Chapter 21
Sugarcane: A Major Source of Sweetness, Alcohol, and Bio-energy

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Abstract Sugarcane is an important tropical crop having C4 carbohydrate metabolism which, allied with its perennial nature, makes it one of the most productive cultivated plants. It is mostly used to produce sugar, accounting for almost two thirds of world production. Recently it has gained increased attention because of its important potential for bio-fuel production. However, sugarcane has one of the more complex crop genomes, which has long hampered the development of sugarcane genetics to support breeding for crop improvement programs. Sugarcane belongs to the genus *Saccharum* L, part of the Poaceae familly (Grasses) and the *Andropogonae* tribe, which encompasses only polyploid species. With the advent of molecular genomics, the sugarcane genome has become less mysterious, although its complexity has been confirmed in many aspects. Shortcuts to genomic analyses have been identified thanks to synteny conservation with other grasses, in particular sorghum and rice. Over time, new tools have become available for understanding the molecular bases behind sugarcane productivity and a renewed interest has surfaced in its genetics and physiology.

21.1 Introduction

21.1.1 Economic, Agronomic, and Societal Importance of Sugarcane

Sugarcane has been the main plant source of sweetener for humans for several millennia. It is able to partition carbon to sucrose in the stem, a vegetative organ, in contrast with other cultivated grasses that usually accumulate their reserve products

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P.H. Moore, R. Ming (eds.), *Genomics of Tropical Crop Plants*

in seeds. This almost unique feature was selected by man who first used its soft watery culm for chewing.

Sugarcane belongs to the genus *Saccharum* L., which is part of the Poaceae family (Grasses) and the *Andropogonae* tribe. The reference, domesticated species for sugarcane is *Saccharum officinarum* (also called noble cane). *S. officinarum* is a group of thick, juicy canes that were initially cultivated in Southeast Asia and the Pacific Islands before spreading over the inter-tropics between 1500 and 1000 BC (Daniels and Roach 1987). In China and India, *S. officinarum* crossed with wild relatives to form the natural hybrids *S. sinense* (Chinese canes) and *S. barberi* (North Indian canes) that were then selected and cultivated. Sugar extraction probably developed in India and China from such hybrids (Daniels and Daniels 1975). Sugar manufacturing appeared in Persia around 500 AD. A few clones of *S. barberi* or hybrids between *S. officinarum* and *S. barberi* were probably taken from India via Persia in the 6th century, arriving in the Mediterranean and Spain by the 8th century. From there the Portuguese took it to Madeira in the 15th century, from whence it spread to other islands and West Africa. Sugarcane reached the Americas in 1493 when Columbus took it to the Dominican Republic, and the Portuguese planted it in Brazil in the early 16th century. In the 16th century, sugar production for world trade progressively changed from cottage industries based on *S. sinense* and *S. barberi*, to plantation and factory industries based on selected clones of *S. officinarum* (Daniels and Roach 1987). Near the end of the 19th century, *S. spontaneum*, a wild species producing no sugar, with thin stalks, as well as a few "North Indian" sugarcanes were used in Java and India in breeding programs aimed at overcoming disease susceptibilities affecting *S. officinarum*. Interspecific hybridization was the major breakthrough in modern sugarcane breeding. Hybridization not only solved many of the disease problems but it also provided increased yields, improved ratooning ability, and adaptability for growth under various abiotic stresses (Roach 1972). All modern sugarcane cultivars have been derived essentially from a few rounds of intercrossing from those first interspecific hybrids (Arceneaux 1967; Price 1965).

Commercially, sugarcane is propagated vegetatively via stem cuttings. Germination of the lateral buds produces new plants that branch into stools consisting of a large number of tillers. Under good growth conditions, the plant will grow 4–5 meters in 12 months, with the extractable culms measuring 2–3 meters and containing 13–16% sucrose. Because it is a perennial crop, after harvest and under the right growing conditions, underground buds will sprout giving rise to a new crop. In most situations, four to six crops are harvested before the yields become economically unsustainable and the field is renewed with the planting of a new crop.

Sugarcane is currently cultivated on more than 20 million hectares in tropical and subtropical regions of the world, producing up to 1.3 billion metric tons of crushable stems. It is mostly used to produce sugar, accounting for almost two-thirds of world production. Recently, it has gained increasing attention since one of its products, ethanol, has been publicized as an important source of renewable bio-fuel, which could turn it into a global commodity and an important energy source. Ethanol is an alcohol that can be produced from a variety of agricultural products and by-products and is probably the best known biofuel. Brazil already diverts half of its sugarcane

production to ethanol production and it will need to build more than 70 new mills and turn more than 2.5 million hectares of land over to sugarcane production to meet the demand for internal ethanol consumption (Pessoa et al. 2005). In addition, new technologies are emerging to convert cellulosic residues like bagasse and other agricultural byproducts, such as sugarcane trash (dry and green leaves and plant tops left in the field during harvest), into valuable commodities that would be degraded into small sugar molecules via either enzymatic or physical-chemical (or both) processes to be fermented into ethanol. These technologies are all in the scale-up phase and in the next few years will become commercial realities, changing the fate of cellulosic residues.

The economic importance of sugarcane and its main products to many countries in tropical and sub-tropical regions of the world has not always been met with significant investments for the research and development of new technologies to support the breeding programs and develop sugarcane genetics. One of the reasons for this is probably the complex nature of the sugarcane genome and the difficulties faced in selecting new, more productive cultivars in long selection programs that could take up to 15 years. With the advent of genomics, new tools have become available and a renewed interest in sugarcane genetics has surfaced (reviewed by D'Hont and Glaszmann 2001; Butterfield et al. 2001; Grivet and Arruda 2001; Ming et al. 2006).

21.1.2 Origin and Diversity of the Sugarcane Complex

The taxonomy of the sugarcane complex, based on morphology, chromosome numbers, and geographical distribution, has been controversial since the original classification of *Saccharum officinarum* by Linnaeus in 1753 (Daniels and Roach 1987; Daniels 1996; Irvine 1999). Recent molecular data are beginning to help trace the domestication and early evolution of sugarcane (review by Grivet et al. 2004, 2006) (Fig. 21.1).

A contribution by various genera other than *Saccharum*, particularly *Erianthus* (2n=20, 30, 40 and 60), *Miscanthus* (2n=38, 40, 76), *Sclerostachya* (2n=30), and *Narenga* (2n=30), to the emergence of sugarcane has been hypothesized by several sugarcane specialists (review in Daniels and Roach 1987). However, recent molecular data do not appear to confirm these hypotheses. Current extant species of the genera *Saccharum*, *Erianthus,* and *Miscanthus* are clearly distinct according to isozyme, nuclear, and cytoplasmic restriction fragment length polymorphism (RFLP) data (Glaszmann et al. 1989, 1990; Burnquist et al. 1992; Lu et al. 1994a; D'Hont et al. 1993, 1995; Besse et al. 1997), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) data (Selvi et al. 2004; Cai et al. 2005), and sequence data (Hodkinson et al. 2002). In addition, repeated species-specific sequences with multiple dispersed loci in the genome were cloned in *Miscanthus* and *Erianthus* and hybridized on the DNA of representatives of traditional cultivars and wild *Saccharum,* and no trace of these *Miscanthus* or *Erianthus* specific sequences was found in any of the individuals tested (Alix et al. 1998, 1999). Restriction fragment analysis of the chloroplast genome (Sobral et al. 1994)

