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Sugarcane

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21 Sugarcane

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21.1 INTRODUCTION

Sugarcane is a tropical grass of the Poacea family and *Saccharum* genus and comprises several species although plants presently cultivated are mostly hybrids derived from *S. officinarum*, *S. spontaneum*, *S. robustum*, *S. sinnensis*, and *S. barberi* (Figueiredo 2008). Sugarcane is originated from Asia; the exact center of origin is unknown, but evidence points to Polynesia, New Guinea, India, China, Fiji Islands, and Tahiti. There are reports that *S. officinarum* was known in 6000 BC in India (Daniels et al. 1975). Sugarcane was already cultivated in the Middle East before the Christian era. Later the Arabs introduced the plant in Europe and from there it spread to America and parts of Africa in the early fifteenth century with the start of colonization (Figueiredo 2008).

Sugarcane is highly efficient in biomass accumulation. Its C₄ carbon metabolism allows for increased photosynthesis at high temperatures and efficient carbon assimilation, which leads to the highest yields produced among grasses. In fact, the “C₄” combination of biochemical and morphological specializations was discovered in sugarcane (Kortschak et al. 1965; Hatch and Slack 1966). Nowadays, sugarcane is grown in more than 100 countries (FAOSTAT 2009), mostly between the parallels 35°N and 35°S, covering an area of about 22 million ha and with a yield of approximately 1.6 billion tons of cane (Table 21.1), which represents 0.45% of the world’s agricultural area and 1.6% of the arable area, with Brazil, India, and China topping the list. Data of 2007 show that these countries account for almost 63% of the world’s cane production; the corresponding figure for Brazil alone is 33% (Table 21.1). In the last 20 years, sugarcane production grew approximately 57% worldwide. In Brazil, the sugarcane production will reach 664 million tons in 2010/2011.

Among the world’s four most productive crops—rice, wheat, maize, and sugarcane — sugarcane produces the greatest crop tonnage and provides the fourth highest quantity of plant calories in the human diet (Ross-Ibarra et al. 2007) even though each of the major cereals occupy a severalfold larger fraction of the world’s arable land. The stalks of most commercial varieties contain 10–16% fibers and 84–90% juice. The latter contains 75–82% water and 18–25% soluble solids of which the greater part (15–24% of the sugarcane juice) is sucrose; 1–2.5% are nonsugars such as amino acids, fatty acids, waxes, and mineral components (Stupiello 1987). Therefore, 1 t of sugarcane yields approximately 130–170 kg of sucrose.

Sugarcane is nowadays a source of food, feed, biofuels, and bioelectricity. Soon, biopolymers will also be added to the product list. Sugarcane ethanol is produced through the fermentation of
Sugarcane sugar. Sugarcane partitions carbon into sucrose that accumulates in the internodes to up to 50% of its dry weight (0.7 M) (Moore 1995). Such high capacity of accumulation in stalks is unique among plants. Most of the ethanol produced in the world derives from plant juices containing sucrose from sugarcane in Brazil and starch from corn in the United States (EIA 2008; UNICA 2009). The United States and Brazil are the top producers of ethanol in the world. The production of bioethanol from sugarcane syrup is well established and nowadays is good enough to provide a product that follows market specification for fuels. The whole process may be considered very robust and does not compete with the food chain because in most of the industrial units the bioethanol is produced with depleted syrup because of simultaneous sugar production. However, in Brazil, where more than 50% of the sugarcane is directed to ethanol production, the whole syrup may be fermented. In addition, there are industrial plants devoted just to ethanol. A close look at the whole production structure allows identification of possible scenarios in which part of the sugarcane bagasse may also be used for bioethanol production when the cellulosic route is sufficiently developed.

### TABLE 21.1
Cane Production of the 20 World Largest Sugarcane Producers in 1987 and 2007

<table>
<thead>
<tr>
<th>Rank in 2007</th>
<th>Country</th>
<th>Cane Production (Mt)</th>
<th>Variation 1987–2007 (%)</th>
<th>Harvested Area (1000 ha)</th>
<th>Average Yield (t/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1)</td>
<td>Brazil</td>
<td>268.5</td>
<td>91</td>
<td>6712</td>
<td>76.6</td>
</tr>
<tr>
<td>2 (2)</td>
<td>India</td>
<td>186.1</td>
<td>91</td>
<td>4900</td>
<td>72.6</td>
</tr>
<tr>
<td>3 (4)</td>
<td>China</td>
<td>52.8</td>
<td>101</td>
<td>1236</td>
<td>86.1</td>
</tr>
<tr>
<td>4 (11)</td>
<td>Thailand</td>
<td>24.4</td>
<td>163</td>
<td>1010</td>
<td>63.7</td>
</tr>
<tr>
<td>5 (6)</td>
<td>Pakistan</td>
<td>43.6</td>
<td>26</td>
<td>1029</td>
<td>53.2</td>
</tr>
<tr>
<td>6 (5)</td>
<td>Mexico</td>
<td>45.9</td>
<td>10</td>
<td>680</td>
<td>74.5</td>
</tr>
<tr>
<td>7 (9)</td>
<td>Colombia</td>
<td>25.0</td>
<td>60</td>
<td>450</td>
<td>88.9</td>
</tr>
<tr>
<td>8 (10)</td>
<td>Australia</td>
<td>24.8</td>
<td>45</td>
<td>420</td>
<td>85.7</td>
</tr>
<tr>
<td>9 (7)</td>
<td>United States</td>
<td>26.5</td>
<td>5</td>
<td>358</td>
<td>77.6</td>
</tr>
<tr>
<td>10 (13)</td>
<td>Philippines</td>
<td>17.2</td>
<td>47</td>
<td>400</td>
<td>63.3</td>
</tr>
<tr>
<td>11 (8)</td>
<td>Indonesia</td>
<td>26.1</td>
<td>–4</td>
<td>350</td>
<td>72.0</td>
</tr>
<tr>
<td>12 (12)</td>
<td>South Africa</td>
<td>21.0</td>
<td>–2</td>
<td>420</td>
<td>48.8</td>
</tr>
<tr>
<td>13 (14)</td>
<td>Argentina</td>
<td>14.5</td>
<td>33</td>
<td>290</td>
<td>66.2</td>
</tr>
<tr>
<td>14 (19)</td>
<td>Guatemala</td>
<td>6.9</td>
<td>174</td>
<td>225</td>
<td>83.6</td>
</tr>
<tr>
<td>15 (16)</td>
<td>Egypt</td>
<td>8.4</td>
<td>92</td>
<td>136</td>
<td>119.6</td>
</tr>
<tr>
<td>16 (23)</td>
<td>Viet Nam</td>
<td>5.5</td>
<td>193</td>
<td>285</td>
<td>56.1</td>
</tr>
<tr>
<td>17 (3)</td>
<td>Cuba</td>
<td>70.8</td>
<td>–84</td>
<td>400</td>
<td>27.8</td>
</tr>
<tr>
<td>18 (17)</td>
<td>Venezuela</td>
<td>8.0</td>
<td>16</td>
<td>125</td>
<td>74.4</td>
</tr>
<tr>
<td>19 (20)</td>
<td>Peru</td>
<td>6.8</td>
<td>21</td>
<td>68</td>
<td>121.7</td>
</tr>
<tr>
<td>20 (24)</td>
<td>Sudan</td>
<td>4.8</td>
<td>58</td>
<td>72</td>
<td>104.2</td>
</tr>
<tr>
<td></td>
<td>Sum/average</td>
<td>887.6</td>
<td>61</td>
<td>19,564</td>
<td>72.9</td>
</tr>
<tr>
<td></td>
<td>World</td>
<td>990.3</td>
<td>57</td>
<td>21,977</td>
<td>70.9</td>
</tr>
</tbody>
</table>


*In parenthesis is rank in 1987. Harvested area and yield data refer to 2007.*
In Brazil, bioethanol production from sugarcane is expected to reach 28.5 billion L in 2010/11. Almost 70% of the ethanol produced is hydrated (5.6% water/volume) and 30% anhydrous. Hydrated ethanol is used in automotive vehicles equipped with engines that run exclusively on ethanol or flex-fuel vehicles. Anhydrous ethanol is mixed with gasoline. Several countries are adding ethanol to gasoline to reduce fossil fuel consumption, increase octane rating and reduce pollution. This trend started in Brazil in 1931 and legislation that establishes a mixture of up to 25% of ethanol in gasoline dates to 1966.

Nowadays almost all of the 35,000 gas stations in Brazil have a hydrated ethanol pump (E100). Initially, cars were designed to run on either gasoline or ethanol. Now, flex-fuel cars are available that run on any mixture of ethanol to gasoline (from 0 to 100%). In 2008, flex-fuel cars represented 82% of the new cars sold, totaling around 23% of the automotive fleet in Brazil (around 6 million units). Motorcycle engines are already available that uses ethanol. New progress on combustion engines is underway to allow buses to also run on ethanol. This is greatly advantageous because the production and use of bioethanol as opposed to gasoline and diesel reduces greenhouse gas (GHG) emissions by 90%. This has been calculated on the basis of the whole sugarcane cycle including planting, harvesting, processing, and transporting of the fuel. And, because sugarcane is a renewable source of energy with a rapid growth and up to six annual harvests without the need for replanting, high dry matter yield per unit of fertilizer applied, minimal needs for pesticides—which require great quantities of fossil fuel for their production—and high CO$_2$ fixing capacity, the use of its ethanol can mitigate global warming.

The success of a biofuel crop is based on its economical and environmental advantages. To achieve sustainability, energy crops should not require extensive use of prime agricultural lands and they should have low-cost energy production from biomass. Basically, the crop energy output must be more than the fossil fuel energy equivalent used for its production. Studies have shown that the output to input ratio of sugarcane first-generation ethanol production is approximately 8–10, compared with 1.6 for maize (Goldemberg 2008). Several crops are being tested for bioethanol production, which can also be produced from starch and sugars from maize, wheat, sugar beet, cassava, and others, but they rarely reach two units of renewable energy produced relative to each unit of fossil fuel energy used. In 2007, the production and use of bioethanol in Brazil reduced GHG in 25.8 million t equivalents of CO$_2$. This corresponds to 360,000 diesel buses/year (Goldemberg 2008).

After juice extraction, the sugarcane stalk residue (bagasse) can be burned in the sugarcane factories for production of steam and electrical energy. Bioelectricity is the most important new product of the sugarcane business. All mills and distilleries in Brazil are self-sufficient in electric energy through co-generation. Approximately one third of the energy is stored in the cane juice, one third in the bagasse, and one third in the trash. Until recently, most of the sugarcane was burned in the fields to facilitated manual cutting, but increased pollution and decreased the energetic efficiency because the straw and leaves were not used for energy generation. Presently, in the state of São Paulo, responsible for 60% of the sugarcane production in Brazil, almost 50% of harvesting is mechanized without burning. Regulation determines that burning should be prohibited by 2014. This trend is spreading to other regions as well.

Bagasse is burned in highly efficient boilers (over 60 bars) that will allow for surplus energy to be commercialized in the electric grid. In 2008, sugar and ethanol mills produced an average of 1,800 MW. With the more efficient boilers being implemented and new investments in co-generation an estimated 11,500 MW can be produced, which is equivalent to 15% of the electricity demanded by Brazil. Bioelectricity brings several advantages, including low environmental impact, carbon credits, and the need for relatively small and low-risk investments. In Brazil, bioelectricity brings an additional advantage because sugarcane harvest takes place mostly in the dry season when hydroelectric mills are at their lowest production.

Alternatively, ethanol can be produced from bagasse and trash lignocellulose by hydrolysis of the cell wall using enzymes, physical, and chemical treatments (Ragauskas et al. 2006). The processing
of lignocellulose and sugar conversion into ethanol is not yet economical but its development is highly desired because it could lead to an increase of 40–50% in ethanol production. Potentially, with the industry of cellulosic ethanol it is expected that the ethanol output might increase from the current 7,500 to 13,000 L/ha. Sugarcane second-generation ethanol has not yet been used commercially but many R&D initiatives are underway.

