PROGRESS IN SUGARCANE MOLECULAR BIOLOGY

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ABSTRACT

Major goals of plant molecular biology research are to develop technology for improving crop traits such as disease and pest resistance and to breed higher yielding or better quality varieties. These goals are approached through developing molecular techniques and tools for identifying and characterizing diseases and pests, applying knowledge required for specific gene isolation, characterization, transfer and expression—i.e., genetic transformation—and through application of knowledge about the genome for improving the efficiencies of conventional breeding and selection programs. Tools are being developed for disease diagnostics to provide greater sensitivity and accuracy in identifying pathogen-infected plant materials. These tools will assist in reducing the spread of diseases and help resolve types of host-pathogen interactions for studies on the genetics of disease resistance. Knowledge about the genetics of disease resistance should assist crop improvement through the traditional breeding and selection methods and eventually offer the capability for improving the crop through map-based cloning of genes of interest for genetic transformation. Methods for the genetic transformation of sugarcane have advanced to a level where it is now possible to consider transformation as a viable method for improving varieties. The first practical transformation appears to be increasing tolerance to herbicides. In the longer term, it appears that transformation with specific promoters will allow tissue-specific expression of genes for controlling pests of roots, leaves or stems. Transformation with genes and regulatory elements from other organisms as well as from sugarcane appears promising.

Genome analyses through restriction fragment length polymorphism and randomly amplified polymorphic DNA technology have shown the conservation in DNA sequences among species of Saccharum and grasses related to Saccharum. This information will allow sugarcane scientists to apply the extensive knowledge about the genetics of maize and sorghum to problems related to the improvement of cane. With respect to the more basic part of genome analysis, data are beginning to show the phylogenetic relationships and probable origins of members of Saccharum. We are learning how many chromosomes constitute the basic "n" number and what these chromosomes look like physically through image analysis. Knowledge about the linkage of molecular markers to loci encoding agronomic traits of interest is beginning to build up. Traits of interest include relatively simple ones such as disease resistance and the more complicated quantitative traits of yield such as sucrose and fiber percent.

Specific enzyme systems involved in sucrose and fiber accumulation are being resolved at the molecular level by isolating, cloning and analyzing the genes involved in these pathways. This information should assist plant breeders in developing varieties for specific conditions or environments such as the need for seasonally early or late ripening. In the more distant future, strategies for increasing sucrose yields or converting sucrose to different natural products of higher value can be expected.

Keywords: Sugarcane, genome analysis, genetic mapping, genetic transformation, molecular diagnostics.

INTRODUCTION

The purpose of this report is to chronicle progress of sugarcane molecular biology between the last ISSCT Congress in Thailand (March 1992) and the present Congress in Colombia (September 1995). During these three years, progress has been communicated by (i) papers presented at the ISSCT-sponsored Molecular Biology Workshop held in Piracicaba, Brazil (April 1993); (ii) papers presented at the series of workshops of The International Consortium for Sugarcane Biotechnology, primarily in association with International Conferences on the Status of Plant Genome Research; and (iii) scientific papers published in journals. Research reported in these three venues reveals the exciting progress being made in sugarcane molecular biology.

MOLECULAR DIAGNOSIS OF DISEASES

The development of molecular techniques such as DNA restriction mapping, DNA hybridization, and DNA and RNA amplification for identifying disease pests has proceeded rapidly. Isolation and characterization of nucleic acids from pathogens allowed the characterization of the causal agent of yellow leaf syndrome (YLS) (Borth et al 1994) and
the development of sequence-specific primers for polymerase chain reaction (PCR) amplification of Fiji disease virus (Smith & van de Velde 1994, Smith et al 1992a), sugarcane mosaic virus (Smith & van de Velde 1994) and sugarcane smut disease (H. Albert, HSPA Expt. Sta., pers. comm. 1995). Reports on the PCR identification of other cane diseases are expected to appear soon. DNA of each pathogen was cloned and labeled for use as probes to detect presence of Fiji disease virus (Smith et al 1994) and Clavibacter xyli subsp. xyli, the causal agent of ratoon stunting disease (RSD) (Chang et al 1994) in cane tissues. Monoclonal antibodies have been used to distinguish among strains of Xanthomonas albilineans, causal agent of leaf scald (Alvarez et al 1995). It is expected that these and related techniques will find practical applications in quarantine programs to prevent the introduction of diseased germplasm and in breeding programs to improve identification of resistance in clones to be used as parents.

