ideal precision planting. Equidistant positioning of plants seems to be the most adequate way to maximize the amount of light reaching the base of the sugarcane plant which is a determining factor for the good tillering required to reach a good stalk population. The most frequently used inter-row spacing is above 1.5 m normally combined with intra-row spacing below 0.5 m, it is far from what could be considered equidistant positioning of plants that would be the adequate way to reduce competition for light, water and nutrients.

The second factor requiring attention is seed quality. Setts should be obtained from a 7 to 8 months seed crop, free from disease and pests. Seed stalks should be cut without damage arising from harvesting and handling in order to get healthy buds. In spite of the fact that good quality setts have paramount effect on crop establishment the seed quality handled by available processes for mechanical planting are far below the optimum.

Setts are placed into the soil through a planting operation that can be manual, semi mechanized or totally mechanized. In the semi mechanized approach seed cane is handled manually but the furrow is opened and closed mechanically using tractor mounted implement. Labor evenly distribute whole stalks at the bottom of the furrows with little mechanical damage and cut them into setts before covering. As labor becomes scarce the planting process in Brazil is shifting from the traditional semi mechanized approach to the totally mechanized one using billet planters. Buds are damaged during the mechanical harvesting, handling and planting operations to the point that 18 to 20 tons of seed cane are required per ha as compared to 10 to12 tons of seed cane per ha required for manual planting. As green cane is implemented mechanical harvesting is required to be able to handle leafy cane at competitive cost. As machines replace manpower during the harvesting season the labor cycle is broken and no manpower is left for planting. It can be concluded that mechanical planting is a natural follower of mechanical harvesting what turns the planting operation a critical factor among the agricultural practices.

2.5 - Harvesting solutions;

Sugarcane harvesting is a significant economical challenge when compared to other crops. Between 30 to 40% of the agricultural cost is related to harvesting and transport. About 400 tons per ha of raw material are to be handled over a full cycle between two successive crop renewals. It means that heavy equipment must be used for sugarcane different from tractors and harvesters used for grain crops that handle less than 10 tons per ha-cycle. On the other hand the total area under sugarcane worldwide is around 3% of the area devoted to grains. It means that little effort could be expected from equipment manufacturers in the way of developing solutions tailored to sugarcane.

Several harvesting approaches have been adopted through the last 50 years in different areas of the world, such as Australia, Cuba and the USA. Most areas are presently converging to the billet type harvesting system even though is not a sustainable solution from the stand point of soil conservation, cane losses and level of extraneous mater collected with the stalks. Adequate solutions are required aiming reduced traffic as well as new harvesting principles capable to process crops progressively more productive and able to collect good quality stalks, leaves and tops with losses well below present levels.

Recommended Readings

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