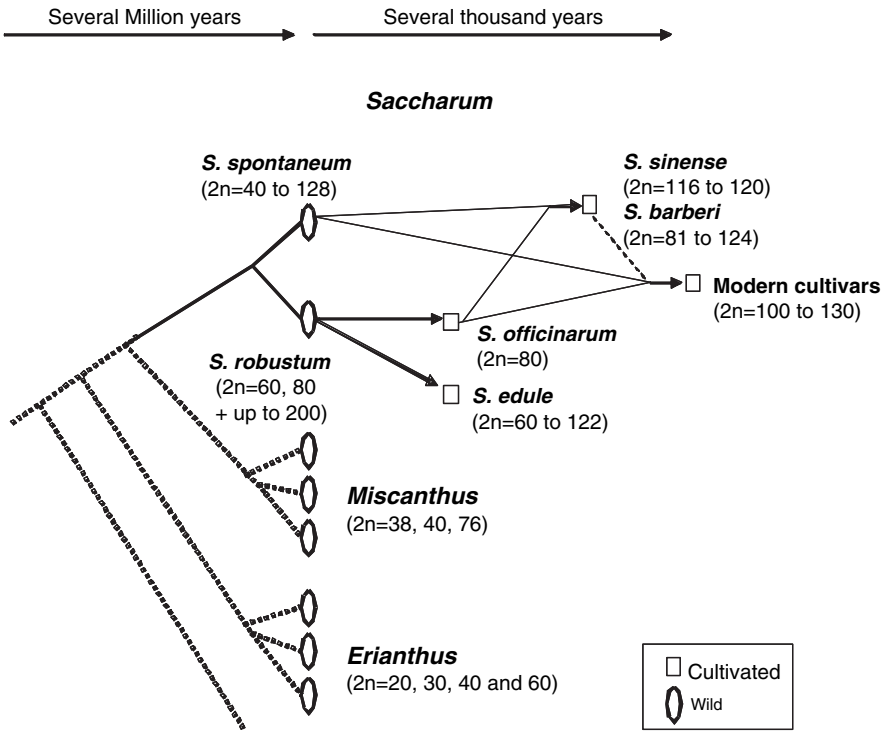

Fig. 21.1 Scenario compatible with available molecular data for sugarcane evolution and domestication (Adapted from Grivet et al. 2005)

and analysis of the nuclear repeated sequences (Alix et al. 1998, 1999) suggested that *Saccharum* is more closely related to *Miscanthus* than to *Erianthus*.

These data support the view that the genus *Saccharum* is a well-defined lineage that has diverged over a long period of evolution from the lineages leading to the *Erianthus* and *Miscanthus* genera (Grivet et al. 2006). Thus cultivated sugarcanes probably emerged from wild *Saccharum* species, and secondary introgressions with other genera are not likely pathways. However, this does not mean that natural intergeneric hybridizations are impossible and may not account for some local peculiarities. Artificial intergeneric hybrids with these genera have been produced (Chen et al. 1993; D'Hont et al. 1995; Piperidis et al. 2000).

The *Saccharum* genus includes six polyploid taxonomic groups often afforded species status: two wild species, *S. spontaneum* (2n=40 to 128) and *S. robustum* (2n= 60, 80 and up to 200); three groups of early cultivars, *S. officinarum* (2n= 80), *S. barberi* (2n=81–124), and *S. sinense* (2n=116–120); and the marginal sterile group, *S. edule* (2n= 2n = 60 to 122) (review in Daniels and Roach 1987; Sreenivasan et al. 1987).

S. spontaneum is characterized by thin stalks with no or very little sugar and has a huge geographic distribution from East Africa to Southeast Asia with a probable

continental Asia origin. *S. robustum* is characterized by long, thick stalks with little or no sugar and has been reported as occurring in natural populations in the Indonesian islands of Kalimantan, Sulawesi, and Maluku, in New Guinea, and in the Bismarck, Solomon, and Vanuatu archipelagos. These two wild species display different structural organizations of their monoploid genome (basic set of chromosome). This was suggested by the presence of polyploid chromosome series based on multiples of eight and ten, respectively. This has been confirmed by cytogenetic mapping of the ribosomal RNAs 45S and 5S by fluorescent in situ hybridization (FISH), which established basic chromosome numbers of x = 8 for *S. spontaneum* (D'Hont et al. 1998; Ha et al. 1999) and x = 10 for *S. robustum* (D'Hont et al. 1998). Molecular diversity is much greater in *S. spontaneum* than in *S. robustum.* Allopatric populations of *S. spontaneum* and *S. robustum* are clearly differentiated at the DNA level. Indeed, *S. spontaneum* samples from Kalimantan and Sumatra and *S. robustum* from New Guinea and Halmahera are strongly differentiated by their nuclear RFLP (Glaszmann et al. 1990; Burnquist et al. 1992; Lu et al. 1994a), cytoplasmic RFLP (D'Hont et al. 1993), AFLP (Selvi et al. 2004) and randomly amplified polymorphic DNA (RAPD) (Nair et al. 1999). Data addressing relationships between sympatric populations of *S. spontaneum* and *S. robustum* are still sparse. In New Guinea, all *S. spontaneum* individuals observed have the same cytotype, 2n = 80. D'Hont et al. (1998) showed that this cytotype is decaploid, with a typical *S. spontaneum* basic chromosome number of x = 8. However, field observations have shown a morphological continuum between extreme types, and some individuals presenting intermediate morphological characteristics between *S. spontaneum* and *S. robustum* are difficult to classify (Henty 1969). Moreover, a small sample of *S. spontaneum* individuals collected in New Guinea appears to be more closely related to *S. robustum* than to any other *S. spontaneum*, based on RFLP with nuclear low copy probes (Besse et al. 1997) and on the hybridization signal intensity of a repeated satellite sequence, SoCIR1 (Alix et al. 1998). This suggests that *S. spontaneum* populations from New Guinea are genetically closer to *S. robustum* than are the *S. spontaneum* populations west of Sulawesi.

Multiple lines of molecular evidence support a direct descent of *S. officinarum*, the domesticated sugarcane characterized by thick stalks, rich in sugar (also call Noble clones), from the wild species *S. robustum*. A single mitochondrial haplotype was detected among a series of *S. officinarum* clones (D'Hont et al. 1993). This haplotype is the most common haplotype detected in a collection of *S. robustum* clones from New Guinea and New Britain. It is also different from the six haplotypes revealed in a collection of *S. spontaneum* individuals sampled over a large geographic area. RFLP analysis of nuclear single copy DNA placed *S. officinarum* cultivars very close to *S. robustum*. The average similarity between a *S. officinarum* clone and a *S. robustum* clone is about the same as the average similarity between two *S. robustum* clones (Lu et al. 1994a). *S. officinarum* has a basic chromosome number of x = 10, as does *S. robustum* (D'Hont et al. 1998), and is octoploid like the most common cytotype (2n = 80) in the *S. robustum* wild species.

S. barberi and *S. sinense* have hybrid origins. RFLP with low copy nuclear DNA (Glaszmann et al. 1990; Burnquist et al. 1992; Lu et al. 1994a; Selvi et al. 2004)

and genomic in situ hybridization (GISH) (D'Hont et al. 2002) clearly show that *S. barberi* and *S. sinense* cultivars are the result of interspecific hybridizations between representatives of the two genetic groups of the *Saccharum* genus, *S. spontaneun* on one side and *S. officinarum* or *S. robustum* on the other. Since the *S. barberi* and *S. sinense* clones have sweet stalks and the region where they were formerly cultivated is outside the natural distribution range of *S. robustum*, the scenario of Brandes (1956) provides the simplest explanation for their origins: *S officinarum* cultivars were probably transported by humans to mainland Asia, where they naturally crossed with local *S. spontaneum* giving rise to *S. barberi* and *S. sinense* in India and China, respectively. It is likely that these clones are early-generation hybrids because no, or very few, interspecific chromosome exchanges were detected using GISH (D'Hont et al. 2002). This contrasts with the observations of higher levels of interspecific chromosome exchange in modern cultivars. The *S. barberi* and *S. sinense* cultivars that were tested have the mitochondrial haplotype of *S. officinarum*, indicating that this species was the maternal parent and wild *S. spontaneum* the paternal parent in the founding crosses (D'Hont et al. 1993). Low copy nuclear RFLP suggests that each morpho-cytogenetic group represents a set of somatic mutants derived from a single founding interspecific hybrid event (D'Hont et al. 2002). The Pansahi group, alias *S. sinense*, is not particularly distinct from the other groups according to nuclear RFLPs. The *S. barberi* and *S. sinense* cultivars are thus all derived from similar processes involving an interspecific hybridization event, followed by morphological and genetic radiation through mutation, which may have occurred in different geographic regions of continental Asia.