CELL AND TISSUE CULTURE

Tissue culture of sugarcane has existed since about 1965. To date, many if not most experiment stations include tissue culture activities in their research program. Interest in tissue culture has focused primarily on how this technology might supplement the traditional breeding and selection programs for developing superior varieties.

Initially, tissue culture projects explored production, identification, selection and evaluation of somaclonal variants and somatic hybrids as methods for varietal development. Recently there have been reports on applying molecular techniques to identify tissue culture-produced somaclonal variants. Somaclonal variants have been identified by means of randomly amplified polymorphic DNA (RAPD) to determine genetic drift during tissue culture (Taylor et al 1995) and to select variants with desirable traits such as disease resistance (Oropeza et al 1995). Although there is a long history of attempting to utilize somaclonal variants in varietal development, progress has been slow, partly because advancement of varieties from tissue cultures needs to be based on the same extensive yield trials required in the traditional breeding and selection programs.

More recent, more successful short-term applications of tissue culture technologies include micropropagation for crop planting (Walker et al 1993), bud culture to eliminate Fiji disease virus (Wagh et al 1995), and in vitro cultures as a tool for germplasm storage (Paulet et al 1993, Taylor & Dukic 1993). Although these applications appear to work they may not find wide acceptance because of the high associated costs. Interestingly, the use of tissue cultures for germplasm storage has proceeded along two completely different systems. In the first, young lateral buds were used to establish mericlone cultures, which were then placed in storage at cool temperatures (18°C) for 12-mo periods between successive transfers (Taylor & Dukic 1993). The second system was based on isolating the apical meristems of in vitro cultured plants, encapsulating the meristems in alginate and freezing the encapsulated meristems to liquid N temperatures (-196°C) (Paulet et al 1993). Both systems claim to minimize tissue culture problems of somaclonal variation and are thus viable, depending on the costs, as alternatives to maintaining germplasm as field-grown clonal material.

Tissue culture technology is required for all current gene transfer systems that are based on the production of suitable target cells followed by the regeneration of transgenic plants. Much of the recent tissue culture research has therefore been directed toward developing various phases of the tissue culture technology for use in genetic transformation. The ideal targets for transformation would be a population of cells, all of which were capable of forming embryos that could be germinated to form plants. Efforts to increase the embryogenic capacity of cell cultures and the length of time the cultures remain capable of regenerating plants have succeeded to a reasonable degree (Brisibe et al 1994, Fitch & Moore 1993).

GENETIC TRANSFORMATION

Genetic transformation of higher plants has proven most efficient in dicots where Agrobacterium has been used routinely as a vector to effect an indirect transfer of a gene via the Ti plasmid. Unfortunately, this indirect method is not possible or at least it is highly ineffective with monocots so that gene transfers in this group of plants have had to involve direct transfer of DNA into the cell nucleus.

Direct transformation requires overcoming the barriers of the plant cell wall and the membranes surrounding the cell and nucleus. The cell wall barrier can be overcome either by enzymatic removal or by physical penetration of the wall. Removal of the cell wall to produce protoplasts that can regrow walls and subsequently regenerate into plants is referred to as protoplast technology. This technology has proven highly successful with dicots but difficult for grasses including sugarcane. The problem is not producing protoplasts, but regenerating plants from them. There have been isolated reports of successful regeneration of cane plants from protoplasts; however, this work has not
succeeded in most labs. Recently methods have been improved for establishing suspension cultures with a high capacity for yielding protoplasts that develop into small colonies, but plant regeneration has not succeeded (Taylor et al 1992a & b, Taylor et al 1994).

Although plants have not been regenerated from protoplasts, work has continued on transforming protoplasts by using an electrical impulse to induce transient membrane pores through which the foreign DNA can enter the cell. This process, called electroporation, has been improved for sugarcane as a transient assay system to study gene-regulatory elements and as a starting point for establishing stable transformation (Rathus & Birch 1992a). This system has resulted in stable transformation of callus (Rathus & Birch 1992b); but until we are able to regenerate protoplasts into plants, this is not a viable method for obtaining transformed plants. Recently it was reported that electroporation can be used with cell cultures or isolated meristems to effect transformation without going through protoplast cultures (Arencibia et al 1992 & 1995). This report is surprising; and if the work can be repeated in other labs, the procedure will assure a more efficient transformation system for cane.