To improve yield and other traits of interest that will allow for a sustained industry of sugarcane and for the development of an energy-cane, research groups in biotechnology, transgenics, sugarcane genomics, statistical genetics for polyploid genomes, and gene discovery are gathering efforts all over the world to devise the mechanisms involved in the regulation of sucrose content, yield, drought resistance, biomass, and, more recently, cell wall recalcitrance. It is important to note that the whole sugarcane genome sequence is unknown and that sugarcane has a highly polyploid giant genome (~10 Gb). Recent works on expressed sequence tags (EST) added value to this crop’s genomics but whole genome sequencing efforts are underway that will make available chromosomal gene structures and allelic variations of sugarcane. The SUCEST (a project that has generated the largest collection of ESTs—http://sucest-fun.org) database consists of 33,620 putative transcripts with a sequence mean size of 864 bp (Vettore et al. 2003) which represents about 30 Mb of sugarcane genome sequence, a small fraction of the complete genome sequence. Only with the recent developments of next-generation sequencing technologies has the identification of genes, alleles, and promoters as well as the definition of the overall structure of the genome been made possible. Also, if sugarcane is to be improved for bioenergy production, a significant number of cultivars and genotypes need to be evaluated at the biochemical and physiological level and molecular biologists have to join efforts with breeders bringing biotechnological tools to the game. Although a lot is known about plant cultivation, the biochemical and genetic characterization of this crop are at early stages. The remainder of this text will focus on the different aspects of bioethanol production using this crop and research developments for the improvement of sugarcane.

### 21.2 SUGARCANE CULTIVATION

Optimum temperature for sugarcane cultivation is between 30 and 34°C. Plant growth is greatly reduced below 21°C for most varieties. However, in the maturation stage, sucrose accumulation is triggered by dry conditions or low temperature, usually with average temperatures below 20°C. Death of leaves may occur below 2.5°C and apical and lateral buds die at −1 to −3.3°C and −6°C, respectively (Alfonsi et al. 1987; Liu et al. 1999). Usually at least 900–1000 mm of rain are necessary for rain fed production (Inman-Bamber and Smith 2005) but the need of irrigation depends also on rain distribution along the season.

Sugarcane is a semi-perennial bushy plant, in which several long stalks germinate from rhizomes or stools. Long alternated leaves are attached to the stalk nodes. The cylindrical stalks may reach 2–5 m tall and accumulate sugars mainly in the internodes. The world average stalk yields are around 70.9 t/ha but this varies with soil, climate, cycle length, and growing conditions (Table 21.1). Under favorable conditions, yield may reach above 200 t/ha but the theoretical yield according to different authors varies from 285–470 t/ha per year (Landell and Bressiani 2008; Waclawovsky et al. 2010).

In commercial fields sugarcane is planted with stem cuttings (seed cane) instead of seeds. Usually 8–12 t of 8- to 10-month-old stalks containing 12–18 buds per meter of row are planted in furrows spaced at 0.8–2.0 m. Small farmers adopt a narrow spacing whereas wider spacing is used in mechanized fields (Anjos and Figueiredo 2008).

The harvest takes place 10–24 months after planting. After harvest the plant sends up new stalks or ratoons that will be cut again usually within one year. Normally, yields decline in subsequent ratoons but two to ten cuttings can be performed before the crop needs to be planted again, depending on the variety, climate, pest and disease incidence, soil type, and management conditions.
Sugarcane is relatively tolerant to soil acidity but limestone application is recommended when soil pH is below 5.5. Because of the high dry matter yields, fertilizer needs of sugarcane are relatively high. The nutrient content of shoots of a crop yielding 100 t of stalks are around 100–154 kg N, 15–25 kg P\(_2\)O\(_5\), 77–232 kg K\(_2\)O, and 14–49 kg S (various authors, compiled by Raij et al. 1997; Cantarella et al. 2007; Rossetto et al. 2008). Fertilization of sugarcane varies widely depending on the country, soil type, and yield potential. Sugar crops account for 7.5 million t of the NPK fertilizers used in 2007–2008, representing 4.5% of the world fertilizer consumption (Heffer 2009). It can be assumed that most of this fertilizer goes to sugarcane because sugar beet cropping area corresponds to 0.11% of that of sugarcane worldwide (FAOSTAT 2009). In Brazil 23% of the N, 8.7% of the P, and 21% of the K fertilizer are used in sugarcane (FAOSTAT 2009). Nitrogen and potassium are the nutrients used in largest amounts. Rates of application of N vary from 60 to 200 kg/ha (Raij et al. 1997; Rice et al. 2006). For P, up to 150 kg/ha P\(_2\)O\(_5\) may be applied in low fertility soils but rates may be much smaller in many regions (Hartemink 2008). The sugarcane plant extracts large quantities of potassium and fertilization may reach, in kg/ha K\(_2\)O, 150 in Brazil and Australia, 175 in Costa Rica, 280 in the United States (Legendre 2001; Rice et al. 2006; Rossetto et al. 2008b).

Despite the high internal requirements of nutrients by sugarcane, the actual chemical fertilizer demand may be low because of recycling of plant and industrial residues because the mills and distilleries export basically carbon, hydrogen, and oxygen in sugar and ethanol. For instance, filter cake, which is generated at a rate of 30–35 kg (18–21 kg dry matter) per tonne of crushed fresh stalks, contains 1–3% P\(_2\)O\(_5\) and is returned to the fields in natural form or composted with bagasse. Vinasse, the fluid residue of ethanol fermentation-distillation, is produced at a rate of 10–15 L/L ethanol and contains an average of 2 g K\(_2\)O/L. Vinasse is applied at rates varying from 50 to 200 m\(^3\)/ha and may supply all of the K needs of the crop that receives the residue. Ashes produced in the furnaces contain several nutrients, including micronutrients, and are also recycled in the fields.

The relatively low amounts of N used in Brazil is taken as evidence that biological nitrogen fixation (BNF) may play a role in nutrition of sugarcane (Urquiaga et al. 1992, 1995; Boddey et al. 2003). Furthermore, Urquiaga et al. (1992) showed that 60–70% of the N accumulated in some sugarcane varieties came from BNF. However, studies carried out in Australia and South Africa failed to show that BNF was a significant source of N to sugarcane (Biggs et al. 2002; Hoeftsloot et al. 2005). Recent findings indicate that selected diazotrophic bacteria may effectively increase yield and supply N for sugarcane (Oliveira 2006; Reis et al. 2008). Although BNF offers a promising way to decrease sugarcane dependence of N mineral fertilizers, much work remains to be done.

Nutrient recycling in the field can also be improved if leaf burning that precedes harvesting is avoided. Manual harvesting that prevails in most of the world, especially in developing countries, is made easy by burning the crop but nutrients such as N and S are lost by volatilization and others are spread away with the ashes. When sugarcane is harvested unburned either manually or with a combine machine, a thick mulch of leaves and tops, equivalent to 8–20 t/ha of dry material remains on the soil, recycling nutrients and organic matter to the soil.

### 21.3 SUGARCANE PESTS AND DISEASES

A number of pests and diseases attack sugarcane. Among the insects the sugarcane borer (Diatraea saccharalis) is the most common, and may affect yield and quality of stalk because of the invasion of fungi and bacteria through the holes opened by the borer. The control of sugarcane borer is carried out with integrated pest management (IPM) that involves scouting of the insect population and biological control with one of the various natural enemies including Cotesia flavipes, Lydella minense, and Paratheresia claripalpis. Insecticides are rarely needed.

Pests that live in the soil, roots or stubble include the spittlebug Mahanarva fimbriolata that sucks the plant, the beetle Migdolus, and several species of termites. The control involves monitoring of infected areas to assess damage levels, management practices such as mechanical destruction
or burning of stubbles, and use of chemical products when necessary. For *M. fimbriolata* biological control with *Metarhizium anisopliae* is also an alternative (Dinardo-Miranda 2008a).

Nematodes of the genera *Meloidogyne* and *Pratylenchus* also attack sugarcane. Resistant varieties usually are not an efficient way to control nematodes but management practices such as crop rotation when sugarcane is replanted, and application of organic matter help to decrease the population of nematodes. In areas where the attack is intense chemical control may be necessary (Dinardo-Miranda 2008b).

Fungi diseases that affect sugarcane include rust (*Puccinia melanocephala*), smut (*Ustilago scitaminea*), eye spot (*Bipolaris sacchari*), red rot (*Colletotrichum falcatum* or *Glomerella tucumanensis*), pokkah-boeng (*Fusarium*), and pineapple disease (*Thielaviopsis paradoxa*). The most important method of controlling fungi diseases is through the use of resistant varieties. Additional measures, which are effective with some diseases, include roguing of diseased stools, burning or plowing out trash or stubbles, and heat treatment of cane seeds (Santos 2008).

Resistant varieties are also the most important method of control of bacteria diseases such as ratoon stunting (*Clavibacter xyli*) and leaf scald disease (*Xanthomonas albilineans*), or viruses such as sugarcane mosaic virus and yellow leaf disease. Periodic surveys of nurseries and heat treatments may also be useful to prevent the spread of some diseases (Almeida 2008; Gonçalves 2008).

Despite the great number of pests and diseases that affect sugarcane, chemical control is applied in less extent than in many other crops because of the effectiveness of biological control methods and especially of resistant varieties. Fungicides and bactericides are seldom used in sugarcane.

### 21.4 SUGARCANE PHYSIOLOGY

In spite of the fact that sugarcane became one of the main sources of sugar and ethanol in the world, its physiology has been poorly studied in relation to other grass species. Sugarcane plants can be obtained from seeds, germination can be easily obtained in vitro and the structure of the seeds and seedlings of sugarcane is typical of other grasses. Seedlings can be an excellent model to study and understand several aspects of gene expression, biochemistry physiology. For biotechnology purposes though, sugarcane seeds are not useful because what we call sugar cane is not a single species, but a polyploid hybrid. This makes the seed method of reproduction inappropriate for crop cultivation. Crops are planted from clones of designed varieties using stem cuttings. The same plant stays in the field for an average of 5 years. Yields decline with age and after a while it becomes uneconomical so the crop must be replanted. One of the most important physiological events related to sugar production is the drought stress that occurs in wintertime. From the agricultural viewpoint, it is well known that the stress is necessary to induce senescence (by ethylene) of the top shoot of the plant and the consequent storage of sucrose in the stem. Accumulation of sucrose is related to photosynthesis, sink-source relationships, flowering, and water stress. Water stress depends on the level and rate it developed. When applied slowly it leads to developmental changes such as a reduction in leaf expansion and the closing of leaf stomata. Photosynthate translocation is not reduced until the stress becomes severe. Combined, the effect of drought is to lead to accumulation of carbohydrates in the leaves and in storage sinks of the sugarcane plant (Hartt 1936). The accumulation of sucrose in storage parenchyma of sugarcane is called ripening. Ripening is caused by the gradual decrease of tissue moisture, reduction of cell expansion, and the formation of new internodes without much inhibition of photosynthesis. Reduced consumption of sucrose for metabolic energy and new cell formation leads to increased sucrose content (Gosnell and Lonsdale 1974). Sugarcane managing includes the use of drought and growth inhibiting stresses to ripen the crop before harvest.

Sugarcane photosynthesis is based on the so-called Kranz syndrome anatomy. Instead of using the C₃ pathway system, in which CO₂ is converted directly by the enzyme ribulose bisphosphate carboxylase (RUBISCO) into organic acids containing three carbon atoms, sugarcane leaves...
incorporate CO$_2$ first into a four-carbon atom compound by a different enzyme (phosphoenol pyruvate carboxylate—PEPc). That is why sugarcane is referred to as a C$_4$ photosynthesis plant. This four-carbon compound (phosphoenol pyruvate) is subsequently processed to produce CO$_2$ in another cell type (the cells of the bundle sheath). The process occurs in the leaves in a much higher pressure of CO$_2$ than in the outside air, and proceeds through the biochemical pathways of C$_3$ photosynthesis, providing sugarcane with an extremely efficient system that processes light and CO$_2$ for production of sugars. This photosynthetic system occurs especially in species that live in environments where there is abundance of light and relatively higher temperatures. The C$_4$ photosynthesis system was discovered in 1966 in Australia in leaves of sugarcane (Hatch and Slack 1966). Later on, other scientists found that plants like maize also display this type of metabolism.

The control of sugar metabolism in sugarcane is associated with the plant hormone ethylene. This hormone cross-talks with several other hormones and also affect the nitrogen metabolism. It is likely that a complex system including multiple genes controlled by environmental factors (mainly water stress), plant hormones that lead to changes in plant metabolism, and ecophysiology (e.g., photosynthesis) are related to the level of production of sucrose and subsequently to biomass and bioethanol.