Few molecular data are available for tracing the origin of marginal group of *S. edule*. This group is grown in subsistence gardens from New Guinea to Fiji for its edible, aborted inflorescence; its large, thick-stalked canes contain no sugar. The mitochondrial haplotype has been established for a single clone. It was the same as the *S. officinarum*, *S. barberi*, and *S. sinense* cultivars and most of the *S. robustum* (D'Hont et al. 1993). An independent investigation based on chloroplast RFLP markers from another clone led to a similar conclusion (Sobral et al. 1994). These sparse data support the hypothesis that *S. edule* corresponds to a series of mutant clones, which were identified in *S. robustum* populations and were preserved by humans.

21.2 Genome Structure and Molecular Diversity of Modern Cultivars

21.2.1 Chromosome Structure

The origin of modern sugarcane cultivars is well known. However, their precise genomic structure has only recently been elucidated, thanks mainly to molecular cytogenetics. Modern cultivars are derived from several artificial interspecific

hybridizations between *S. officinarum*, used as the female, and *S. spontaneum* and, to a lesser extent, *S. barberi* as the pollen donor. F1 hybrids were then backcrossed to *S. officinarum* to recover a high-sugar-producing type species. This process was accelerated through the selection of hybrids derived from the 2n transmission of *S. officinarum* chromosomes (Bremer 1961). All present-day cultivars are derived from the interbreeding of these first interspecific hybrids. Altogether, it is estimated that 19 *S. officinarum* clones (four with high frequency), a few *S. spontaneum* (two with high frequency) clones, and one *S. barberi* clone were involved in these interspecific crosses (Arceneaux 1967).

Modern cultivars are thus highly polyploid and aneuploid, with about 120 chromosomes. GISH studies of chromosome preparations demonstrated that 15–25% of their chromosomes were inherited from *S. spontaneum*, and that the recombination between homoeologous chromosomes is possible (D'Hont et al. 1996; Piperidis and D'Hont 2001; Cuadrado et al. 2004). In cultivar 'R570', for example, 10% of the chromosomes are inherited in their entirety from *S. spontaneum*, 80% are inherited entirely from *S. officinarum,* and 10% are the result of recombination between chromosomes from the two ancestral species. In addition, as a consequence of the different basic chromosome numbers of *S. officinarum* and *S. spontaneum*, two distinct chromosome organizations coexist in current cultivars. The genome structure of a typical modern cultivar is represented in Fig. 21.2.

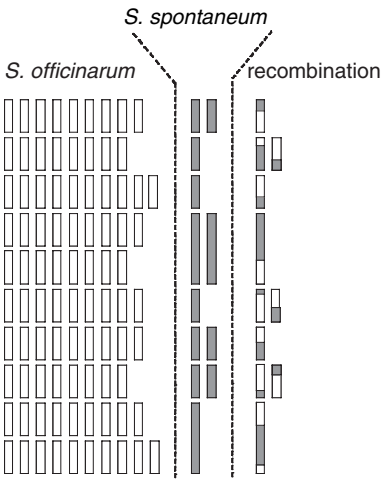

Fig. 21.2 Schematic representation of the genome of modern sugarcane cultivars as deduced from FISH and GISH experiments. Modern cultivars are highly polyploid and aneuploid with around 120 chromosomes. They are derived from interspecific hybridization between *S. officinarum* (2n=8x=80) and *S. spontaneum* (2n=5x to 16x=40 to 128). 10% to 20% of their chromosomes are inherited in their entirety from *S. spontaneum* (Grey bars); 70% to 80% are inherited entirely from *S. officinarum* (white bars) and around 10% are the result of recombination between chromosomes from the two ancestral species. In addition, as a consequence of the different basic chromosome numbers of *S. officinarum* (x=10) and *S. spontaneum* (x=8), two distinct chromosome organizations coexist in current cultivars

In diploids, genome sizes are generally given for the (non-replicated) gametic genome size (1C value), which in diploids corresponds to the size of the basic set of chromosomes (monoploid genome). However, in polyploids such as sugarcane, the gametic genome size (1C) value does not correspond to the size of the basic set of chromosomes. For this reason, in polyploids it seems more appropriate to refer to the genome size of (non-replicated) somatic cells (2C) or to the size of the monoploid genome.

The genome size in *S. officinarum* (2n=8x=80) has been estimated as 7.68 pg, which corresponds to 7440 mega base pairs (Mbp) for a somatic cell and to 926 Mbp for the monoploid genome (x=10) (D'Hont and Glaszmann 2001). For a *S. spontaneum* with 2n=8x=64, the genome size has been estimated as 6.30 pg, which corresponds to 6,080 Mbp for a somatic cell and to 760 Mbp for the monoploid genome (x=8) (D'Hont and Glaszmann 2001). With 760 to 926 Mbp, the size of the *Saccharum* monoploid (basic) genome is roughly double the monoploid genome size of rice (389 Mbp), similar to that of *Sorghum bicolor* Moench (760 Mbp), and significantly smaller than maize (2500 Mbp). The genome size of somatic cells (2C) of the typical modern cultivar R570 (2n= about 115) has been estimated as 10,000 Mb (D'Hont 2005).

21.2.2 Molecular Diversity

Low copy nuclear RFLP analysis showed that modern sugarcane cultivars are highly heterozygous, presenting multiple alleles at each locus (Lu et al. 1994b). Despite the low number of *S. offcinarum* involved in the interspecific hybridization at the origin of modern cultivars, more than 80% of the markers present in the whole *S. officinarum* samples studied by Jannoo et al. (1999a) and Selvi et al. (2005) were also found in modern cultivars. This is due probably to a high heterozygosity related to polyploidy (Jannoo et al. 1999a). Although the cultivars appear closely related to *S. officinarum* clones, the minor chromosome complement inherited from *S. spontaneum* seems to constitute the principal component of cultivar diversity (Lu et al. 1994b; Jannoo et al. 1999a). The sub-tropical cultivars seem to have retained a larger number of *S. spontaneum* markers than the tropical cultivars (Jannoo et al. 1999a; Selvi et al. 2005), reflecting the selection for different environments and resulting in numerous thinner stalks in subtropical regions and thicker stalks in tropical regions. SSR (microsatellite) markers are now routinely used in breeding programs for cultivar identification and progeny validation.

21.2.3 Linkage Disequilibrium

The small number of meiotic divisions since the first artificial crosses that gave rise to modern cultivars provided little opportunity to recombine founder chromosomes. Moreover, there were not many of these chromosomes, as there were only a few

founder individuals involved in the origin of modern cultivars. Consequently, a high level of linkage disequilibrium is still expected among modern cultivars. This was suggested by Lu et al. (1994b) and confirmed in a sample of Mauritian cultivars in which some chromosome haplotypes are significantly conserved over regions as long as 10 cM (Jannoo et al. 1999b). This is an important finding because it may offer original and powerful perspectives to identify and locate genes that are involved in traits of interest. Polyploidy may greatly hamper this task, but the huge level of linkage disequilibrium, compared to that estimated for the human genome, for example, may offer some advantages. In particular, the density of markers needed for genetic mapping may be quite low. Recent work based on AFLP and diversity arrays technology (DArT) are paving the way to this type of application (Raboin, Pauquet, and Butterfield personal communication).