Direct penetration of the cell wall and membranes with microprojectiles coated with the DNA of interest and propelled with a blast from a gun cartridge or compressed gas has proven more successful than the protoplast route to effect sugarcane transformation (Birch & Maretzki 1993). Although cane transformation with microprojectile bombardment or the "gene gun" method is moderately successful, the procedures employed are far from routine. Variables include: the target tissue receiving the transgenes, the reporter gene to indicate that DNA insertion occurred, the selectable marker gene and selective medium to eliminate the nontransformed cells, the gene of interest encoding an agronomic trait, and the promoters used to drive the expression of each gene.

- The tissues used for obtaining stable transformation have been apical meristems (Gambley et al 1993 & 1994), embryogenic callus (Bower & Birch, Chowdhury & Vasil 1992, Fitch et al 1995, Gallo-Meagher & Irvine 1995), and a sieved "green callus" (Sun et al 1993), which appears to be nonelongated green shoots. Embryogenic callus appears to be the most useful target tissue.

- For visualizing or reporting successful transfer of DNA—either GUS, the product of the β-glucuronidase gene uidA (Gallo-Meagher & Irvine 1993), or luciferin, product of the luciferase gene luc (Gambley et al 1994)—have been used. In general the GUS reporter has been used more widely (Bower & Birch, Chowdhury & Vasil 1992, Fitch et al 1995, Gambley et al 1993, Sun et al 1993).

- For stable transformation it is usually necessary to cull the transformed cells from those that have not been transformed before plant regeneration. The most widely used selectable markers have been the gene npt-II encoding neomycin phosphotransferase (Bower & Birch, Fitch et al 1995), the gene bar encoding phosphinothricin acetyltransferase (Chowdhury & Vasil 1992, Fitch et al 1995, Gallo-Meagher & Irvine 1995, Sun et al 1993), and a mutant acetolactate synthase gene, als (Fitch et al 1995). Selection media containing the antibiotic geneticin is used to select for transformation with npt-II, the antibiotic chlorosulfuron, the herbicide bialaphos (Gallo-Meagher & Irvine 1995) or its active ingredient, phosphinothricin (Sun et al 1993), has been used to select for transformation with bar, and the sulfonyl urea herbicide Oust (sulfometuron methyl) was used to select for transformation with als. Selection is not considered necessary for transformation of apical meristems (Gambley et al 1993 & 1994) because each meristem should have at least some transformed cells. Nevertheless, in transforming these large blocks of tissue there is a high probability of obtaining chimeras of transformed cells and wild-type cells that may have to be segregated to produce fully transformed plants.

- The first gene of agronomic interest to be transformed into cane cells (Sun et al 1993) and regenerated into plants (Chowdhury & Vasil 1992, Gallo-Meagher & Irvine 1995) is the bar gene encoding resistance to the herbicides bialaphos and glyphosate ammonium. The coat protein gene of sugarcane mosaic virus (SCMV) showed transient expression in sugarcane protoplasts (Smith et al 1992b) but has not yet yielded SCMV-resistant plants. Work is under way with the bt gene of Bacillus thuringiensis to develop resistance to certain insect pests.

- The promoters used to drive the reporter, selectable marker and gene of interest have been as varied as the other parameters involved in sugarcane transformation. Direct comparison of promoters to elicit transient GUS expression in cane protoplasts (Rathus et al 1993) showed the artificial promoter EMU to be much more active than the cauliflower mosaic virus CaMV 35S or the maize alcohol dehydrogenase Adh 1 promoters. Transient GUS expression in immature sugarcane leaves was greatest when the uidA gene was under the control of the maize ubiquitin, ubi, promoter followed by EMU, the rice actin, act 1, and then the CaMV 35S promoter (Gallo-Meagher 1993).
& Irvine 1993). Together these studies show the relatively low activity of the standard dicot promoter CaMV 35S compared to the monocot promoters abi and EMU. Current studies are seeking to identify tissue-specific promoters to direct expression of transgenes to specific organs of the plant.