Physiologically, the accumulation of sucrose in stems of sugarcane appears to be directed for flowering. In 1998 Carlucci et al. reported results of a cultivar (IAC 52–150) growing in Piracicaba, SP, Brazil, that flowers if grown under long days (sugarcane flowers with a 12- to 12.5-h photoperiod). Induction of flowers occurred in March, when humidity was high and flowers started to develop in May, reaching up to 70 cm in June. Flowering initiation is thought to have its optimum between 18 and 31°C. In fact, the difference between maximal and minimal temperatures is crucial. Pereira et al. (1986) and Carlucci et al. 1988 found that flowering was intense when this difference was of 10°C and that flowering did not happen when the maximal−minimal temperature difference was on the order of 14°C. According to these authors, the combination of extreme temperature differences with water stress during the flower induction period negatively affects flowering, retarding or preventing it completely. Thus, flowering seems to be controlled by a combination of factors including temperature and water. Because of that, sugarcane plants hardly flower in the Southeast of Brazil (ca. 24° of latitude), where most of the sugarcane crops are planted. On the other hand, this is probably the reason why a period of water stress is desirable for high accumulation of sugar. The stress is likely to delay flowering initiation, but not induction, which probably leads to a change in the pattern of source-sink relationship, provoking an accumulation of sugar to prepare the plant for flowering. Therefore, avoiding or delaying flowering is very important from the agricultural viewpoint as the sucrose that is stored in the parenchyma cells of the stems is the reserve of carbon that the plant will use to produce the flowers. Thus, by harvesting, farmers intervene in the flowering process, preventing the plant from using the sucrose stored for that purpose and extracting it for sugar or ethanol production. The lack of low temperature or water stress becomes critical when plants are cultivated in the Amazon, in the North of Brazil because it makes it difficult for most varieties to accumulate sucrose; therefore, that region has no favorable conditions to grow sugarcane.

21.5 SUGARCANE GENOME AND GENOMICS INFRASTRUCTURE

Genomics is increasingly recognized as a powerful approach to address scientific questions in biology. It establishes the nucleotide sequence of an organism and as a result enables gene content prediction. The adaptation of an organism to the environment, its performance and its phenotype are a result of multiple gene products interactions. Moreover, knowledge transfer from one model organism to another of yet less information is made possible with comparative analysis. Sugarcane biology will greatly benefit from nucleotide sequence determination, as it will foster a systems biology approach by understanding genome structure and regulatory networks. The challenge in determining the sugarcane genome sequence is the complexity of its genome structure as a polyploid and
understanding the balance between alleles. Sugarcane cultivars are known to be a hybrid resulting from crosses between two polyploid genomes \( S. \) *spontaneum* \((x = 8; \ 2n = 40–128)\) and \*S. officinarum* \((x = 10; \ 2n = 80)\). Modern cultivars are polyploid and aneuploid which renders allelic variation/assortment a key aspect in breeding programs (D’Hont 2005; D’Hont et al. 1996).

Collectively, the Saccharum complex is diverse in genome content and organization. Sorghum, Miscanthus, and Erianthus are closely related species and represent genetic reservoirs for exploitation of genetic diversity. Molecular phylogenetic studies within the Saccharinae group indicate that Miscanthus sensu stricto and Saccharum are sister groups, while Sorghum and Erianthus share a close relation. Monophyly supports Miscanthus and Saccharum relation, but a distinct clade named Miscanthidium is identified (Hodkinson et al. 2002).

An International Genome Sequencing Initiative has recently been formed to produce a draft sequence from several sugarcane cultivars so that tools are developed for understanding genome ploidy variation, enabling gene discovery and generating a knowledge base molecular infrastructure (http://bioenfapesp.org). Basic research will benefit not only from gene discovery but also from the identification of regulatory sequences involved in sucrose metabolism, carbon partitioning in the plant and responses to restrictive water supply. Breeding programs will have access to the development of new molecular markers. The sugarcane monoploid genome is estimated to be about 1 Gb, comparable in scale to the human and maize genomes. The ground basis to tackle the sugarcane genome are available resources such as the EST collections (see below), array hybridization profiles generated by SUCEST-FUN (described below), a collection of bacterial artificial chromosome (BAC) clones from R570 cultivar, and the recently released Sorghum genome (Paterson et al. 2009). Genetic maps are currently being improved by the inclusion of repetitive sequences such as microsatellites and resistance gene analogs (RGAs) (Rossi et al. 2003) thus, increasing resolution. Another class of repetitive sequences is transposable elements (TEs), which are composed in sugarcane by a heterogeneous universe of molecular entities previously described by Araujo et al. (2005). TE selected BAC clones have been sequenced and specific insertion polymorphism studies provide information concerning their association to genetic diversity. The ultimate goal in generating the sugarcane genome sequence is to contribute with a large scientific community effort to improve sugarcane breeding and develop a systems biology-based approach in sugarcane. Initially, shot-gun sequencing and a draft assembly of 1000 BAC clones will provide resources for basic biological processes including access to promoter regions and the possibility of comparative studies among grasses and, specially, cultivars of interest. Furthermore, the sequencing initiative is expected to provide tools for the identification of functional modules of gene variation.

The sugarcane directed genome sequence will provide valuable tools for understanding genome polyploidy variation when compared to Sorghum and other Poaceae species (Miscanthus, Erianthus, and Oryza). Sugarcane is a domesticated crop that originated from New Guinea (Asia) about 6000 AD. No more than a hundred years ago modern cultivars have been produced from the cross of two closely related species and have progressively replaced the Noble clones spreading in all of the sugarcane producing areas of the globe. Since then, breeding programs are devoted to addressing the main questions that any crop under a heavy agricultural system is subjected to: growth habit and harvest index; adaptation to photoperiod; resistance to diseases and abiotic stresses (mainly water supply and temperature), flowering and genetic erosion (loss of variability to adapt). Sugarcane varieties are basically maintained from vegetative propagation of selected clones. These clones would in principle keep their agricultural traits. A sugarcane cultivar issued from a breeding program is productive for approximately 15 consecutive years after which cultivar replacement is needed because of loss of quality traits most probably because of genetic erosion and/or instability or changes that occur in pest and disease agents to overcome plant resistance.

The genome of hybrids is highly polyploid and aneuploid (Grivet et al. 1996). Efforts on mapping genes and molecular markers to generate physical maps have been described but because of the genetic complexity of sugarcane, its genome is poorly understood (Ming et al. 1998; Hoarau et al. 2001; Lima et al. 2002; Pinto et al. 2004; Garcia et al. 2006; Raboin et al. 2008). Modern
sugarcane cultivars are complex interspecific hybrids with a chromosome number ranging from 100 to 130, of which 15–25% comes from *S. spontaneum*. Considering monoploid genomes, the DNA content is ~930 Mb for *S. officinarum*, ~750 Mb for *S. spontaneum* and approximately 1000 Mb for sugarcane hybrids (D’Hont 2005). At Clemson University Genomic Resources a BAC library prepared from the R570 sugarcane cultivar is available and is represented by 103,296 BAC clones with an average size of 130 kbp (CUGI 2009). This library has been screened for resistance gene analogs (A. D’Hont, personal communication), *adh* locus (Janoo et al. 2007), sorghum euchromatic regions (A. Paterson, personal communication), transposable elements and genes associated to sucrose content and drought responses. These trends render it feasible to undertake a pilot project to sequence the sugarcane genome and address questions related to gene allelic variation and regulatory regions.

A combined approach of new sequencing technologies such as 454 pyrosequencing and Sanger reads will give access to the sugarcane genome sequence uncovering the genetic basis structure sustaining the biological processes. Not only will regulatory regions associated to specific genes of interest be discovered but also gene prediction models compared to sorghum, rice and maize. Breeding strategies can benefit from the comparative genome sequence of homologous regions between R570 and SP80-3280 (the cultivar most represented in the EST collections), thereby allowing for rapid translation of the sequence data into genetic markers. Regulatory sequence variation is also to be uncovered through this comparative approach and, in combination with the expression profile analysis, relevant insights on the evolution of these regions and the contribution of transposable elements will come to light. In a broader view, BAC sequencing will add to the understanding of chromosomal differentiation among Poaceae.

A long-standing goal in polyploid genomes is to understand the relative contribution of each allele to a particular phenotype in a given cultivar. The key problem in achieving this goal is identifying allelic variation and subsidizing breeding programs to quickly select it from among a segregating population. Also, accumulating multiple genes into plant varieties is not yet widely used for polyploid plants. Because of the lack of understanding of the genetic basis of heterozis, genome sequencing of “gene of interest” containing regions will advance knowledge on genome structure creating the molecular basis to explore the genetic diversity among cultivars and breeding populations segregating for characters of interest.

One innovative approach is to understand the relative contribution of TEs to genetic variation in the sugarcane polyploid genome. These mobile elements are ubiquitous among living organisms and constitute intermediate-repeat DNA long considered as selfish (or junk) DNA (Doolittle and Sapienza 1980; Orgell and Crick 1980). Contrary to that, and as previously proposed by McClintock (1984), a new biological concept is arising for transposable elements where, despite their mutagenic capacity, they actively contribute to changes in the gene expression profile and may ultimately result in species divergence (Cordaux et al. 2006; Jordan 2006; Cropley and Martin 2007; Xiao et al. 2008). Their contribution to eukaryotic genome structure is usually associated with gains of nuclear interspersed sequences such as noncoding repetitive DNA between and sometimes within coding units. One means of understanding the contribution of a particular class of TE to the genome is to first identify the gene pool present, its relative amplification across contrasting varieties and its expression pattern. Much of the initial work will be to create a ground basis for identifying TE families associated to particular traits (brix, drought, high CO₂ environment, and regeneration capability). This recognition will impact on balancing selection for (or against) the presence of a given TE family. Clearly, a prerequisite in sequencing polyploid genomes is to be familiarized with its repetitive DNA elements. For sugarcane, 21 families have been identified and further studies were carried out on two retrotransposons (*Hopscotch*-like and SURE) and two transposons (*Mutator*-like and hAT-like). *Hopscotch*-like and *Mutator*-like elements contain lineages that are represented by highly repeated unit spread along the chromosomes with no particular clustering evidenced at telomeric or centromeric regions. Independent of their amplification profiles both high and low copy number elements are expressed in different tissues of sugarcane.
21.6 SUGARCANE BREEDING

There are basically six species within the *Saccharum* genus: *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*). Because genomes of all of these species may be involved in some form of modern cultivars, sugarcane is considered to have one of the highest genetic complexities among cultivated species.

The genetic breeding of sugarcane in Brazil and worldwide has been explained in great detail in several articles and books (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; Matsuoka et al. 1999a, 1999b; Landell and Alvarez 1993; Landell and Bressiani 2008). The present text will concentrate on the work of Matsuoka et al. (1999a, b). Matsuoka et al. (1999a) showed in detail how sugarcane breeding began in Brazil in the nineteenth century. The first reports that sugarcane seeds (not the stem) could result in offspring occurred in Barbados in 1858 (Deerr 1921; Stevenson 1965). However, it is presumed that the breeding actually started in 1885 in Java, from the germination of *S. spontaneum*. But a few years earlier in Brazil, Peixoto Lima (1842) had stated in thesis defense that sugarcane would reproduce from seeds obtained by crossing (i.e., by sexual reproduction). This fact, combined with several others, seems to indicate that Brazil was among the pioneers in getting new commercial varieties from seeds.

From the beginning of the twentieth century, there was an increasing concern about the poor sugarcane productivity as well as the increase of pests (Deerr 1921; Aguirre Jr 1936; Edgerton 1955; Dantas 1960; Stevenson 1965; Andrade 1985). As a result, there was an intense exchange of germplasm between different countries, the farmers being primarily responsible for this (Matsuoka et al. 1999a). However, at times when the producers felt pressured by crises in the sector, there were initiatives toward the setting up of experimental stations (Geran 1971).

At present, Brazil has four main sugarcane breeding programs: (1) the sugarcane program from the Agronomic Institute of Campinas (IAC), which started in 1933; (2) the RIDESA Interuniversity Network Sugarcane Genetic Breeding Program, made up of federal universities that started in 1971 as PLANALSUCAR; (3) the Sugarcane Technology Center (CTC), which began work in 1968 as COPERSUCAR; and (4) Canavialis, which began in March 2003 and was recently acquired by Monsanto. Syngenta also started a sugarcane breeding program in Brazil.