21.3 Genetic Mapping and Synteny

21.3.1 Genetic Maps

Polyploidy dictates particular constraints for mapping, which have been theoretically developed by Wu et al. (1992). When polyploidy is high and pairing is polysomic or irregular, such as in sugarcane, multiple bands are identified by a DNA probe or a pair of primers, and alleles with different dosage level segregate. In this context, alleles that are present as single copies are much more informative for the construction of genetic maps than any others. Using molecular marker technologies such as RAPD, AFLP, and RFLP markers, partial genetic maps have been produced for *S. spontaneum* (da Silva et al. 1993, 1995; Al-Janabi et al. 1993; Ming et al. 1998 *S. officinarum* (Guimarães et al. 1999; Ming et al. 1998 *S. robustum* (Guimarães et al. 1999; Ming et al. 1998 and modern cultivars (D'Hont et al. 1994; Grivet et al. 1996; Hoarau et al. 2001; Rossi et al. 2003; Aitken et al. 2005; Reffay et al. 2005; Raboin et al. 2006 (http://tropgenedb.cirad.fr/).

Co-dominant markers such as RFLP and SSR can reveal several alleles of the same locus and are thus very useful in the identification of co-segregating groups that correspond to hom(oe)ologous chromosomes. Developing a saturated, low-density genetic map for sugarcane requires much more work than for a diploid; for a given level of molecular diversity, the effort required to simultaneously distinguish ten or so haplotypes is much greater than that needed to distinguish only two. At the moment, none of the published genetic maps of sugarcane is saturated. About one half of the genome is estimated to be tagged onto the most refined maps (Rossi et al. 2003; Aitken et al. 2005). As for maps of current cultivars, marker coverage is uneven, with *S. spontaneum* chromosomes being covered more densely than those of *S. officinarum*.

At meiosis mainly bivalents are observed in *S. officinarum*, *S. robustum*, *S. spontaneum,* and interspecific cultivated clones (review by Sreenivasan et al. 1987). Mapping data suggest that pairing behavior probably does not fit any pre-established,

clear-cut scheme, such as complete disomy or complete polysomy. In *S. robustum* (MOL5829, 2n=80), a high proportion of preferential pairing (50%) in the few co-segregation groups already defined was reported (Al-Janabi et al. 1994; Ming et al. 1998). In *S. officinarum,* some preferential pairing was also observed, whereas no preferential pairing was found in *S. spontaneum* (Al-Janabi et al. 1994; Ming et al. 1998). In the cultivar R570, Grivet et al. (1996) and Hoarau et al. (2001) observed a general polysomy with several cases of preferential pairing and suggested the possibility of complete local disomy. In R570, the preferential pairing detected concerned chromosomes of *S. officinarum, S. spontaneum,* as well as interspecific-recombinant origin. Jannoo et al. (2004) took advantage of a particular single copy probe (BNL 12.06) revealing 11 alleles by RFLP in cultivar R570. They determined the doses of the various BNL12.06 RFLP alleles among 282 progeny of R570 and estimated the mutual pairing frequencies among the corresponding homo- or homoeologous chromosomes using a maximum likelihood method. The result is an atypical picture, with pairing frequencies ranging from 0 to 40% and differential affinities leading to the identification of several chromosome subsets. It highlights a continuous range of pairing affinities between chromosomes and pinpoints a strong role of individual chromosome features, partly related to their ancestral origin, in the determination of these affinities (Jannoo et al. 2004).

21.3.2 Tagging Genes of Interest

An important application of genetic maps is the location on the genome of loci that contribute to the variation of phenotypic traits. Only three major genes have been mapped until now, two rust resistance genes (Daugrois et al. 1996; Raboin et al. 2006) and one gene responsible for stalk color (Raboin et al. 2006). Quantitative trait loci (QTL) detection is complicated by the potential for segregation of several (potentially up to 12 in a modern cultivar) alleles at a locus and by the lack of preferential pairing. As a consequence, different parental alleles are not mutually exclusive alternatives. For the subset of polymorphic alleles that show simplex segregation ratios, the effect of an allele can be estimated from the average phenotypic difference between the two possible genotypes (presence versus absence).

A QTL experiment was conducted with two interspecific *S. offinarum* x *S. spontaneum* crosses to investigate variation in the sucrose content of the stalk populations (Ming et al. 2001, 2002a). Many independent segregating alleles were identified and could be assigned to eight distinct loci. In several cases, the presence of several alleles at a locus that contributed to the variation of a trait, demonstrated that strong interaction effects between alleles could lead to an important buffering effect. Large-scale QTL mapping was also conducted for the modern cultivar R570 (Hoarau et al. 2002). The effects of individual QTLs were small for all of the traits investigated, always accounting for less than 7% of the phenotypic variance, and the size of the effects was not conserved across crop cycles. Additional QTL experiments using modern cultivars were conducted, revealing, in general, rather small

effects of individual QTLs (Reffay et al. 2005; McIntyre et al. 2005). Polyploidy is going to challenge the application of markers in sugarcane more than in any other crop, and improved biometrical methods are needed to extract full information from the QTL-detection experiments.

Multiplex segregation at QTL loci may be partly responsible for phenotypic buffering, which is an important factor in the success of many autopolyploid crops. Non-additive gene action in multiple dose QTLs may also have contributed to evolutionary opportunities. For example, if a single copy of a gene/QTL is physiologically sufficient, the additional copies may be free to collect mutations, often becoming nonfunctional, perhaps occasionally resulting in a distinctive new function that improves fitness.

21.3.3 Synteny with Other Grasses

Due to its high polyploidy and the absence of diploid close relatives, the advantage of investigating synteny conservation between sugarcane and other grasses, in particular with other members of the Andropogoneae tribe, was realized early. The first comparisons were made with maize, which had, at that stage, a more advanced map. Gaut et al. (2000) confirmed synteny to be conserved (D'Hont et al. 1994; Grivet et al. 1994; Dufour et al. 1997), although quite perturbed by the duplicated structure of the maize genome and the presence of many rearrangements. Rice also showed relatively global simple synteny relationships with sugarcane with, however, many rearrangements explained by the large distance between the two species (Glaszmann et al. 1997). Rice remains an interesting model for sugarcane because the sequence of its genome is available (International Rice Genome Sequencing Project 2005) and because large numbers of rice mutants are being collected. To date, of the species studied, sorghum appears to have the simplest synteny relationship with sugarcane (Grivet et al. 1994; Dufour et al. 1997; Glaszmann et al. 1997; Guimarães et al. 1997; Ming et al. 1998), and thus more adapted to help with sugarcane studies (Asnaghi et al. 2000). Corresponding QTLs controlling plant height, stalk number, and flowering were found in sorghum and sugarcane (Ming et al. 2002b; Jordan et al. 2004). The availability of the entire sorghum genome sequence in the near future (Sorghum Genomics Planning Workshop Participants 2005) will be of great interest to sugarcane genomics, in particular for map-based gene isolation.

Co-linearity has been employed to help develop a fine map around a gene conferring resistance to brown rust (*Bru*1), which is the focus of a map based cloning approach using the cultivar R570. Sorghum and rice regions orthologous to the sugarcane target area were identified and markers derived from sorghum genetics (Boivin et al. 1999; Bowers et al. 2003), physical maps (http://genome.arizona.edu/genome/sorghum.html), and a comparison of sugarcane cDNAs with the rice orthologous sequence (http://www.genome.arizona.edu/fpc/rice/), were used to saturate the sugarcane map in the target region (Asnaghi et al. 2000, 2004; D'Hont unpublished data).

21.4 BAC Library Development and Utilization

A bacterial artificial chromosome (BAC) library of 103,296 clones, with a mean insert size of 130 kbp, has been constructed for the sugarcane cultivar R570 (Tomkins et al. 1999). On the basis of the monoploid genome size of sugarcane the coverage is estimated to be 14x. However, since sugarcane is highly polyploid and heterozygous, this represents only the coverage of 1.3x of the total genome of this sugarcane cultivar.