**GENOME ANALYSES**

Application of molecular biology to genome analysis of sugarcane began in 1988 with more hope than promise that the technology could be developed and directed toward goals of documenting varieties, clarifying phylogenetic relationships, assessing genetic diversity in the germplasm pool, detecting major genes and resolving complex genetic traits. By the time of the last Congress we were beginning to see that at least some of the goals were achievable. During the interval between the Congress in Thailand and this one, we see progress toward all five of the early goals and have research beginning in new directions not envisioned a few years ago.

The earliest step toward realizing the potential of genome analysis was the development of protocols for mapping polyploids such as sugarcane, without information about the ploidy level or type (Wu et al 1992). When work began with restriction fragment length polymorphism (RFLP) analysis of sugarcane, it was soon realized that although the majority of DNA fragments of the genome existed in multiple copies, some existed as a single copy. The single-copy DNA fragments could be observed on electrophoretic gels as bands that segregated in a 1:1 ratio among the progeny of parents polymorphic for that band. The single-copy DNA fragments, referred to as single-dose restriction fragments (SDRFs) (Sorrells 1992, Wu et al 1992) have proved to be necessary and sufficient for mapping genomically complex plants. SDRFs were used to develop the first genetic map of a *Saccharum* sp, specifically for SES 208, a clone of *S. spontaneum* with 2n = 64 chromosomes (Da Silva et al 1993). Research revealed that this clone is an autopolyploid, most likely an octaploid, with considerable DNA homology with barley, maize, oats, wheat and rice (Da Silva et al 1993). The same population was also analyzed using PCR to amplify single-dose DNA polymorphisms, confirming that this clone is an autopolyploid (Al-Janabi et al 1993). The RFLP and PCR data were combined to produce a genome map containing 208 single-dose (SD) arbitrarily primed PCR polymorphisms, 234 SD RFLPs, 41 double-dose (DD) and 1 triple-dose (TD) RFLP in 64 linkage groups (Da Silva et al 1995). The DD and TD RFLP fragments were used to identify 8 chromosome homology groups. The integrated map was estimated to have markers averaging 6 cM between them and thus cover 93% of a genome approx. 33 cM in size.

The conclusion that clone SES 208 is an autopolyploid was based primarily on the fact that all linkages of the more than 200 mapped RFLP markers were in coupling phase. Given that *Saccharum* is recognized as a highly polymorphic genus with a wide range in chromosome numbers, it is not certain that the other species of the genus or even other members of *S. spontaneum* are autopolyploids. Linkage analysis of SD RAPD markers detected in progeny of a *S. officinarum* x *S. robustum* intercross showed that repulsion-phase linkages could be detected at about the 25% level in each genome (Al-Janabi et al 1994). These genomes thus appear as possible segmental allopolyploids, indicating that polyploidy in *Saccharum* probably evolved in different ways a number of times.

Initial mapping work was conducted on SES 208 because haploid lines and progeny of a backcross family from a haploid were available as genetically simplified materials. An alternative approach for mapping in a simplified system was to identify and map with DNA probes specific to the species *S. spontaneum* and *S. officinarum* in the progeny of a selfed hybrid variety recognized as an aneuploid (D'Hont et al 1994). A set of 53 maize probes, covering the whole maize genome, was hybridized to 348 RFLP bands, which mapped to 25 co-segregation groups. Of these groups 18 involved *S. spontaneum*-specific markers proposed to represent 7 chromosomes of *S. spontaneum* (D'Hont et al 1994). Co-segregation of the markers indicated a large degree of synteny between *S. spontaneum* and maize. The synteny clusters of *S. spontaneum* and *Sorghum bicolor* were located on the genomic map of the 10 maize chromosomes by shared loci (Griyet et al 1994). This map revealed common large chromosomal rearrangements among these three species of the tribe Andropogoneae. Distances between markers were similar in maize and sugarcane, but *S. spontaneum* displayed reduced recombination. RFLPs specific to the two species *S. spontaneum* and *S. officinarum* were then used to map a modern hybrid (Griyet et al 1995). A map of 428 markers on 99 co-segregation groups was assembled into 10 basic linkage groups on the basis of probes in common.