Despite there being large differences in the details about how the breeding programs in Brazil (and the world) carried out their activities, there are some points in common which will be highlighted here. In essence, the breeding is based on the selection and cloning of superior genotypes in segregating populations, which are obtained from the sexual crossing between different individuals. The success rate of these processes depends on several factors including the adequate choice of the parents to maximize the chance of response to selection; use of adequate experimental designs; and the correct choice of the traits to be evaluated. Most of the traits considered in the selection process have a quantitative nature and are controlled by quantitative trait loci (QTL), such as soluble solid rate; sucrose content; diameter and number of stalks; fiber content; flowering; precociousness; resistance to pests and diseases, etc.

Each year, breeding programs generate segregating populations formed by thousands of seedlings. The number of seedlings varies according to the program and depends on economic and technical factors. These segregating populations are then submitted to selection in different schemes, which are presented below.

21.6.1 GENERATION OF VARIABILITY

The genetic variability available for selection comes from sexual crossing and can be done in different ways (Matsuoka et al. 1999a, 1999b): (1) biparental crossing, in which crossings are made using two known parents, some of which may be used exclusively as a female; (2) polycrossings,
when many genotypes are intercrossed, in which case seeds are gathered from the inflorescence of all parents involved, which prevents the identification of the pollen source; and (3) free pollination, in which seeds are harvested from plants growing freely.

Crossings should be planned in such a way as to maximize the probability of genotype selection that can be released as commercial cultivars. Thus, an alternative that is greatly used is to choose parents with good economically interesting performance traits (Matsuoka et al. 1999a), which naturally occurs for commercially used cultivars. It is noteworthy that this can lead to a narrowing of the genetic basis (Lima et al. 2002).

Because sugarcane is a type of allogamy, the crossings should be planned so as to avoid the occurrence of interbreeding between relatives. This can be achieved based on the genealogies of materials as well as genetic divergence obtained with molecular markers (Lima et al. 2001). Other criteria should also be used such as trait complementarity; ability to combine material during the crossings; and the capacity for each material to produce good populations throughout time.

Some authors mention the use of estimates of the genetic parameter named heritability that in its restricted form takes into account the sexual phase. This can be considered in the prediction of crosses, because heritability indicates how the interest traits are transmitted to the offspring. Some results indicate that the predominant gene action in the Brix content is additive, whereas for the other components the output is not additive (Hogarth 1980; Wu et al. 1980). Hogarth (1977) cites that for stem production, the dominant variance has shown the same magnitude as the additive, where the epistatic variance is predominant for stem weight. Hogarth (1987) showed high heritability values for the fiber content trait. For volume and number of stems, the dominant variance proved to be important (Hogarth et al. 1981). Bressiani (1993) reported high heritability values for length of stem and Brix in Brazilian conditions. For rust, the heritability has been high (Hogarth et al. 1983; Bressiani and Sanguino 1994), which also usually happens with other diseases (Matsuoka et al. 1999a), indicating that the selection of parents may be resistant effective. However, there are examples of transgressive segregation.

### 21.6.2 Initial Stages of Selection

There are many variations on how the breeding programs develop this stage, especially in terms of selection rates, group size, number of locations, and replications (Simmonds 1979; Skinner et al. 1987; Matsuoka et al. 1999a). In general, a low intensity selection is applied in the early phases, selecting only for traits with high heritability. This is followed by an increase in selection intensity as the experimental precision increases, and cultivation recommendation only when there are many experimental results in different places and years of cultivation.

Broad sense heritability, which should be considered in these phases, has several different values reported in literature. For example, in relation to vigor, some studies indicate low heritability, although several breeding programs have obtained good results with intense selection for this trait (Skinner et al. 1987). These authors report different heritability values for various traits, such as sugarcane yield; Brix yield per hectare; number and diameter of stems; and resistance to rust and smut. These results suggest that in early stages selection is more effective for Brix and for resistance to rust and smut, but these data should be interpreted with caution given the low number of available estimates (Matsuoka et al. 1999a).

Another very important feature to be considered in these phases is the genotype regrowth ability. Despite the small number of available estimates in this respect, many programs consider this aspect in the selection process (Giamalva et al. 1967; Watkins 1967; Giroday 1977; Mariotti 1977; Bond 1978; Skinner et al. 1987; Arizona 1994; Matsuoka et al. 1999a), which can lead to many genotypes in the early phases being discarded.

Several selection strategies for these steps have been used and are briefly described below.
• **Bunch planting:** According to Ladd et al. (1974), this is a recommended method when there is a large number of individuals from a previously untested crossing, because the simultaneous evaluation of many stems in the same area is possible. Normally, 5–10 seedling bundles are planted (Mangelsdorf 1953) and it is expected that natural selection eliminates the inferior ones, based on competition (Urata 1970), although this seems questionable, because it is not known whether this may actually occur in heterogeneous bunches. Matsuoka et al. (1999a) pointed out that the main disadvantages are the inability to select more than one stem per bunch, as the mixture prevents individual identification and makes it difficult to assess tillering, which is positively correlated with budding and yield. Skinner et al. (1987) argue that there are examples of success of the bunch planting, although there are no conclusive data about its superiority.

• **Family selection:** It is based on the fact that for the important traits, the heritability between families is higher than the heritability between individual plants. As a result, several breeding programs prefer to carry out the selection between the families, choosing those with higher phenotypic averages. Note that this procedure also serves to identify superior crosses (Skinner et al. 1987). Matsuoka et al. (1999a) mentioned that this procedure can lead to the disposal of superior individuals in families with a low average, but with high variance.

• **Selection of individual plants:** Many breeding programs carry out this type of selection, using only the traits of high heritability as a criterion in this phase, such as Brix and resistance to flowering and disease. Latter (1964) mentions that in some cases this selection strategy leads to better results than the selection between families and also reduce the risk of discarding superior individuals in families with a low average. According with Matsuoka et al. (1999a) the economic feasibility of assessing each plant individually, rather than evaluating a large number of seedlings should be taken into consideration. In practice, the individual evaluations are usually possible, because in the early phases of selection many genotypes are discarded visually based only on their vigor (Skinner et al. 1987; Matsuoka et al. 1999a).

### 21.6.3 Evaluation of Clones in Field Trials

In this phase, the genotypes selected are compared based on experiments using appropriate statistical designs. Because of the high number of genotypes being evaluated and the small number of stems to be used in experiments, the plots are usually small, e.g., a 5-m-long row per replication, place and time of evaluation. It is very common in this step to use increased complete randomized blocks (Federer 1956). Other traits that were not evaluated in the previous phase are now considered, and assessments of the regrowth capacity are also commonly made. Because of limited experimental precision, the values of heritability are still low and therefore the selection should not be too intense (Matusuoka et al. 1999a). Many sugarcane breeding programs in the world have applied approximately 10–30% intensities (Skinner et al. 1987).

### 21.6.4 Field Trials for Precommercial Genotypes

The clones selected as above are then evaluated in experiments with many replications, in several environments, and throughout various rattons. Such experiments in Brazil are usually installed in randomized block designs. After intensive evaluations through several cuts, environments and years, the new cultivars can be recommended for commercial use. An interesting feature of this phase is that sugarcane growers become greatly involved because most experiments are installed in commercial farms and evaluated under conditions that are very similar to those in actual cultivation.
21.7 SUGARCANE IMPROVEMENT TOOLS: MARKER-ASSISTED BREEDING AND TRANSGENICS

Sugarcane improvement has been achieved through the classical route for over 2 centuries but it is a costly and slow process. Currently, it takes around 12 years for a new cultivar to be released. One strategy to speed up this process is the use of genetic maps and molecular markers through marker-assisted breeding. Another strategy is the use of transgenics.

21.7.1 GENETIC MAPS AND MOLECULAR MARKERS

Most genetic designs used for the construction of genetic linkage maps use populations derived from crosses between inbred lines (e.g., using backcross or selfing). The statistical and genetics methods used in this case are already established and implemented in several softwares, such as Mapmaker/EXP. However, to obtain such strains for sugarcane is impractical, especially because of the large inbreeding depression that occurs when selfing occurs. In this case, the mapping populations are F1 generations obtained from crosses between non-inbred individuals (Lin et al. 2003).

For sugarcane (and several other species for which there are no available inbred lineages, such as fruit and eucalyptus), an alternative that was widely used in the past is called double pseudo-testcross. This strategy is the construction of two individual maps (one for each parental), by the polymorphic identification of single dose markers for each parental (Grattapaglia and Sederoff 1994; Porceddu et al. 2002; Shepherd et al. 2003; Carlier et al. 2004). On the basis of this approach, linkage maps for *S. officinarum* (“LA Purple”) and *S. robustum* (“Mol 5829”) were constructed using random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP) markers in a single dose (Guimarães et al. 1999). Other situations were also considered, but rarely the kind of crossing used involved commercial parents (Al-Janabi et al. 1993; da Silva et al. 1993; Sobral and Honeycutt 1993; da Silva et al. 1995; Grivet et al. 1996; Hoarau et al. 2001). However, from both a biological and statistical point of view, the integration of the information contained in these individual maps into a single integrated map is desirable. This can only be done with the presence of heterozygote markers between parents, which are used to establish linking relationships between the markers individually segregated in each parental (Barreneche et al. 1998; Wu et al. 2002; Garcia et al. 2006; Oliveira et al. 2007).

The construction of integrated genetic maps, using different types of molecular markers with different segregation, has great advantages because it allows the linking map to be saturated and extends the characterization of polymorphic variation throughout the genome. Codominant markers may be useful to bring together cosegregation groups in their respective homology groups, specifically for polyploid species such as sugarcane (da Silva et al. 1993; Grivet et al. 1996). Moreover, the more precise localization of QTL is helped by the availability of an integrated genetic map (Maliepaard et al. 1997). However, there may be different numbers of alleles, in heterozygote parents for each segregating loci, turning linkage analysis more complex, because the linkage phases in the parentals may be unknown a priori, making it difficult to detect recombination events (Maliepaard et al. 1997; Wu et al. 2002).

Wu et al. (2002) proposed a statistical method on the basis of maximum likelihood analysis that allows the simultaneous estimation of recombination fractions and linking phases between loci in mapping populations derived from crossings between non-inbred individuals (F1 generation). This method works toward the construction of an integrated genetic map, which is the result of the combination of various pieces of information generated by different types of molecular markers, whose information content varies. Garcia et al. (2006) and Oliveira et al. (2007) constructed integrated genetic maps, consisting of 357 markers distributed throughout 131 co-segregation groups from the crossing between two precommercial sugarcane cultivars (SP80-180 × SP80-4966). The results were better than those obtained when the same data were analyzed using the JoinMap program, which indicates, in this case, better efficiency in the estimation of linking and linking phases of the
method proposed by Wu et al. (2002). To do so, a software called OneMap, developed specifically for this purpose (Margarido et al. 2007) was used. It can also be used for other outcrossing species. Despite the superiority of this new analysis approach in relation to previous methods, in the case of sugarcane, it is still possible to use markers with 1:1 and 3:1 segregations, which are known to be less informative than other types (as for example, those that segregate 1:2:1 and 1:1:1:1 in the case of diploid species). This makes it difficult to get linkage groups and also to order markers within these groups. This leads to less saturated maps with less genome coverage. Furthermore, the integration of maps of the genitors is not always possible.

The first sugarcane genetic maps were built using RAPD and RFLP type molecular markers. Currently, mainly gene or genomic microsatellite molecular markers are used in the setting up of genetic and molecular maps. Microsatellites or SSRs (simple sequence repeats) have become widely used in plant marker studies. These markers are conventionally tandem repeats of small nucleotide sequences of one to six bases in length. The variation in the number of repetitions results in polymorphic loci that are extremely useful, especially in mapping studies. The ability of SSRs to reveal high allelic diversity is particularly useful in the discrimination between genotypes. The successful use of this marker in other species such as barley (Saghai-Maroof et al. 1994; Russell et al. 1997), rice (Wu and Tanskley 1993), wheat (Röder et al. 1995), apple (Szewc-McFadden et al. 1996), and avocado (Lavi et al. 1994), stimulated the application of this technique to more genetically complex species such as sugarcane (Cordeiro et al. 2000).