This BAC library is currently being used to develop a physical map of the region bearing the rust resistance gene *Bru*1 in cultivar R570 and to perform comparative genomic studies within sugarcane as well as with other grasses.

Two homoeologous BAC clones (97 kb and 126 kb), one derived from *S. spontaneum* and one from *S. officinarum,* corresponding to a region that has already been studied in several cereals (Ilic et al. 2003), were sequenced and compared (Jannoo et al. 2007). The results indicated that the two *Saccharum* species diverged by 1.5–2 mya from one another and 8–9 mya from sorghum. The two sugarcane homoeologous haplotypes showed perfect co-linearity and also high homology along the non-transcribed regions, apart from the insertion of a few retro-transposable elements. The gene distribution highlighted high synteny and co-linearity with sorghum and rice, and partial co-linearity with each homoeologous maize region, which became perfect when the sequences were combined. This first analysis of sugarcane haplotype organization at the sequence level suggested that the high ploidy in sugarcane did not induce generalized reshaping of its genome, thus challenging the idea that polyploidy quickly induces generalized rearrangement of genomes. These results also consolidated the fact that sorghum is a choice model for sugarcane.

21.5 Functional Genomics

21.5.1 EST Development

The large sugarcane genome, in which, on average, each single-copy gene is represented by ten alleles, represents a challenge for genetic analysis. Expressed sequence tag" (EST) collections may contribute significantly to identify candidate genes associated with important agronomical traits (i.e., tolerance to abiotic and biotic stress, mineral nutrition, and sugar content, amongst others) and more generally to provide relevant information for functional and evolutionary analyses.

Several sugarcane EST collections have been developed (Carson and Botha 2000, 2002; Casu et al. 2001, 2003; Ma et al. 2004; Bower et al. 2005), the largest one being the Brazilian sugarcane EST project, SUCEST (Vettore et al. 2003). All of the publicly available sugarcane sequence ESTs were assembled into tentative consensus sequences (virtual transcripts), singletons, and mature transcripts, referred to as the Sugarcane Gene Index (SGI; http://compbio.dfci.harvard.edu/tgi/cgi-bin/tgi/gimain.pl?gudb=s_officinarum). The SUCEST collection of ESTs was assembled

into 43,141 putative unique sugarcane transcripts referred to as sugarcane assembled sequences (SAS) http://sucest.lbi.ic.unicamp.br/public/; (Vettore et al. 2003).

A comparative analysis between the SASs and the DNA and protein sequences from the model eudicotyledon plant *Arabidopsis* thaliana, model monocotyledon rice, and a set of other angiosperms was undertaken. Three main classes of sequences were identified, a core set of eudicot - monocot sequences that may represent angiosperm basic functions (75% of the SASs), monocot-specific sequences (14% of the SASs), and sequences restricted to sugarcane (13.5% of the SASs). A significant proportion of the monocot-specific sequences were found to represent fast-evolving sequences integrated in members of conserved angiosperm gene families. This observation was particularly relevant since a high rate of evolution may be related to a functional diversification that could be involved in the differentiation of specific evolutionary lineages. New protein architecture formed by monocot-specific motives or domains that were recruited into conserved eudicot-monocot proteins and long non-coding RNA (~500 nucleotides) were also identified among the monocot-specific sequences (Vincentz et al. 2004; Vincentz, unpublished results). The divergence between monocot and eudicot may therefore rely partly on functional diversification (generating new protein functions) from duplicated copies of conserved gene families. The extent to which the sugarcane-specific SASs represent novelties restricted to this organism remains unclear.

Several studies have described the SUCEST sequences and discussed their putative roles, in particular for genes that correspond to the general metabolism of sugarcane, signaling growth, development and stress responses (Grivet and Arruda 2001; Arruda 2001). Over 30% of the identified transcripts corresponded to genes with no sequence similarity to known genes. The identification of novel gene functions in such a complex organism is a formidable task. Ongoing efforts to associate putative functions with the sugarcane genes include gene expression profiling of tissues, identification of genes associated with sucrose content in cultivars showing contrasting Brix values, comparing different varieties submitted to biotic and abiotic stress, as well as mapping efforts to associate genes with phenotypes, as described above.

21.5.2 Tissue Profiling

The use of gene chips and cDNA micro-arrays allows both temporal and spatial gene expression data to be obtained. Custom designed cDNA micro-arrays constructed using the cDNA collection from the SUCEST database (http://sucest-fun.org), were used to determine the distribution of gene transcripts in sugarcane tissues and to define tissue-specific activities and ubiquitous genes. Using cDNA micro-arrays containing 1,280 distinct elements, the individual gene expression variation of plants grown in the field and transcript abundance in six plant organs (flowers, roots, leaves, lateral buds, 1st [immature] and 4th [mature] internodes), was analyzed (Papini-Terzi et al. 2005). The expression of 217 genes was found to be tissue-enriched, while 153 genes showed expression levels that were highly

similar in all the tissues analyzed. A virtual profile matrix was constructed where the tissue expression levels can be compared amongst 24 tissue samples. Most of the genes characterized coded for signal transduction components, hormone biosynthesis, transcription factors, stress and pathogen response-related genes. A catalogue of sugarcane signal transduction and other regulatory genes can be found at the SUCAST Database (http://sucest-fun.org). The database integrates the gene catalog, the information generated by the phylogenetic categorization of the sugarcane kinome, tissue matrix, sequencing data, and analyses by the SUCEST Project. Tissue expression information can also aid in the identification of gene promoter sequences.

21.5.3 Exploitation of EST Resources for Functional Analysis

21.5.3.1 Sugar Synthesis, Transport, and Accumulation

Sucrose synthesized in sugarcane leaves is stored in the culms. Sugarcane has the striking ability of accumulating high levels of sucrose that can reach up to about 0.7 molar in mature internodes (Moore 1995), which corresponds to approximately 50% of the stalk dry weight. This physiological specialization makes sugarcane an interesting model for studies on sugar synthesis, transport, and accumulation.

Carbohydrates are synthesized in sugarcane leaves by CO_2 fixation during photosynthesis (Moore 1995; Lunn and Furbank 1999; Grof and Campbell 2001). In C4 plants, such as sugarcane, CO_2 is fixed by phosphenolpyruvate carboxylase in mesophyll cells, producing oxaloacetate, which is reduced to malate. In the bundle sheath cells, the malate is decarboxylated, the CO_2 being released and re-fixed by Rubisco in the photosynthetic carbon reduction (PCR) cycle. The resulting glyceraldehyde 3-phosphate molecules are the substrate for a multi-step pathway leading to the synthesis of sucrose, in which the activities of fructose-1,6-bisphosphatase (FBPase) and sucrose-phosphate synthase (SPS) play a major control role. Sucrose is thought to be synthesized exclusively in the mesophyll, and then transferred to phloem cells, where it is transported to the stem parenchyma cells (Grof and Campbell 2001). Besides these leaf reactions, Grof and Campbell (2001) highlighted three major rate limiting or co-limiting steps: the rate of transport to the stalks, including phloem loading; the rate of transport into the parenchyma cells and into their vacuoles; and the rate of sucrose mobilization used for the vegetative growth. It is supposed that once these limitations are solved, commercial yields could be doubled (Grof and Campbell 2001 and references cited therein).