Using isozymes to document varieties at the genetic level has been suggested since 1969 and has been reported recently as accomplished (Eksomtram age et al 1992, Gallacher et al 1995). In one study 97 of 100 hybrid clones selected at random could be separated on the basis of only 11 bands derived from 3 enzyme systems (Gallacher et al 1995). Under the study conditions, the other 17 enzyme systems tested failed to provide polymorphic data for variety
separation. In a second isozyme study, 82 clones of three species and hybrids of *Saccharum* were documented with 21 bands from 7 enzyme systems (Eksomtramage et al. 1992). The increase in polymorphism in the second study could be the result of the wider genetic difference among the clones tested or it could be due to experimental differences in the isozyme assays. In both experiments the level of polymorphism was sufficient to be of value in precise identification of clones. Additional polymorphism is readily obtained with molecular markers such as RFLPs and RAPDs, which have been suggested as methods for documenting varieties (Fukui et al. 1994).

RFLP analysis of nuclear DNA, using maize low-copy DNA probes, clearly distinguished similarities and differences among species of *Saccharum* and single accessions of *Erianthus* and *Miscanthus* (Lu et al. 1994b). The average genetic similarities with *S. officinarum* and *S. barberi* were high (65-66%), but low (31-38%) within *S. spontaneum* and *S. robustum* (Lu et al. 1994b). When the RFLP analysis was done on a collection of 40 hybrid varieties, the genetic similarity was 61%, slightly more variable than in *S. officinarum* (Lu et al. 1994a). This supports the idea that the germplasm pool of *S. officinarum* is much more uniform than that of *S. spontaneum* and that the variation in hybrid varieties is primarily due to the variation contributed by *S. spontaneum*. Similarity of germplasm among a different set of hybrid varieties was 80-100% based on RAPDs, but was only 26-85% based on simple sequence repeats (SSRs), indicating the greater variability with SSRs than with RAPDs (Harvey et al. 1995).

Plants have, in addition to a large nuclear genome, a small cytoplasmic genome consisting of the DNA of the chloroplasts (cpDNA) and mitochondria (mtDNA). The cytoplasmic genome, which is maternally transmitted to progeny of a cross, thus offers insight into maternal lineage and has potential for evaluating phylogenetic relationships. Total DNA of a group of 55 clones of *Saccharum* spp and hybrids plus one each of *Erianthus* and *Miscanthus* was digested with one or two restriction enzymes and probed with two cpDNA clones from wheat and four mtDNA clones from wheat and sunflowers to evaluate phylogenetic RFLP relationships among them (D'Hont et al. 1993). There was no variance for mtDNA among the accessions of *S. officinarum*, hybrid varieties and all but two *S. robustum* accesses (D'Hont et al. 1993). However, there were eight different mtDNA patterns, which could partially define groups in the larger collection of species (D'Hont et al. 1993). There was only one difference in the cp genome among the plants; all 55 *Saccharum* clones tested showed a single pattern, which differed from the pattern showed by *Erianthus* and *Miscanthus* (D'Hont et al. 1993). The cpDNA data indicate different origins for these two groups, but it is too limited to determine whether there are undetected differences with *Saccharum*. The phylogenetic relationship of cpDNA among sugarcane and related Andropogoneae grasses was expanded to a collection of 32 genotypes representing 8 genera and 19 species evaluated with 12 recombinant cpDNA clones from rice and 21 different restriction enzymes (Sobral et al. 1994). The combination of probes and restriction enzymes gave a total of 62 polymorphic sites to describe the genotypes. These site mutations placed the 32 genotypes into 9 different chloroplast groups: 7 from within the Saccharinae subtribe and the 2 outgroups, maize and *Sorghum*. There was very little variation in the cp-genome of *Saccharum*, which separated *S. spontaneum* from all other *Saccharum* by a single-site mutation (Sobral et al. 1994). Because the level of polymorphism detected by RFLP analysis was low, two potentially polymorphic regions of the cp-genome and two potentially polymorphic regions of the mt-genome were sequenced to allow finer resolution of cytoplasmic genome differences (Al-Janabi et al. 1994b). Despite the increased resolution, there was very little difference within the *Saccharum* complex (Al-Janabi et al. 1994b). Collectively, the data from the various studies indicate a narrow cytoplasmic genome for sugarcane.