Nowadays, the search for SSRs is being carried out in expressed sequence tags (ESTs) deposited in public databases, as this alternative is a simpler, faster and more economical strategy for the development of SSRs. EST analysis is a simple strategy to study a portion of the expressed genome, even in organisms with large, complex and highly redundant genomes, such as sugarcane. The basic strategy for obtaining the EST is a fast and efficient method for genome sampling of gene active sequences. As genetic markers, the EST-SSRs have been evaluated in several studies and tend to be considerably less polymorphic than the markers generated from genomic sequences for rice (Cho et al. 2000), sugarcane (Cordeiro et al. 2001; Pinto et al. 2006), wheat (Eujayl et al. 2002), and barley (Thiel et al. 2003).

The analysis in sugarcane of 8678 EST sequences revealed approximately 250 SSRs, the majority made up of perfect trinucleotide repeats where (GCC) \(n\), (CGT) \(n\), (CCT) \(n\) motifs were the most common (Cordeiro et al. 2001). All selected EST-SSRs were polymorphic in the co-related Erianthus and Sorghum genera. The lowest value for the polymorphic information content (PIC) was obtained among the varieties of sugarcane (0.23), increasing between the species S. officinarum and S. spontaneum (0.62) and reaching the highest value (0.80) among the genera Erianthus and Sorghum. Because of the narrow genetic base of the varieties of sugarcane, the use of EST-SSR can assist in the characterization of the genetic variability available in the germplasm collections of related genera used in introgression programs. Thus, the introgression limitation of the Erianthus genome in sugarcane (Saccharum) can be overcome by the use of EST-SSR in identifying the portion of the Erianthus genome in intergeneric hybrids (Cordeiro et al. 2001).

The application of ESTs was shown to be a successful and efficient means of identifying sugarcane genes. A study by Carson and Botha (2000) showed that of all cDNA clones from the leaf identified in the search for homology, 38% showed significant similarity with known gene sequences. This value can be compared with that observed in the analysis of cDNA libraries from the endosperm and seed corn (39.3%, Shen et al. 1994) and even better than the results obtained using a cDNA library from maize leaves (20%, Keith et al. 1993), tissues of different growth stages of rice (25%, Yamamoto and Sasaki 1997) and portions of RNA from seeds, roots, leaves and inflorescences of Arabidopsis (32%, Newman et al. 1994).

### 21.7.2 Transgenics

Sugarcane biotechnology started in the 1960s with callus induction and rooted callus recovery (Nickell 1964) followed by callus regeneration (Barba and Nickell 1969; Heinz and Mee 1969). The
first genetically modified clones were obtained by biolistics (Bower and Birch 1992). Transgenic plants have since then been obtained that incorporate agronomic traits of interest (Gallo-Meagher and Irvine 1996; Arencibia et al. 1997, 1999; Enríquez-Obregon et al. 1998; Ingelbrecht et al. 1999; Zhang et al. 1999; Falco et al. 2000; Butterfield et al. 2002; Falco and Silva-Filho 2003; Leibbrandt and Snyman 2003; McQualter et al. 2004; McQualter et al. 2005; Vickers et al. 2005a, b; Wang et al. 2005; Snyman et al. 2006) including drought tolerance (Zhang et al. 2006; Molinari et al. 2007). Several plant tissues can be used to produce callus (Liu 1981 1993; Irvine 1987). Somatic embryogenesis is the most studied (Guiderdoni et al. 1995; Manickavasagam and Ganapathi 1998) and regeneration can be direct (Manickavasagam and Ganapathi 1998) or through the induction of embryogenic callus from immature leaf explants (Guiderdoni 1988). Embryogenic callus can be maintained for months without loss of regeneration capacity (Fitch and Moore 1993). Regeneration efficiency can be optimized for transformation as shown recently (Lakshmanan et al. 2006; Snyman et al. 2006).

Agrobacterium tumefaciens-mediated transformation has also been used in sugarcane (Arencibia 1998; Enríquez-Obregon et al. 1998; Elliott et al. 1998; Manickavasagam et al. 2004). With adequate manipulation of in vitro culture conditions and adequate A. tumefaciens cell lines the method can lead to the transference of relatively long DNA fragments, little rearrangements, low copy number and low costs. Sugarcane cultivars differ in their regeneration capacity and the methods must be optimized. Many cultivars that regenerate have already been described (Falco et al. 2000; Lima et al. 2001; Falco and Silva-Filho 2003; Cidade et al. 2006). Although the methods are available, so far there are no genetically modified cultivars released for trading. Transgene expression is largely unstable and many groups are searching for gene promoters that may lead to stable expression in mature plants. Examples of plant promoters useful for transformation but that vary in their efficacy are CaMV35S, nopaline, and octopine from A. tumefaciens, Ubi1, Emu, Act1, but there is no guarantee of tissue specificity (Last 1991; McElroy et al. 1991; Zheng et al. 1993; Green 2002; Neuteboom 2002; Christensen and Quail 1996). Ubi-1 is the most used in sugarcane for constitutive expression (Lakshmanan 2005) and some studies point to more adequate promoters (Liu 2003; Braithwaite 2004) but still there is little guarantee of targeting the gene expression for specific tissues (Benfey 1989; Neuteboom 2002). It is important to note that constitutive expression of a transgene can lead to phenotypic anomalies and that in the case of drought tolerance a drought inducible promoter can greatly ameliorate the effects (Liu et al. 1998; Huang 2001). Sugarcane promoters active in culms have been described recently (Hansom 1999). Research is also focusing on post-transcriptional silencing events that may render transgene expression unstable.

21.8 SUGARCANE FUNCTIONAL GENOMICS AND BIOINFORMATICS

No matter which route one chooses for sugarcane improvement, target genes for genetic manipulation or use as markers need to be identified. The analysis of the sugarcane transcriptome has been extensively used for many years in a search for genes associated to agronomic traits of interest (Menossi et al. 2008). Transcriptomics complements gene marker identification and either technique can be excellent tools for breeders that wish to improve sugarcane through transgenics or classic breeding. Also, the use of the transcriptome can aid in the identification of markers for QTL mapping and expression-QTL (eQTL), which in sugarcane is difficult until statistical genetic tools are available for highly polyploid genomes. Techniques that have been used for transcriptomics in sugarcane include EST sequencing, nylon-based cDNA macroarrays, cDNA microarrays, long oligonucleotide microarrays (Agilent Technologies®) and short oligonucleotide microarrays (Affymetrix®).

Transcriptome studies in sugarcane were first undertaken by EST sequencing (Carson 2000; Carson and Botha 2002; Casu et al. 2003, 2004; Ma et al. 2004; Vettore et al. 2003; Bowers et al. 2005). The largest collection of ESTs was generated by the SUCEST Project (http://sucest-fun.org), a consortium of over 100 Brazilian laboratories that generated approximately 238,000 ESTs.
from 26 diverse cDNA libraries which were clustered into 43,000 transcripts or SAS (Sugarcane Assembled Sequences).

The information generated in EST projects has been used in comparative mapping of the family of grasses, using common markers that hybridize with sugarcane, rice, corn, hexaploid wheat, barley and sorghum. However, the molecular information developed to date for sugarcane is minimal when compared to the information that is necessary to identify and characterize the loci that encode the important agronomic characteristics. Table 21.2 lists the genetic maps available for sugarcane. To date, approximately 400 genetic markers have been developed (Cordeiro et al. 2002; Pinto et al. 2006; Oliveira et al. 2009) and used in the construction of the first functional sugarcane genetic map (Oliveira et al. 2008).

All of the EST collections were clustered by the Center for Genomic Research (TIGR) as the Sugarcane Gene Index 2.1, and more recently by the Computational Biology and Functional Genomics Laboratory at the Dana-Farber Cancer Institute as the Sugarcane Gene Index 2.2. In the SUCEST-FUN database (http://sucest-fun.org) a tool is available where clusters generated by the SUCEST collection and the Sugarcane Gene Index collection can be cross-referenced. The SUCEST-FUN has been developed in the concept of the mediator approach that incorporates concepts from Data Warehouse and Federation approaches. It is a flexible data integration that assembles heterogeneous distributed data sources, experimental data, resources, the application of scientific algorithms and computational analysis. Bioinformatics and the management of scientific data are critical to the support of life sciences discoveries. Nowadays, an explosion of available biological data and research has risen up, most of it compound and stored in dozens of smaller databases. Scientists are not currently able to easily identify and integrate autonomous data sources and exploit this information because of the variety of semantics, interfaces, and data formats used by the underlying data sources. The SUCEST-FUN Database is, therefore, being developed to give access to gene expression studies and make available tools that will allow a Systems Biology approach in sugarcane and the identification of regulatory networks.