It is known from several plant species that sugar transport relies on both symplastic and apoplastic steps (Patrick 1997; Lalonde et al. 2004). An evaluation of the SUCEST database revealed full-length genes encoding nine monosaccharide and four dissacharide transporters (Felix 2006). Casu et al. (2003) performed an EST survey comparing transcripts from immature and mature internodes. Several transcripts encoding proteins homologous to known sugar transporters were found, and all of them were more abundant in the mature internodes. Micro-array and northern blot analyses showed that a putative sugar transporter, type 2a, was highly

expressed in mature internodes and absent in mature leaf and root. However, the sugar transported by this protein remains elusive. Rae et al. (2005) cloned the sugarcane *ShSUT1* gene, which encodes a transporter that may play a role in the transfer of sucrose from the vascular tissue to parenchyma cells of internodes. Since sink strength regulates photosynthesis in sugarcane (McCormick et al. 2006), the evaluation of sugar transporter genes in transgenic sugarcane plants will certainly help to assess their role in sugar accumulation in the internodes.

To analyze the genes expression during culm development, a collection of 7,409 ESTs from maturing sugarcane stems in combination with a smaller collection (1,089) of ESTs from immature stems (Casu et al. 2001, 2003, 2004) were analyzed by bio-informatics and using cDNA micro-arrays, allowing for the identification of genes that were differentially regulated with respect to stem maturity. These studies indicated that genes associated with sucrose metabolism were not abundantly expressed in stem tissues and that genes related to the synthesis and catalysis of sucrose were down-regulated as the stem matured and the sucrose concentration increased. In a similar approach, the use of sugarcane GeneChips from Affymetrix (Lockhart et al. 1996) containing approximately 6,024 distinct *S. officinarum* genes, led to the discovery that genes involved in cellulose synthesis, cell wall metabolism, and lignification were developmentally regulated during culm maturation (Casu et al. 2007).

Gene activity associated with internode development was also compared between a non-sucrose accumulating genotype from the species *S. robustum* and two high sucrose accumulating genotypes (an *S.officinarum* genotype and a hybrid cultivar) (Watt et al. 2005). Using nylon arrays containing 88 ESTs, gene expression variations associated with stalk development and those pertaining to sucrose accumulation were investigated. In mature internodes of all three genotypes, transcript-relative activities were found to decrease for the cell wall biosynthesis genes and increase for the sucrose metabolism-related genes. The most notable differences were represented by increased activity of sucrose synthase-1 and sucrose phosphatase. Mature and immature culm samples were also analyzed using cDNA micro-arrays containing 1,228 elements in common with the array used by Papini-Terzi et al. (2005) (mentioned above), plus an additional 317 elements including 229 kinases representatives of the sugarcane kinome. Sucrose accumulating internodes (sink tissues) were collected from field grown plants contrasting for Brix. In some cases, samples were collected throughout the year (Papini-Terzi et al. 2007). Genes identified as developmentally regulated during culm maturation included hormone signaling (auxin, ethylene, jasmonate, salicylic acid), stress responses, sugar transport, lignin biosynthesis and fiber content.

To identify genes associated with sucrose content, a strategy introduced by Jansen and Nap (2001), which involved the large-scale analysis of gene expression in a segregating population, was applied to sugarcane populations segregated for soluble solids content (Brix). Using cDNA micro-arrays containing 4,715 genes, 62 genes, mostly unrelated with sucrose metabolism, were identified as associated with sucrose content (Casu et al. 2005). In a similar work, cDNA micro-arrays containing 1,545 elements were used, and over 100 genes found to be differentially expressed

for high sugar, as compared to low sugar genotypes (Papini-Terzi et al. 2007). The differentially expressed genes identified belonged to several functional categories including calcium signaling, stress responses, transcription, and ubiquitination. The categories with the highest number of hits included protein kinases from the SNF-related family of kinases, auxin hormone signaling, the Cyp family of cytochrome P450 monoxygenases, and other stress-related genes.

The expression profile of genes associated with signal transduction was also evaluated in leaves from high sugar and low sugar sugarcane plants from an F1 progeny selected from a cross between the sugarcane varieties SP 80–180 and SP 80–4966 (Felix 2006). Twenty-four genes were differentially expressed between high and low sugar plants. Five had higher transcript levels in high sugar plants, including an omega-3 fatty acid desaturase putatively involved in methyl jasmonate (MeJa) signaling, a putative receptor-like serine/threonine kinase, and an Myb domain transcription factor. Most of the genes had higher expressions in low sugar genotypes, such as those encoding three 14-3-3 like proteins and an SNF1-related protein. A homologue of this protein phosphorylates the enzyme SPS in vitro (Sugden et al. 1999), making it a putative target to interact with 14-3-3 proteins, which in turn reduces the SPS activity (Toroser et al. 1998; Huber et al. 1998).

The efficiency and control of carbon fixation and allocation, which is affected by sink strength (Watt et al. 2005), may be regulated at the source tissues. Ma et al. (2004) investigated gene expression in source tissues using EST analyses, and more recently, serial analysis of gene expression (SAGE) was used (Calsa and Figueira 2006). Sugarcane, as well as maize and sorghum, was considered to operate under the NADP-malic enzyme (NADP-ME) pathway (Bowyer and Leegood 1997), although a highly expressed photosynthesis-related phosphoenolpyruvate carboxykinase (PEPCK) had already been detected and validated in maize leaf bundle sheath cells (Furumoto et al. 1999, 2000). C4 grasses such as sugarcane, maize, and sorghum, contain anatomical and physiological adaptations to optimize CO_2 fixation for carbohydrate biosyntheses (Brown et al. 2005). Basically, three C4 photosynthetic primary carbon cycle pathways have been described that differ in the four-carbon organic acid intermediate transported from the mesophyll to the bundle sheath cells (malate and/or aspartate), in the three-carbon acid returned to the mesophyll cells (pyruvate or alanine), as well as the decarboxylation enzyme present in the bundle sheath cells, which can be either NADP-ME, NAD+malic enzyme (NAD-ME), or (PEPCK) (Taiz and Zeiger 1998). The combined SAGE and real time quantitative PCR (RT-qPCR) results (Calsa and Figueira 2006) suggested that PEPCK decarboxylation appeared to predominate over NADP-ME in mature field-grown sugarcane leaves, in contrast to the conventional NADP:ME model accepted for sugarcane, although both may occur.

21.5.3.2 Responses to Environmental Challenges

Plants react to changes in the environment through an array of cellular responses that are activated by stress stimuli, leading to plant defense and/or adjustment to adverse conditions. Physiological changes elicited by external signals can be modulated by

transcriptional regulation resulting in the induction or repression of target genes. Studies have been conducted to unravel the responses of sugarcane to biotic and abiotic stress and the role of phytohormones in these processes.

Drought

Drought is a condition of special interest with respect to sugarcane, since water scarcity conditions prevent the expansion of this culture to vast areas in tropical regions. To identify differentially expressed genes in response to hydric stress, cDNA microarrays representing 1,545 genes were used (Rocha et al. 2007). Among the differentially expressed genes, regulators of drought-responsive genes such as the WRKY and MYC transcription factors (Abe et al. 1997) were identified. Cold and drought signaling overlap and many of the responses are mediated by the phytohormone ABA. Accordingly, low temperature-induced (LTI) proteins were seen to be up-regulated in response to lack of water in sugarcane, and genes induced by ABA have also been found to be induced by drought, including two delta-12 oleate desaturases, one S-adenosylmethionine decarboxylase, and a protein phosphatase ABI1/ABI2 (Tahtiharju and Palva 2001) that regulates stomatal closure. A sugarcane transcription factor homologous to rice DREB2 was induced and may represent an important transcription factor for the regulation of sugarcane drought responses, since the over-expression of DREB2A in *Arabidopsis* led to the development of plants tolerant to drought (Sakuma et al. 2006). Other genes identified include an S-adenosylmethionine decarboxylase, known to accumulate in response to salinity and drought (Li and Chen, 2000) and fatty acid desaturases (FAD2), directly related to drought tolerance (Zhang et al. 2005; Im et al. 2002).