The application of genome analyses toward identifying and characterizing major genes and quantitative trait loci (QTL) has lagged behind the other applications of genome analyses for at least two reasons:

- Because cane is a high polyploid, it has been difficult to identify traits encoded by major genes; we consider that a majority of the agronomic traits is encoded by several genes.
- In addition most sugarcane scientists recognize the large effect of the environment on phenotype expression and consequently the need for extensive testing of varieties across a range of environments before obtaining a valid assessment of the phenotypic trait.

Although progress in identifying molecular markers linked to loci encoding phenotypes of agronomic interest has been slow, preliminary reports are encouraging. The first abstracts of reports have appeared at the most recent International Consortium of Sugarcane Biotechnology and the International Plant Genome Meeting. Liu & Paterson (unpub.) report that sorghum RFLP markers linked to the traits of Brix, flowering, height and rhizomatous habit in sorghum were correlated with the same traits in progeny of two crosses of *S. officinarum* x *S. spontaneum*. Honeycutt

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et al (1995) report co-segregation of specific markers with the traits of percent flowering, smut disease susceptibility, stalk girth, cane tonnage and percent pol in the family of a cross of S. officinarum x S. robustum. Given that this work was on a single population, which was small as was the number of phenotypic measurements, this report must be considered preliminary. Msomi & Botha (1995) used an alternative approach to finding markers linked to QTL. Their approach, termed bulk segregate analysis (BSA), was to seek DNA polymorphisms between DNA pools of individuals differing in the presence/absence of high fiber. The authors report finding 8 DNA polymorphisms between the high- and low-fiber pools (Al-Janabi et al 1994a). One of the polymorphisms used in a simulation study gave a significant enrichment in the population for clones with high fiber. We can be fairly confident that over the time period between this and the next ISSCT Congress, more definitive identification of molecular markers linked to loci encoding important agronomic traits will become available for sugarcane.

Research in cytogenetics is revealing important information about the sugarcane genome. Computer-aided imaging technology was used to resolve the quantitative karyotype of the haploid clone AP85-361 (2n=32), derived from another culture of SES 208 (2n=64), a clone of S. spontaneum from northern India (Fukui et al 1994). AP85-361 was shown to have 32 chromosomes, of which 4 were satellite-chromosomes. On the basis of physical features the chromosomes were divided into 8 groups of 4 nearly identical structures. This indicates that AP85-361 is a tetraploid (2n=4x=32), with the base no. x=8. This conclusion supports the findings with the molecular marker mapping of SES 208. Fluorescently labeled, highly repetitive elements have been used to identify specific chromosomes, and species-specific DNA has been labeled to distinguish between the chromosomes from each species contributing to a hybrid (D'Hont et al, unpublished results). The use of these techniques, in combination with genome mapping, is beginning to give us a good picture of the genetic and physical structure of the sugarcane genome.

SPECIFIC GENE CHARACTERIZATION

Just 3 years ago the first sugarcane gene was isolated and characterized: phosphoenolpyruvate (PEP) carboxylase (Albert et al 1992). Since then there have been at least four more gene sequences deposited in the GenBank data base: a ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit gene (Tang & Sun 1993), two glucose transporter genes (Bogus & Thom 1993) and a cytosolic fructose-1,6-bisphosphatase (C. Grof, pers. comm.). In addition there have been reports of a partial sequence of a sucrose synthase gene (Kumar et al 1992), the distribution of the messenger RNAs for producing sucrose synthase (Buczynski et al 1993), and isolating the cell membrane sucrose carrier protein (Getz et al 1994).

Much of this initial research has involved genes encoding the enzymes important in sucrose metabolism, partitioning and storage. Future work is expected to continue emphasizing sucrose accumulation processes because of their importance to cane yield. Research progress in isolating and characterizing the sugarcane genes encoding acid invertases and other metabolic enzymes should be published soon. The success in this early work has been based primarily on using heterologous cDNAs, published DNA sequences and antibodies to enzymes purified from other plants. Progress using these approaches is expected to accelerate as information builds up on the molecular genetics of other Gramineae such as maize and sorghum. Research is expected to expand to include characterizing the genes involved in disease resistance.