The first transcriptomics tools developed made use of existing cDNA clones to produce macroarrays. Macroarrays have been used to define gene expression patterns in immature and mature leaf, immature and mature internodes (Carson and Botha 2002; Carson et al. 2002), sugarcane responses to cold (Nogueira et al. 2003), tissue profiling of transposable element transcripts (Araujo et al. 2005), stem development (Watt et al. 2005), methyl jasmonate responses (De Rosa Jr et al. 2005), the response of sugarcane leaves to ethanol application (Camargo et al. 2007), sink-source activity alterations (McCormick et al. 2006, 2008) and ABA/MeJA-activation of sugarcane transcription factors (Schlogl et al. 2008). Data mining of the SUCEST database led to the identification of 276 sequences homologous to TEs in 21 different families of which 54% correspond to classical transposons and 46% to retrotransposons (Rossi et al. 2001). Retrotransposons mobilize themselves through an RNA intermediate and thus are now considered one of the major forces driving genome expansion in plants (Piegu et al. 2006) while transposons usually move using either a cut/paste or a copy/paste mechanism. Expression profiling of 162 clones (Araujo et al. 2005) showed that callus was the tissue with most expressed TE families. Although it has been proposed several times that tissue culture somaclonal variation could be a result of TE activity, this was the first report that demonstrated that callus is indeed a tissue where different TEs are expressed at the same time, not necessarily in the same cell. One largely unanticipated result was the revelation that within a family there are lineages with varying copy number in the genome (Rossi et al. 2004; Saccaro-Junior et al. 2007). Adding to that, some of the transposon lineages were associated with previously described "domesticated" versions of a transposase (Bundock and Hooykaas 2005; Cowan et al. 2005). Regardless of the mechanism by which a transposable element moves, the field of genetic mobile elements is now flourishing with hypotheses of their impact on genome structure, gene regulation and even function leaving their once considered "junk DNA" status as a secondary role (Casacuberta and Santiago 2003; Kashkush et al. 2003; Bundock and Hooykaas 2005).
<table>
<thead>
<tr>
<th>Mapping population</th>
<th>Progeny</th>
<th>Marker</th>
<th>SD</th>
<th>DD</th>
<th>DM</th>
<th>Linked markers</th>
<th>N0 CG</th>
<th>*Map coverage</th>
<th>Map Software</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP068 × SES208</td>
<td>90</td>
<td>RFLP</td>
<td>216</td>
<td>-</td>
<td>-</td>
<td>188</td>
<td>44</td>
<td>1361</td>
<td>Mapmaker</td>
<td>Da Silva et al. 1993</td>
</tr>
<tr>
<td>ADP068 × SES208</td>
<td>88</td>
<td>RAPD</td>
<td>208</td>
<td>-</td>
<td>-</td>
<td>176</td>
<td>42</td>
<td>1500</td>
<td>Mapmaker ver 1.0</td>
<td>Al-Janabi et al. 1993</td>
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<tr>
<td>SP701006</td>
<td>32</td>
<td>RFLP</td>
<td>253</td>
<td>-</td>
<td>-</td>
<td>94</td>
<td>25</td>
<td>-</td>
<td>Mapmaker ver 1.0</td>
<td>D’Hont et al. 1994</td>
</tr>
<tr>
<td>R570</td>
<td>77</td>
<td>RFLP, Isozyme</td>
<td>505</td>
<td>-</td>
<td>-</td>
<td>408</td>
<td>96</td>
<td>2008</td>
<td>Mapmaker ver 3.0</td>
<td>Grivet et al. 1996</td>
</tr>
<tr>
<td>La Purple × Mol 5829</td>
<td>84</td>
<td>RAPD</td>
<td>279</td>
<td>-</td>
<td>-</td>
<td>161</td>
<td>50</td>
<td>1152</td>
<td>Mapmaker ver 2.0</td>
<td>Mudge et al. 1996</td>
</tr>
<tr>
<td>La Purple× Mol 5829</td>
<td>100</td>
<td>RAPD, RFLP, AFLP</td>
<td>341</td>
<td>-</td>
<td>-</td>
<td>283</td>
<td>74</td>
<td>1881</td>
<td>Mapmaker ver 2.0</td>
<td>Guimarães et al. 1999</td>
</tr>
<tr>
<td>R570</td>
<td>112</td>
<td>AFLP</td>
<td>939</td>
<td>-</td>
<td>-</td>
<td>883</td>
<td>120</td>
<td>5849</td>
<td>Mapmaker ver 3.0</td>
<td>Hoarau et al. 2001</td>
</tr>
<tr>
<td>R570</td>
<td>112</td>
<td>AFLP, EST-RFLP, EST-SSR</td>
<td>939+347</td>
<td>-</td>
<td>-</td>
<td>883 + 282</td>
<td>128</td>
<td>&gt; 5849</td>
<td>Mapmaker ver 3.0</td>
<td>Rossi et al. 2003</td>
</tr>
<tr>
<td>Green German ×</td>
<td>85</td>
<td>RFLP</td>
<td>434</td>
<td>132</td>
<td>-</td>
<td>289</td>
<td>75</td>
<td>2466</td>
<td></td>
<td>Ming et al. 2002</td>
</tr>
<tr>
<td>IND81-145</td>
<td></td>
<td></td>
<td>395</td>
<td>54</td>
<td>-</td>
<td>257</td>
<td>70</td>
<td>2172</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIN84-1× Muntok Java</td>
<td></td>
<td></td>
<td>308</td>
<td>86</td>
<td>-</td>
<td>194</td>
<td>71</td>
<td>1395</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IJ76-514 × Q165</td>
<td>227</td>
<td>AFLP, RAE, SSR</td>
<td>967</td>
<td>36</td>
<td>123</td>
<td>1074</td>
<td>136</td>
<td>9058.3</td>
<td>TeiMap ver 2.0</td>
<td>Aitken et al. 2005</td>
</tr>
<tr>
<td>IJ76-514 × Q165</td>
<td>227</td>
<td>AFLP, SSR</td>
<td>240</td>
<td>-</td>
<td>234</td>
<td>534</td>
<td>123</td>
<td>4906.4</td>
<td>JoinMap ver 3.0</td>
<td>Aitken et al. 2007</td>
</tr>
<tr>
<td>SP80-180 × SP80-4966</td>
<td>100</td>
<td>AFLP</td>
<td>441</td>
<td>677</td>
<td>-</td>
<td>3571</td>
<td>1311</td>
<td>2602.41</td>
<td>One Map</td>
<td>Garcia et al. 2006</td>
</tr>
<tr>
<td>R570× MQ76-53</td>
<td>198</td>
<td>AFLP, RFLP, SSR</td>
<td>1057</td>
<td>-</td>
<td>-</td>
<td>424</td>
<td>86</td>
<td>3144</td>
<td>Mapmaker ver 3.0</td>
<td>Raboin et al. 2006</td>
</tr>
<tr>
<td>M134/75 × R570</td>
<td>227</td>
<td>AFLP, SSR</td>
<td>557</td>
<td>-</td>
<td>79</td>
<td>474</td>
<td>95</td>
<td>6200</td>
<td>Mapmaker/exp v3.0</td>
<td>Aljanabi et al. 2007</td>
</tr>
<tr>
<td>SP80-180 × SP80-4966</td>
<td>100</td>
<td>AFLP, EST-SSR, EST-RFLP</td>
<td>800</td>
<td>869</td>
<td>-</td>
<td>664</td>
<td>192</td>
<td>6261.1</td>
<td>One Map</td>
<td>Oliveira et al. 2007</td>
</tr>
<tr>
<td>La Striped × SES 147B</td>
<td>100</td>
<td>AFLP, SRAP, TRAP</td>
<td>i</td>
<td>j</td>
<td>33</td>
<td>146</td>
<td>49</td>
<td>1732</td>
<td>JoinMap ver 3.0</td>
<td>Awala et al. 2008</td>
</tr>
</tbody>
</table>

*Refers to the map information of the respective parental. Bold parents correspond to the parental map constructed.

**Cumulative length in centimorgans (cM). SD, single-dose markers (simplex marker), marker present only once in the genome segregating in a 1:1 ratio; DD, double-dose marker (double simplex marker), marker present in one copy in both parental genomes, segregating in a 3:1 ratio; DM, duplex marker, marker present twice in one parental genome segregating in a 11:3 ratio (x = 8) or in a 7:2 ratio (x = 10).
The advent of microarrays increased significantly the number of studies on gene expression profiling. Carbohydrate metabolism has been extensively studied in sugarcane using cDNA microarrays to define gene expression associated with sucrose content. Microarrays were used to profile the developing culm (Casu et al. 2003, 2004) leading to the identification of sugar transporters highly expressed in the maturing stem and the coordinated expression of enzymes involved in sucrose synthesis and cleavage. Transcripts associated with fiber metabolism and defense and stress mechanisms were the most highly expressed transcripts in maturing stem. Stress responses were also defined in roots (Bower et al. 2005) using the same arrays. Customized microarrays containing signal transduction components were used to profile the individual variation of plants cultivated in the field and transcript abundance in six plant organs (flowers, roots, leaves, lateral buds, and 1st and 4th internodes) leading to the identification of genes ubiquitously expressed or tissue-enriched (Papini-Terzi et al. 2005). The same arrays were also used to study signal transduction-related responses to phytohormones and environmental challenges in sugarcane (Rocha et al. 2007) including drought, methyl jasmonate, abscisic acid, insect (Diatraea saccharalis), and endophytic bacteria (Gluconacetobacter and Herbaspirillum) elicited responses. Thirty genotypes contrasting for sucrose content were profiled and over 300 genes associated to sucrose content were discovered (Papini-Terzi et al. 2009). In parallel, sugarcane plantlets were treated with sucrose to define genes directly responsive to this sugar. Interestingly, a large overlap was observed between gene expression responsive to drought and sucrose indicating a common controlling mechanism behind these processes that may rely on protein kinases of the SnRK/SNF1 family. Hormonal regulation associated to sucrose content was also revealed that included ethylene, auxins, jasmonates, abscisic, and salicylic acid. Additionally, sugarcane has been expression-profiled under the effect of elevated CO2 (de Souza et al. 2008). This is an important issue related with sugarcane physiological responses to the global climatic changes (GCC). GCC can be characterized by an elevation of the atmospheric CO2 concentration (because of fossil fuel usage), which leads to the elevation of temperature and consequently changes in the climate. An experiment performed with sugarcane plants growing in elevated CO2 showed that they respond by increasing photosynthesis (CO2 assimilation) and growth (50% more biomass). This culminated with the production of more sucrose and fiber (ca. 29% each). The authors evaluated the gene expression changes associated with the treatment and found carbon metabolism to be affected. Genes related to electron transport in the chloroplasts were more expressed. On the other hand, no effects on CO2 assimilation were observed, suggesting that sugarcane leaves will respond to the elevation of CO2 by improving their light harvesting system to use the excess of CO2 (de Souza et al. 2008).

A large part of the gene expression data is publicly available. Most published sugarcane microarrays were catalogued on public repositories such as Gene Expression Omnibus (GEO-NCBI), Center for Information Biology Gene Expression Database (CIBEX), Microarray Gene Expression Data Society (MGED), and ArrayExpress (Tables 21.3 and 21.4).

<p>| TABLE 21.3 |
| Summary of Sugarcane Microarray Data |</p>
<table>
<thead>
<tr>
<th>Number of platforms</th>
<th>Total</th>
<th>SUCEST-FUN</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of platforms</td>
<td>6</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Number of series</td>
<td>17</td>
<td>12</td>
<td>5</td>
</tr>
<tr>
<td>Number of samples</td>
<td>226</td>
<td>182</td>
<td>44</td>
</tr>
</tbody>
</table>

Because of rising oil prices and environmental concerns regarding nonrenewable fuels, ethanol has become an alternative to gasoline for auto engines. The Brazilian Government started a program to replace ethanol for gasoline in 1975; in 2008 the use of ethanol surpassed that of gasoline as fuel for cars and light vehicles. Because of the flex-fuel technology that allows engines to operate with any proportion of ethanol and gasoline, since 2008 cars that run on ethanol became dominant in Brazil; in 2009/2010 57% of the sugarcane crushed in Brazil was used for ethanol and 43% for sucrose (Unica 2009) and of the 413 mills operating in Brazil, 398 produce ethanol (MAPA 2009). Except for Brazil, in most countries ethanol comprises a relatively small proportion of the sugarcane industry output and is directed to liquor or chemical purposes; however, it is expected that the use of ethanol as fuel will be the main driver for the expansion of sugarcane production in the world in the near future (Figure 21.1). Presently, approximately 90% of the fuel ethanol production in the world comes from the United States and Brazil.

Table 21.4: Summary of All Sugarcane Microarray Experiments

<table>
<thead>
<tr>
<th>Platform</th>
<th>Samples</th>
<th>Number of Differentially Expressed Genes</th>
<th>Treatment/Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane nylon arrays (1536 genes)</td>
<td>12</td>
<td>34</td>
<td>Cold stress-induced</td>
</tr>
<tr>
<td>Sugarcane EST nylon arrays (1536 genes)</td>
<td>12</td>
<td>26</td>
<td>MeJA-induced</td>
</tr>
<tr>
<td>Affymetrix sugarcane genome array (6024 genes)</td>
<td>12</td>
<td>119</td>
<td>Stem development</td>
</tr>
<tr>
<td>SUCASTv1 (2208 genes)</td>
<td>16</td>
<td>14</td>
<td>Phosphate starvation</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>74</td>
<td>Leaves sugar accumulation</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>13</td>
<td>ABA</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>42</td>
<td>MeJA</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>11</td>
<td>N₂-fixing endophytic</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>80</td>
<td>Drought</td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>172</td>
<td>Mature/Immature Internode high/low brix</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>126</td>
<td>Internode high/low brix 1,5,9</td>
</tr>
<tr>
<td>SUCASTv3 (1920 genes)</td>
<td>26</td>
<td>216</td>
<td>Six different tissues</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>24</td>
<td>Leaves high/low brix</td>
</tr>
<tr>
<td>SUCASTv2 (1920 genes)</td>
<td>28</td>
<td>216</td>
<td>Six different tissues</td>
</tr>
</tbody>
</table>


21.9 CONVENTIONAL BIOETHANOL PRODUCTION

Because of rising oil prices and environmental concerns regarding nonrenewable fuels, ethanol has become an alternative to gasoline for auto engines. The Brazilian Government started a program to replace ethanol for gasoline in 1975; in 2008 the use of ethanol surpassed that of gasoline as fuel for cars and light vehicles. Because of the flex-fuel technology that allows engines to operate with any proportion of ethanol and gasoline, since 2008 cars that run on ethanol became dominant in Brazil; in 2009/2010 57% of the sugarcane crushed in Brazil was used for ethanol and 43% for sucrose (Unica 2009) and of the 413 mills operating in Brazil, 398 produce ethanol (MAPA 2009). Except for Brazil, in most countries ethanol comprises a relatively small proportion of the sugarcane industry output and is directed to liquor or chemical purposes; however, it is expected that the use of ethanol as fuel will be the main driver for the expansion of sugarcane production in the world in the near future (Figure 21.1). Presently, approximately 90% of the fuel ethanol production in the world comes from the United States and Brazil.