Cold

Cold stress, which includes low temperatures above (chilling) and below (freezing) 0°C, causes severe losses of most crop plants, due to the formation of extracellular ice (Xin and Browse 2000). Sugarcane is considered to be a cold-sensitive crop (Tai and Lentini 1998), and although sugarcane fields are restricted to tropical and subtropical regions, cold stress is not unusual in these areas, decreasing sugar productivity. Nogueira et al. (2003) evaluated the gene expression profile in sugarcane plantlets exposed to 4°C. Thirty-four cold-inducible genes and 25 cold-repressed genes were found. Based on these data, a model of sugarcane response to cold stress was proposed. In their model, several transcription factors, such as an ABI3-interacting protein 2, an OsNAC6 and an OCSBF-1, regulate the transcription of proteins involved in the protection against oxidative stress, sugar transporters, protein degradation and cell wall synthesis. These genes could be good targets for study and to possibly improve sugarcane cold tolerance.

Phosphorus Deficiency

Phosphorus (P) is an essential nutrient because it is used in a large number of biological processes, from nucleic acids biosynthesis to the regulation of enzyme

activities. Plants take up P as inorganic phosphate (Pi), and have developed several strategies to cope with the low availability of Pi in the soil, which is usually in the range from 2 to 10 mM (Raghothama 1999). Sugarcane is a crop that performs well in acid soils, indicating the use of strategies to overcome P deficiency. To access the expression profile of genes in response to P starvation, Rocha et al. (2007) evaluated sugarcane plantlets grown in the absence of P. Surprisingly, only genes repressed due to P starvation were found. The expression profile obtained pointed to changes in protein N-glycosylation and redox status due to an altered expression of an N-acetylglucosamine-1-phosphate transferase and two thioredoxins. Genes homologous to an MYB transcription factor and an ethylene insensitive-like (EIL) transcription factor putatively involved in the ethylene response were also repressed. These data indicated that under low levels of the nutrient, sugarcane roots might be under severe metabolic restraint, in line with the observations in *Arabidopsis*, where genes related to photosynthesis were repressed in response to Pi starvation (Wu et al. 2003).

Herbivory

Insect pests frequently challenge sugarcane productivity. Even though, over the last few decades, highly productive sugarcane cultivars with enhanced insect pest resistance have been developed in conventional breeding programs, modern cultivars appear to retain a lower degree of resistance when compared to wild-type genotypes. The availability of insect-control genes that could be genetically engineered to obtain pest resistant varieties is of significant interest. In a search for sugarcane orthologs of genes that are potential targets for the management of insect resistance, Falco et al. (2001) identified, among the SUCEST sequences ESTs coding for proteinase inhibitors, alpha-amylase inhibitors, lectins, chitinases, and polyphenol oxidases. In this study, putative systemic and constitutive wound response proteins were identified.

The sugarcane borer *Diatraea saccharalis* is the major sugarcane pest in Brazil, causing plant death due to apical bud death (dead heart) in up to four-month-old plants and damage to lateral bud development, aerial rooting, weight loss, and stalk breakage in older plants. The attack also allows for infection by opportunistic fungi, which results in production losses for both the sugar and alcohol industries (Braga et al. 2003). The expression profile of a variety highly susceptible to the borer was obtained in response to the insect attack using cDNA micro-arrays (Rocha et al. 2007). The expression data indicated a strong induction of a pathogenesis-related protein similar to thaumatin, 24 h after the onset of this stress. These proteins are important for plant defense mechanisms and may present anti-fungal action, endo-β1,3-glucanase activity, and trypsin or α-amylase inhibitory activity (Grenier et al. 1999; Franco et al. 2002). Further characterization of this sugarcane thaumatin-like protein should be carried out to define its activity and the defense mechanism that it may trigger against the sugarcane stalk borer.

Endophytic Bacteria

In Brazil, the long-term continuous cultivation of sugarcane with low N fertilizer inputs, without apparent depletion of the soil-N reserves, led to suggestions that N_2-fixing bacteria associated with the plants might be the source of agronomically significant N inputs for this crop. Years of study led to the conclusion that the diazotrophs that infected the interior of the plants, such as the 'endophytic diazotrophs' were responsible for the increased nitrogen contribution to Brazilian soils (Boddey et al. 2003). Diazotrophic acetobacters were also isolated from sugarcane roots or soil collected from four regions in Queensland, Australia (Li and Macrae 1991). However, biological nitrogen utilization seems to be restricted to some cultivars and regions. In South Africa for instance, it was shown that biological nitrogen fixation did not contribute to the nitrogen demand of a commercially grown cultivar (Hoefsloot et al. 2005).

In Brazil, sugarcane culture benefits considerably from its association with N_2-fixing endophytic bacteria (*Herbaspirillum seropedicae / Herbaspirillum rubrisubalbicans* and *Gluconacetobacter diazotrophicus*). Unlike rhizobium/leguminosae symbiosis, where the bacteria are restricted to nodules, *Herbaspirillum* spp. and *G. diazotrophicus* are endophytic, and colonize the intercellular spaces and vascular tissues of most plant organs, without causing damage to the host (James and Olivares 1998; Rheinhold-Hurek and Hurek 1998). These bacteria possibly promote plant growth by nitrogen fixation and also by the production of plant hormones (Sevilla et al. 2001). Despite the non-pathogenic aspects of this interaction, plants should limit bacterial growth inside their tissues to avoid disease development (Olivares et al. 1997). It is believed that sugarcane plants recognize these microorganisms and activate defense responses until the establishment of an efficient association (Vinagre et al. 2006). Using cDNA micro-arrays, four resistance gene analogs were found to be responsive to the endophytic association (Rocha et al. 2007). Plant disease resistance genes mediate specific recognition of pathogens via the perception of avirulence gene products (review by Ellis et al. 2000). Two resistance gene analogs were induced on account of the association with both *Herbaspirillum* and *Gluconacetobacter diazotrophicus*. Inoculation with *Gluconacetobacter* also led to the induction of a salicylic acid biosynthesis gene. Salicylic acid accumulates in plant tissues in response to pathogen attack, and is essential for the induction of systemic acquired resistance and for some responses mediated by resistance genes (Gaffney et al. 1993; Delaney et al. 1994; Mur et al. 1997). The expression of a PP2C and five transcription factors was altered when the plants were cultivated in association with endophytic bacteria. Amongst these, there were two zinc-finger transcription factors, one of which was up regulated by inoculation with either *Gluconacetobacter* or *Herbaspirillum*. A possible role for phosphatases and zinc-finger transcription factors in response to endophytic bacteria has also been pointed out by the in silico analysis of ESTs, that identified a SAS corresponding to these categories, exclusively or preferentially expressed in the cDNA libraries constructed from plants inoculated with *Gluconacetobacter* and *Herbaspirillum* (Vargas et al. 2003).

21.5.3.3 Phytohormone Signaling

Hormones such as methyl-jasmonate (MeJA) and abscisic acid (ABA) are key regulators of mechanisms that integrate plant responses to internal and external stimuli. Gene expression changes in response to these hormones have been evaluated in sugarcane (Bower et al. 2005; De Rosa et al. 2005; Rocha et al. 2007). MeJa induced several genes encoding protein homologues related to phytohormone signaling, including an MYB transcription factor and a receptor-like protein in sugarcane roots (Bower et al. 2005) and a zinc finger protein, a heat shock factor, and a protein kinase in young sugarcane leaves (De Rosa et al. 2005). In a survey of most of the sugarcane homologues for known genes related to hormone signaling, Rocha et al. (2007) found that MeJa induced the expression of protein kinases, an MYB transcription factor, and an NAC protein, and repressed another protein kinase. ABA induced the expression of genes encoding homologues to two receptor Ser/Thr kinases, a phosphatase and a small GTPase, while a protein kinase homologue was repressed. Schlögl et al. (2006) evaluated the expression profile of the whole set of known b-ZIP transcription factors in response to ABA and MeJa. Two bZIPs were induced by ABA and four were repressed, while two others were induced by MeJa.