OUTLOOK

Research on sugarcane molecular biology has been in place only a few years, but it is already making significant progress on several fronts including genetic transformation, genome analyses and developing knowledge on specific gene systems. It is expected that progress will accelerate rapidly—both because of the effort spent on sugarcane and the transfer of knowledge from intensely studied grass species. By the next ISSCT Congress, we can expect molecular marker maps for S. officinarum and commercial hybrids. By then genomic maps will also include loci of genes responsible for specific enzymes or phenotypes such as disease resistance. As more saturated genomic maps are developed for sugarcane and phenotypic traits are placed on the maps, it will become possible for breeders to select parents or progeny on the basis of DNA homology instead of scoring for the phenotype. Ultimately it will be possible to isolate specific genes based on their map locations and to engineer these genes into different varieties to correct important deficiencies.
REFERENCES


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Les principaux buts des recherches en biologie moléculaire sur les plantes sont de développer des technologies permettant d'améliorer les caractères des plantes cultivées telles que leur résistance aux maladies et aux ravageurs et de développer des cultivars plus productifs et de meilleure qualité. On approche de ces buts en développant des techniques moléculaires et des outils permettant d'identifier et de caractériser les maladies et les ravageurs; on applique ainsi les connaissances requises pour isoler un gène spécifique, le caractériser, le transférer et l'exprimer, c'est à dire lui appliquer la transformation génétique; on applique aussi dans ce but nos connaissances sur le génome pour améliorer l'efficacité de la sélection variétale conventionnelle et les programmes de sélection. On développe maintenant des outils pour diagnostiquer les maladies et pour rendre plus sensible et précise l'identification des pathogènes de plantes. Ces outils aideront à réduire la propagation des maladies et à résoudre les modes d'interactions dans les études de résistance aux maladies. Les connaissances sur ce dernier point devraient faciliter l'amélioration des plantes au moyen des méthodes traditionnelles de sélection et au bout du compte devraient fournir les moyens d'améliorer la plante grâce au clonage de gènes piloté par la cartographie moléculaire en vue de leur utilisation en transformation génétique.

Ces dernières méthodes ont progressé chez la canne à sucre jusqu'à un niveau tel qu'il est maintenant possible d'envisager la transformation génétique comme une méthode viable d'amélioration des cultivars. La première
transformation pratique apparaît être l'obtention d'une tolérance accrue aux herbicides. A plus long terme, il apparaît que la transformation avec des promoteurs spécifiques permettra l'expression de gènes spécifiques d'un tissu pour contrôler les ravageurs des racines, des feuilles ou de la tige. La transformation avec des gènes et des éléments régulateurs provenant à la fois d'autres organismes et de la canne à sucre apparaît prometteuse.

Les analyses du génome au moyen des RFLP et des RAPD a démontré la conservation des séquences de l'ADN parmi les espèces de Saccharum et parmi les graminées apparentées. Cette information permettra aux chercheurs d'appliquer à l'amélioration de la canne à sucre les vastes connaissances acquises sur le maïs et le sorgho. Quant à l'analyse plus fondamentale, celle du génome, des résultats commencent à démontrer les relations phylogénétiques et l'origine probable de certains Saccharum. Nous apprenons combien de chromosomes représente le nombre de base "n" et à quoi ils ressemblent physiquement par le biais de l'analyse d'image. On commence à accumuler les connaissances sur la liaison des marqueurs moléculaires avec les loci contrôlant les caractères agronomiques intéressants. Ces derniers comprennent des caractères relativement simples comme la résistance aux maladies et les caractères quantitatifs les plus complexes du rendement comme le pourcentage de saccharose et de fibres.

Les systèmes enzymatiques spécifiques impliqués dans l'accumulation du saccharose et des fibres sont en cours de résolution au niveau moléculaire par isolement, clonage et analyse des gènes concernés dans ces processus. Cette information pourrait aider le sélectionneur à développer des cultivars adaptés à des conditions ou à des environnements particuliers, comme une maturité précoce ou tardive. Dans un futur plus éloigné, on peut s'attendre à des stratégies permettant d'augmenter les rendements en sucre ou de le transformer en divers produits naturels de plus grande valeur.

Mots clés: Analyse du génome, carte génétique, transformation génétique, diagnostics moléculaires.