Sugarcane is used for ethanol production by fermentation followed by distillation of sucrose or molasses. The theoretical yield of ethanol is 617 L/t of sucrose but, at the normal operating conditions of ethanol distilleries, the yield is usually 510–530 L/t sucrose. The average yield of ethanol in Brazil is 82–85 L ethanol/t of fresh cane crushed (Boddey et al. 2008). Anhydrous bioethanol production processed from sugarcane juice in an autonomous distillery is comprised by the following main steps: sugarcane cleaning; extraction of sugars; juice treatment, concentration and sterilization; fermentation; distillation and dehydration; and purification as described in the following items.
21.9.1 Extraction, Juice Treatment, Concentration, and Sterilization

Sugarcane is first cleaned in wet or dry cleaning system, which removes 70% of the dirt, before entering the mills, in which sugarcane stems are processed and juice and bagasse are obtained. Mill efficiency is around 96%. Sugarcane juice passes through screens and hydrocyclones that remove dirt, sand and fibers; then phosphoric acid is added to increase phosphate content, and the mixture is heated from 30 to 70°C before addition of lime. The limed juice is heated from approximately 70 to 105°C before the flash tank, where air bubbles are removed from the juice. The degasified juice receives a flocculant polymer and is decanted, aiming at removal of insoluble impurities, including calcium phosphates formed during the liming step. The mud obtained in the decanters is filtered (producing filter cake) and the liquid phase returns to the process just after the liming step.

The clarified juice obtained in the decanters goes through multiple effect evaporators (MEE) to achieve adequate sugars concentration. Only part of the clarified juice must be concentrated. The final juice is made up of clarified and concentrated juice, and contains about 22 wt % sucrose.

To promote sterilization before fermentation, the juice is heated up to 130°C, cooled down to 28°C and fed to the fermentation reactor.

21.9.2 Fermentation

The sterilized juice is added to the fermentor along with the yeast media, which is made up of a yeast suspension containing about 28% yeast (vol. basis) and comprises 25% of the reactor volume. Fermentation is carried out at 28°C; ethanol content of the wine can reach 13°GL (approximately 10.5% ethanol on a mass basis, that means around 100 g/L). To achieve this high ethanol content, batch fermentation may have to be carried out for up to 15 h and alternative cooling methods, such as a steam jet system or an absorption machine, are necessary to provide water at temperatures low enough to maintain reactor cooling (Dias et al. 2007), because fermentation is negatively affected by high temperatures. In an integrated process taking into account hydrolyzed lignocellulosic material from sugarcane bagasse or straw, the fermentor could be fed with a blend of molasses and hydrolyzed.

The wine obtained in the fermentor is centrifuged for the recovery of yeast cells. The yeast milk obtained in the second centrifuge contains about 70 vol % yeast, so water is added to this milk to

produce a mixture containing 28 vol % yeast that are fed to the reactor for another batch of fermentation. Sulfuric acid is added to the yeast medium to avoid bacterial contamination.

Gases released during fermentation are collected and washed to recover ethanol in an absorption column. The alcoholic solution obtained is mixed with the centrifuged wine and fed to the distillation columns.

21.9.3 Distillation and Dehydration

Product purification takes place in double-effect and extractive distillation systems. A series of distillation and rectification columns are used for hydrous bioethanol production (93 wt % ethanol). Hydrous ethanol in vapor phase is dehydrated to produce anhydrous ethanol (AE, 99.5 wt % ethanol) in the extractive distillation process with monoethylene glycol. In the double effect distillation, column reboilers and condensers are integrated, thus diminishing energy consumption on the distillation.

21.9.4 Simulations for Increasing Efficiency of Hydrated Bioethanol Production

Product purification is a critical step during ethanol production because it is an energy intensive operation. Ordinary distillation is used to produce hydrous bioethanol (approximately 93 wt % ethanol), which is used as fuel in ethanol-based or flex-fuel engines. The conventional configuration of the distillation process used in Brazilian refineries consists of a series of distillation columns (A, A1, and D) and rectification (B and B1) columns. This configuration is depicted in Figure 21.2.

In conventional bioethanol production (first generation), wine obtained from fermentation of sugars containing between 7 and 12 wt % ethanol is the feedstock to obtain ethanol. However, wine produced in the fermentation process with an extractive vacuum flash chamber can achieve 50 °GL (Atala and Maugeri 2006), what may lead to a significant reduction in the separation costs.

21.9.4.1 Ordinary Distillation

Because distillation operations require significant amounts of energy and have a great importance in bioethanol production, the simulation of an operation unit has to be as representative as possible.

FIGURE 21.2 Configuration of conventional distillation process.
For this reason, simulation of conventional distillation process was carried out in Aspen Plus® considering nonequilibrium stage model. In this model, conservation equations are written for each phase independently and solved together with transport equations that describe mass and energy transfers in multicomponent mixtures; also it is assumed that equilibrium occurs only in the vapor–liquid interface. Besides, in this way, empirical correcting factors, such as efficiencies used in the equilibrium model, are no longer necessary (Pescarini et al. 1996).

Results of the simulation considering nonequilibrium stage model were compared to those obtained using equilibrium stage model with constant plate efficiencies of 55, 70, 85, and 100%. It was observed that, to obtain hydrous ethanol (93%), the idealized equilibrium stage model (efficiency of 100%) predicts an energy requirement that corresponds to 80% of that given by nonequilibrium stage model (~7,000 kJ/kg of hydrous ethanol). In addition, simulations showed that equilibrium stage model with an efficiency of 70% provide results quite similar to nonequilibrium stage model for the conventional distillation process.

### 21.9.4.2 Multiple Effect Distillation

An alternative to the conventional distillation process is the multiple effect operation of the distillation columns. The operation in different pressure levels gives rise to different temperature levels on condensers and reboilers of the different columns, thus it is possible to integrate the equipment and reduce steam consumption on reboilers.

To optimize bioethanol production, simulations using Aspen Plus were carried out with five different configurations: conventional fermentation and distillation (CFCD), vacuum extractive fermentation and conventional distillation (VFCD), vacuum extractive fermentation and conventional distillation (VFCD), vacuum extractive fermentation and double effect distillation (VFDD), and vacuum extractive fermentation and triple effect distillation (VFTD).

Vacuum extractive fermentation consists of a fermentation reactor coupled to a vacuum flash evaporator, which allows ethanol produced to be simultaneously removed from the reactor. In this study, ethanol concentration in the reactor was kept at low levels (around 8 wt % ethanol) whereas in the flash chamber, wine with 36 wt % ethanol was obtained.

The double effect configuration was similar to the conventional configuration, however, the distillation columns operate under vacuum (19–25 kPa), while rectification columns operate under atmospheric pressure (101–135 kPa). In this way, different temperature levels are observed between columns “A” reboiler and “B” condenser (65 and 78ºC, respectively), allowing thermal integration of these equipments and consequently reducing energy consumption on the distillation stage.

In the triple effect configuration, the distillation columns operate under vacuum (19 – 25 kPa), and the liquid phlegm stream produced on column D is split in two: one of them is fed to a rectification column operating under nearly atmospheric pressure (column “B,” 70 – 80 kPa) and the other is fed to a rectification column which operates under relatively high pressure (column “B-P,” 240 – 250 kPa).

Regarding thermal energy, results showed that the configuration that presents the lowest energy demand is the triple effect configuration (VFTD), providing a reduction in energy consumption of 44 % when compared to the VFCD process and 77 %, when compared to the CFCD process, which is the configuration most commonly used in Brazilian biorefineries.

### 21.9.5 Simulations for Increasing Efficiency of Anhydrous Ethanol Production

Anhydrous bioethanol, suitable to be used as a gasoline additive or as raw material for production of different renewable materials, must contain at least 99.3 wt % ethanol. Because water and ethanol form an azet trope with 95.6 wt % ethanol at 1 atm, alternative separation processes are necessary to produce anhydrous bioethanol. The most used processes in Brazilian biorefineries are azotropic distillation with cyclohexane and extractive distillation with monoethylene glycol (MEG).
21.9.5.1 Azeotropic Distillation

In azeotropic distillation for bioethanol production, the entrainer (cyclohexane) is added to the binary mixture, producing a heterogeneous azeotrope with water and ethanol. In this process, two distillation columns are used: azeotropic and recovery.

Simulation of the azeotropic distillation process is often complex and extremely sensitive to project parameters and specifications, mainly because of the formation of a second liquid phase inside the azeotropic column. Higler et al. (2004) reported that column efficiencies between 25 and 50% are not uncommon when a second liquid phase is present.

To study the formation of the second liquid phase inside the column, simulations of the azeotropic distillation process with cyclohexane for anhydrous bioethanol production were carried out using the software Aspen Plus with three configurations.

In the configuration that presented the best results, hydrous bioethanol is mixed with the recycle stream of the recovery column and fed to the azeotropic column. Entrainer is comprised of the organic phase obtained in the decanter and a solvent make-up stream. Anhydrous bioethanol is produced on the bottom of the azeotropic column, and the ternary azeotrope in the top. After cooling, the ternary azeotrope splits into two liquid phases in the decanter. The aqueous phase is fed to the recovery column, producing pure water on the bottom.

For all configurations studied, it was observed that the mixture inlet stage in the azeotropic column determines the beginning of a second liquid phase, because this stage is the one that first presents two liquid phases. For this reason, it is important to define the inlet stage near to the bottom of the column; therefore the best feed inlet stage is the last one that allows column convergence.

21.9.5.2 Extractive Distillation

In extractive distillation, also known as homogeneous azeotropic distillation, a separating agent, called solvent or entrainer, is added to the azeotropic mixture to alter the relative volatility of the components in the original mixture.

In the conventional extractive distillation process, solvent is fed to the first column (extractive column), above the azeotropic feed. Anhydrous ethanol is produced on the top of the extractive column, while in the bottom a mixture containing solvent and water is obtained. The solvent is recovered in a second column (recovery column), cooled and recycled to the extractive column (Higler et al. 2004).

Conventional extractive distillation processes used in the industry for the separation of ethanol–water mixtures use monoethyleneglycol (MEG) as a solvent in the distillation column, which is a toxic component.

Energy consumption on column reboilers for the optimized configuration is 1057 kJ/kg of anhydrous ethanol, which is a reasonable value when compared to other distillation-based dehydration processes. Bioglycerol is not harmful to humans or the environment, and its availability has increased in the last years because it is a byproduct of the biodiesel production process.

21.9.6 Simulations for Increasing Efficiency of Extraction Processes

The need to decrease residues generation and the pursuit of cost reduction in bioethanol production has motivated the investigation of more efficient processes to produce this biofuel. One of the proposed options is the use of reaction-separation systems, such as the continuous flash fermentation.

Cardona and Sanchez (2007) point out the reaction–separation integration is a particularly interesting choice for the intensification of ethanol production. The removal of ethanol from the culture broth diminishes the inhibition effect on the growth rate. The continuous extractive fermentation has shown several advantages, such as low vinasse generation and fresh water consumption because of the possibility of feeding molasses at higher concentrations, which reduce costs in waste treatment, and the potentiality of eliminating one distillation column from the process. It is worthwhile
mentioning that the conventional mode of operation produces 13 L of vinasse/L of ethanol (Navarro et al. 2000). Vinasse is one of the main polluting byproducts of alcoholic fermentation because of its low pH, high solids content, etc. Further details of the technical features of extractive processes can be found in Silva et al. (1999) and Atala and Maugeri Filho (2006).

The extractive process was developed by Atala (2004), as cited by Mariano et al. (2008) in an application for the butanol purification. This configuration consists of three interconnected units: fermentor, filter (tangential microfiltration for cell recycling) and vacuum flash vessel (for the continuous removal of ethanol from the broth).

The concentration of sugarcane molasses in the feed stream is from 180 to 330 kg/m³ of total reducing sugar, and the 
Saccharomyces cerevisiae
in the steady state reaches 30 kg/m³. The low ethanol concentration is maintained at 40–60 kg/m³. These characteristics of operation guarantee higher yield (10 kg/m³ per hour) than in fed-batch and continuous modes of operation. Figure 21.3 depicts the extractive process formed by a fermentor, pumps, flash vessel, and a membrane system. The kinetic model takes into account the substrate and product inhibitions, the volume occupied by the cells, intracellular ethanol and terms of cell death (considering that continuous processes are operated for long periods). The term \( \rho \) is a relation between dry cell mass and the volume of wet cells, and \( \gamma \), the relation between the inter- and extracellular ethanol concentrations. Having a higher ethanol concentration in the fermentation stage is an interesting alternative, and has a significant impact on the whole process costs because of the reduced effort required in the ethanol purification.