21.5.3.4 Transposon Expression

Retrotransposons mobilize themselves through an RNA intermediate and 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. Recently, a hypothesis on the impact of transposable elements (TE) on genomic structure, gene regulation, and even on function has been proposed (Casacuberta and Santiago 2003; Kashkush et al. 2003; Bundock and Hooykaas 2005). Twenty-one different families of TEs were identified in the SUCEST collection, of which 54% correspond to classical transposons and 46% to retrotransposons (Rossi et al. 2001). Further studies to validate the expression profile of the TE families identified were developed, which confirmed that the callus is the tissue with more expressed TE families (Araujo et al. 2005). Although it has been proposed several times that tissue culture somaclonal variation could be a result of TE activity, this is the first report that demonstrates that callus is indeed a tissue where different TEs are expressed at the same time. Focus on particular sugarcane families highlighted the existence of lineages of elements with diverse levels of representation in the genome (Rossi et al. 2004).

21.6 Genetic Engineering

Genetic transformation has been extensively used to produce commercial varieties of a number of different crops such as soybeans, corn, and cotton, expressing traits such as herbicide and insect resistance, resulting in improvements in the farmers'

incomes and a decrease in the use of pesticides (http://www.isaaa.org) over the last 10 years. Besides delivering such successful agricultural products, this technology also offers the possibility of studying the thousands of plant genes (with known and unknown functions) that have been produced by numerous genome programs conducted throughout the world (Dong et al. 2005). As a general rule these new genes are silenced or over-expressed, creating opportunities to study their function in the plant and to produce new phenotypes not possible through conventional breeding (Galun 2005; Mullerr 2006).

The first examples of the expression of exotic genes in sugarcane plants were obtained by the insertion of genes conferring antibiotic and herbicide resistance (Bower and Birch 1992; Gallo-Meagher and Irvine 1996). For many years now, the genetic transformation of sugarcane has been a reality in different laboratories around the world. The production of herbicide-resistant plants is now a common practice (Falco et al. 2000; Manickavasagam et al. 2004) and agronomical performance and inheritance studies of plants containing this trait have been performed in the field (Leibbrandt and Snyman 2003; Butterfield et al. 2002). Insect-resistant sugarcane plants were first produced by transformation with a truncated version of the *Bacillus thuringiensis cryIAb* gene and the plants produced very low amounts of the protein, presenting some larvicidal activity (Arencibia et al. 1997). Braga et al. (2001, 2003) reported the production of a number of transgenic events resistant to sugarcane borer in two commercial sugarcane cultivars. The gene used was a reconstructed version of *cryIAb* and the plants showed high resistance under greenhouse and field conditions. Recently, a truncated version of the *B. thuringiensis cryIAc* gene was expressed in sugarcane plants by Weng et al. (2006), and significant protein levels and insect resistance were obtained from at least two sugarcane clones. Other strategies have been used to obtain insect-resistant sugarcane plants using genes from various sources. Nutt et al. (1999) and Nutt (2005) obtained transgenic sugarcane plants expressing either the potato proteinase inhibitor II or the snowdrop lectin gene, which were able to reduce the weight of the cane grub larvae feeding on them. Transgenic plants containing the snowdrop lectin gene were also reported by Chen et al. (2004) with no information on insect resistance studies. Falco and Silva-Filho (2003) expressed the soybean Kunitz and Bowman-Birk trypsin inhibitors in sugarcane, obtaining a reduction in growth of the sugarcane borer larvae feeding on transgenic plants, with no mortality. Different groups have reported on resistance to viral diseases in sugarcane: for sugarcane mosaic virus (SCMV) (Joyce et al. 1998; Ingelbrecht et al. 1999); for Fiji disease virus (FDV) (McQualter et al. 2001); and for sugarcane yellow leaf virus (SCYLV) (Rangel et al. 2003). Even though sugarcane genetic engineering has demonstrated high potential, there has been no commercial release of transgenic sugarcane either due to intellectual property considerations or more probably to industrial concerns over public perception. These constraints on commercial release are likely to continue in the near future. The ease with which many sugarcane genotypes can now be transformed, together with the identification of the sequences of thousands of genes that this

plant expresses, raise the possibility of altering the expression of specific genes in the plant and identifying the effects this modification has on the plant's phenotype. The development of RNAi and anti-sense technology allows for gene down-regulation even in a high polyploid situation. Non-flowering plants of a heavily flowering sugarcane variety were produced through the anti-sense expression of a single candidate flower development gene found in the Sucest database (Figueiredo 2003). Flowering is undesirable in sugarcane commercial fields. Wu and Birch (2007) showed that the expression of a heterologous sucrose isomerase gene directed towards the vacuole of transgenic sugarcane plants resulted in plants capable of doubling the total sugars stored in mature culms. In these plants, the amount of stored sucrose was the same as in control non-transgenic plants and the increase in total sugar was due to the accumulation of isomaltulose, a sucrose isomer. The transgenic plants with enhanced sugar accumulation also showed increased photosynthesis, sucrose transport and sink strength. Down-regulation and over-expression of the genes involved in carbohydrate metabolism is the topic of many studies, with the aim of increasing the content of sucrose and other metabolites.

In the last few years, sugarcane has also turned into a target for the production of novel products such as biopolymers (McQualter et al. 2005) and as a producer of pharmaceutical proteins with different properties (Wang et al. 2005). The plant is well-suited to these approaches due to some of its characteristics such as vegetative propagation, the absence of flowering in most commercial cultivars, the production of a large biomass, the large amount of carbon partitioned into sucrose (up to 42% of the stalk dry weight), and a mobile pool of hexose sugars throughout most of its life. The production of biopolymers was obtained by McQualter et al. (2005) in sugarcane plants, accumulating up to 7.3% and 1.5% dry weight of p-hydroxybenzoic acid in leaf and stem tissue, respectively. This product was quantitatively converted to glucose conjugates by endogenous uridine diphosphate-glucosyltransferases and presumably stored in the vacuole. Initial steps to produce pharmaceutical proteins were taken when Wang et al. (2005) successfully produced the human granulocyte macrophage colony-stimulating factor (GM-CSF) in sugarcane. Unfortunately, in the field, the plants were able to accumulate only 0.02% of the total soluble protein as GM-CSF.

In monocots, the most frequently used promoters are the maize ubiquitin Ubi-1 and the rice actin act1. Even though they maintain a relatively constant expression pattern, they show distinct expression patterns in different species, cell types, and cultivation conditions (Neuteboom et al. 2002). The identification of sugarcane tissue-specific promoters is an important step that will allow controlled gene expression so that novel products can be accumulated in the desired part of the plant, such as in the leaves or stem parenchyma cells. A few promoters from sugarcane genes have been tested in the past, with limited success (Birch et al. 1995; Wei et al. 1999, 2003; van der Merwe et al. 2003). Again, the large number of sugarcane genes available, coupled with studies to understand when and where they are expressed, become important tools to identify regulatory sequences that can be used to drive specific genes.

21.7 Perspectives

Sugarcane Mendelian genetics has literally started with the advent of molecular investigation techniques. The first monofactorial segregations and the first genetic linkage were oberved less than twenty years ago (Glaszmann et al. 1989). Many evolutionary questions have been addressed, confirming hypotheses or concluding earlier debates and occasionally throwing new light and improving overall understanding. The genome has become less mysterious, although its complexity has been confirmed for many aspects. Shortcuts have been identified thanks to synteny with other grasses, and the availability of the sorghum sequence will shortly initiate a new round of progress. The remarkable effort made with sugarcane ESTs is yielding a wealthy catalog of genes, whose documentation starts to open new perspectives for breeding better-adapted sugarcane. The worldwide realization of the central importance of bio-energy will undoubtedly foster attention on sugarcane physiology and genetics.

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