### 21.10 CELLULOSIC ETHANOL PRODUCTION

Plants are structurally sustained by their walls, which are composites analogous to liquid crystals. Cellulose microfibrils are deposited in large quantities in the vascular tissues and fibers. These microfibrils are covered by hemicellulosic polysaccharides, which in the case of sugarcane are arabinoxylan and \( \beta \)-glucan. The deposition of cellulose is one of the most efficient packing processes in nature. Glucose chains linked by \( \beta \)-1,4-glycosidic linkages are packed together so that very little water is left among the polymers. These interactions and the lack of water prevent the access of enzymes to the glycosidic linkages, avoiding the attack of microorganisms and consequently defending plant tissues against pathogen attack.

As a result of this efficient packing, the plant cell wall is, by far, the most adequate form in which to store carbon and energy in high quantities but at the same time protect against the attack of microorganisms. The potential for production of biomass will therefore increase as a plant makes proportionally more wall. On the other hand, this highly packed form of storage imposes a tremendous barrier to access the energy stored. Enzyme hydrolysis is probably the most efficient way to gain access to the energy stored in glycosidic linkages. What is lacking, though, is the necessary
knowledge about how the enzymes of sugarcane or microorganisms attack the wall. It is crucial to find ways to activate these enzymes in vivo so that energy access is gained more easily.

When sugarcane is harvested, sugars are extracted in water by pressing stem tissues. This extracts approximately one third of the energy contained in the plant as soluble sugars. Leaves and other plant parts that are left in the field after stem harvesting correspond to another one third of the sugarcane biomass energy; eventually part or all this material may be collected and used to produce energy. The remaining one third of energy is composed of the bagasse that is already available at the mill facilities and is used to produce vapor, mechanic, and electrical power; in some sugarcane and ethanol plants in Brazil surplus energy is sold and fed to the electric grid. Although part of the bagasse is currently used for production of thermo electricity, the current efficiency of this process is far beyond the potential of producing energy from the hydrolysis of biomass either with acids or with enzymes (Cortez et al. 2008).

The process of production of free fermentable sugars from hemicelluloses and cellulose is the basis of cellulosic ethanol production. This can be achieved by physical, chemical or biochemical procedures. The great challenge is finding the combination of processes that could produce free fermentable sugars in an economically viable way.

There is great potential for an increase in bioethanol production and process optimization, considering process improvements and the use of sugarcane bagasse as raw material in the hydrolysis process. The evaluation of the energy consumption of the integrated production of ethanol from sugarcane and sugarcane bagasse remains one of the main obstacles for the technical and economical feasibility of the hydrolysis process. Figure 21.4 shows a possible pathway for the integrated process, which can be easily incorporated in existing units as soon as the sugarcane hydrolysis process is sufficiently developed.

A process that may be used to produce bioethanol from lignocellulosic materials, the Organosolv process with dilute acid hydrolysis, is being tested under semi-industrial scale in Brazil for the production of 5000 L/day of ethanol (Rossell et al. 2005). A typical large scale plant, that is, one that produces 1000 m³/day of anhydrous bioethanol crushing approximately 12,000 t sugarcane/day, was considered for the conventional bioethanol production from sugarcane juice. Sugarcane bagasse is one of the main byproducts of conventional bioethanol production from sugarcane juice.

![Figure 21.4 Integrated bioethanol production from sugarcane and sugarcane bagasse.](image-url)
In this example 75% of sugarcane bagasse produced in the mills is being used as raw material for bioethanol production in the integrated process. The figure represents a feasible configuration in the years to come, when sugarcane burning is abolished and sugarcane straw is efficiently recovered from the field.

Although polysaccharides can be easily hydrolyzed with acids producing free sugars, when this is done, part of the free monosaccharides produced will be destroyed by the acid and form furfurals, which are toxic to yeast (Cortez et al. 2008). As a result, acid hydrolysis has never become economically viable in industry. An alternative is the use of enzymes, which are much more precise, to break the glycosidic linkages among monosaccharides. However, the problem in this case is to prompt enough water within the microfibrils so that hydrolysis can occur. Hydrolases can be used directly on the fiber residue and a blind search may lead to good extracts from microorganisms. However, if one understands what linkages have to be broken to loosen the wall, much less energy can be used in the process, making it more efficient. The use of polysaccharide hydrolases obtained from microorganisms, insects and the plants themselves, is the strategy that is now being developed in most initiatives to produce ethanol from biomass and sugarcane (Buckeridge et al. 2010). This is a very complex task and will probably be achieved by the combination of the strategies of pretreatment of biomass using physical and chemical methods, together with enzymatic hydrolysis.

21.11 FUTURE PROSPECTS: RESEARCH AND DEVELOPMENT FOR PRODUCTIVITY AND SUSTAINABILITY

Since 1975 R&D has improved the productivity of ethanol from sugarcane more than 80% (from 4200 to 7650 L/ha). The main contributions to these productivity gains came from the development of new varieties through classical breeding, improvements in the fermentation processes and improvements in the agricultural processes. R&D has also been instrumental in obtaining more efficient use of energy in ethanol mills, reducing GHG emissions from the agricultural processes and reducing water, negative environmental impacts and defensive use.

In almost all studies of sugarcane physiology and biochemistry, the ability of the plant to produce sucrose has been the main target of research. This is plausible as this plant is so important to sugar and ethanol world production. As a consequence, several authors have focused their lines of research in sugar biochemistry and source-sink relationships. Moore (2005) has reviewed sugarcane physiology from the viewpoint of systems biology. He proposes that any scientific approach should target the interactions among the several network connections that lead to higher sugar accumulation. These networks include the layers of gene transcription, which leads to protein production and interactions and subsequently leads to metabolic changes in plant tissues. A few relevant questions can be asked such as (1) How is photosynthesis connected to sucrose biosynthesis? (2) How is carbon partitioned between nonstructural and structural carbohydrates? (3) How are the carbon pathways connected with nitrogen metabolism? (4) How is all this integrated or how do hormones perform the cross talk so that communication among biochemical, physiological and ecophysiological stimuli is controlled? and finally (5) Can we alter physiology and metabolism regulation to achieve the attributes of an Energy-Cane? Tools are needed if we want to improve sugarcane either through marker-assisted breeding or transgenic approaches and the sequencing of the sugar cane genome will be an important step toward the development of the biotechnology for this crop. Furthermore, these questions are very complex but interdisciplinary work and integrated databases may solve some of these problems. This is so also because the morphology and physiological performance of the plant is an emergent property of the integration of all of these factors. On the other hand, such an approach can be considerably simplified by using modularity (Wagner et al. 2007). Understanding how plants use the same module in different parts of its body will probably be key not only to understanding how these modules connect, but also to applying what is learnt from how different modules work, by using synthetic biology.
Synthetic biology may also be important to developing alternative uses of ethanol from sugarcane. There are many possible and perhaps suitable ways to use well the available biomass for energy generation in a broader scenario. In fact, many possible pathways to obtain biofuels may be designed based on the available feedstock. The primary use of sucrose for ethanol production is essentially an economical decision and the combination either sequentially or serially of alternative processes is an option to improve feedstock for biofuels and energy production. For first-generation biofuel, the knowledge is in place for the direct use of sugarcane syrup to produce ethanol even when it is depleted through sugar manufacture in integrated bioethanol and sugar production units (in this case it is postulated to be the one and half generation process). The use of lignocellulosic material, in this case either sugarcane bagasse or microalgae from CO₂ as a feedstock, is generally defined as second generation. On the other side, the use of the biomass gasification to further produce liquid biofuels is framed as the third generation pathway. The technology is always improving and in the last years the learning curve has offered already a potential for short term competitive costs for all idealized pathways. It is expected that plastics from sugarcane will be soon available and diesel from the ethylic route will be the next option.

Sugarcane research has been increasing over the years. Figure 21.5 shows the evolution of the number of scientific papers published annually on sugarcane-related subjects, classified according to the country of origin. Brazil is the leader in publications, a trend that started with the public release of the SUCEST EST sequencing data in 2001. In 2008, Brazilian publications accounted for over half of the articles indexed by the ISI/WoS System, 22% of which were originated from the State of São Paulo in Brazil, which also is responsible for 60% of the sugarcane production in Brazil. Recently, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), one of the largest funding agencies in Brazil started the Bioenergy Program BIOEN (http://bioenfapesp.org) aiming at integrating comprehensive research on sugarcane and other plants that can be used as biofuel sources, stimulating innovative and path changing research in cooperation with research institutions and private companies from other states of Brazil and from other countries.

Research on sugarcane aims at two broad objectives: increasing productivity and increasing sustainability. In Brazil productivity gains have been intense, especially since the establishment of the PROALCOOL Program in (1975) The average yield has increased from 46 to 75 t/ha, mostly through the development of customized varieties, using classical breeding, and through improvements in the agricultural process. As mentioned above, Paul Moore estimated from first principles that the potential yield for sugarcane is expected to be 472 t/ha (Waclawowsky et al. 2010). Considering that yields as high as 212 t/ha have been observed there seems to be room for improvement.
for improvement. Genomics and a systems biology approach might bring contributions to these developments.

A systemic approach seems to be one of the principal routes to be taken to understand functioning of the sugarcane plants, their integration with the environment and also the production processes that lead from the crop to ethanol. The bioenergy-producing system imposes an immense challenge that includes not only the scientific approaches of agriculture, physics, chemistry, biology, and engineering but also humanities approaches related to economy, health, and sociology. Producing bioethanol in the twenty first century is not an ordinary task as it has to be sustainable from all points of view. For that to happen, top level research will have to be undertaken. This will certainly include the use of synthetic biology strategies that will probably produce much better adapted plants with minimal environmental and social impacts and the development of new methods for converting sugars to biofuels and cellulose to sugars and then to biofuels. While the fermentation of sugar to ethanol is a process that has been well known for centuries, the production of other biofuels remains a scientific and technical challenge. A recent development has been that of engineering microbes to process sugars and secrete certain fuels, including gasoline, diesel, jet fuel, and others. Lee et al. (2008) reviewed several possibilities of metabolic engineering strategies for increasing yields of some biofuels. The technology depends on R&D in metabolic engineering and synthetic biology that might create new means for metabolic engineers to better understand how to adjust the cell pathways to create phenotypes with sufficient efficiency for the production of economically viable biofuels.

Sustainability issues became more prominent as biofuels came to be recognized as serious alternatives to oil, implying the possibility of large scale production. In this theme, land, water, and fertilizer use are relevant topics, as is the precise determination of GHG emissions reduction. In Land Use Change studies, a relevant challenge is to establish the behavior of soil organic carbon (SOC). This knowledge is essential for the correct determination of the GHG emissions balance for sugarcane ethanol. The behavior of SOC depends on the specifics of the land use change action. Table 21.5 shows the expected changes in SOC content related to sugarcane in the Brazilian Atlantic Region (Mello et al. 2006). Large gains in carbon sequestration seem to be obtainable through management modifications and by adequate choice of land. Of course, the contrary is true: for example, planting sugarcane in established forests will cause carbon emissions, as pointed out by Searchinger et al. (2008). It should be mentioned that most of the sugarcane expansion verified in Brazil has been over degraded pastures, and not over forest land (Goldemberg 2008). Much more R&D is necessary, mostly because the behavior of SOC is strongly dependent on specific characteristics of the crop, as shown by Anderson-Teixeira et al. (2009). Water usage is another issue that relates to the sustainability theme. Gerbens-Leenes et al. (2009) reviewed the literature and estimated the Water Footprint (WF) for several biofuels. They pointed out that the WF for

<table>
<thead>
<tr>
<th>Land-Use Change from</th>
<th>Land-Use Change to</th>
<th>Total Area (Mha)</th>
<th>Potential for SOC Sequestration (Tg/year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane, harvested with burning</td>
<td>Machine harvesting</td>
<td>3.3</td>
<td>5.35</td>
</tr>
<tr>
<td>Degraded pasture</td>
<td>Sugarcane without burning</td>
<td>1.93</td>
<td>0.19–1.54</td>
</tr>
<tr>
<td>Sugarcane harvested with burning</td>
<td>Reforestation</td>
<td>1.93</td>
<td>1.27</td>
</tr>
</tbody>
</table>

sugarcane ethanol is 108 m$^3$/GJ, second only to ethanol from sugar beet (59 m$^3$/GJ) and potato (103 m$^3$/GJ), however it must be remembered that both sugar beet and potatoes have poor GHG and energy balance for ethanol. One difficulty with this kind of estimate is that actual data depend strongly on specific properties of the crop and its management. All of these themes present challenges that require more R&D.

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