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- Programme
- **Abstract**s

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GENETICS, PHYSIOLOGY, MAPPING AGRONOMIC TRAITS AND DISEASE RESISTANCE TRAITS

QTL mapping of sugar cane resistance to brown spot (Cercospora longipes, Butler)

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Brown spot caused by the fungus Cercospora longipes, Butler, is widely spread in many sugar-producing countries. However, no molecular studies are available to date to identify quantitative trait loci (QTL) for resistance to this disease. A genetic linkage map was generated in a segregating population of 227 progeny derived from a cross between resistant (M 143/75) by susceptible (R 570) varieties to facilitate the identification of such QTLs. The amplified fragment length polymorphism (AFLP) and microsatellites (SSR) markers were used to generate 722 polymorphisms in the segregating population, scored as present in M 134/75 alone and absent in R 570. Thirty-six AFLP primer combinations screened against the 227 progeny generated 556 polymorphisms and 65 SSR primers screened added 166 additional polymorphic alleles. The 722 polymorphisms were used to calculate SDMs, utilized to construct a genetic linkage map of cultivar M 134/75. Out of the 722 polymorphic markers, 607 were SDMs segregating 1:1. Linkage analysis assigned 524 SDMs (404 AFLP and 120 SSRs) to 98 LGs containing at least two linked markers with a total of 83 markers remained unlinked. A framework map of variety M 134/75 consisted of 524 single does markers (segregating 1:1 as present:absent) was constructed. Twelve genomic regions were associated with the resistance to brown spot. Nine of the putative QTLs were found on different linkage groups and three were unlinked. Out of the 9-mapped putative QTL, 5 were identified by AFLP markers, while the remaining four by SSR markers. The percentage of phenotypic variation explained by the linear regression on individual markers ranged from 4.2-11.1%. This study provides a framework for future investigation of brown spot disease resistance in sugar cane.

Keywords: Cercospora longipes, Molecular markers, linkage mapping, polyploidy, quantitative resistance, sugarcane

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Genetic of sugarcane resistance to smut (Ustilago scitaminea): characterisation of pathogen diversity, QTL mapping in a bi-parental progeny and associations study among modern cultivars

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The worldwide genetic structure of U. scitaminea was investigated (1) to describe the global population structure of the fungus and (2) to infer its reproduction system. A total of 142 single teliospore isolates from 15 countries worldwide were analysed using 17 polymorphic microsatellite loci. All isolates but one were homozygous for all loci, indicating that selfing is the highly preferential reproductive mode of U. scitaminea. In America and Africa, genetic diversity was found to be extremely low and all isolates belonged to a single inbred lineage. This inbred lineage was also found in some parts of the Asian continent where most U. scitaminea genetic diversity was detected. These observations support the hypothesis that the fungus originated in Asia.

In order to identify the mendelian factors involved in sugarcane resistance to smut, two strategies have been implemented (1) QTL mapping in a bi-parental progeny derived from a cross between a resistant cultivar 'R 570' and a highly susceptible clone 'MQ 76/53', evaluated in Reunion island for resistance to smut (2) Association study in a population of cultivars, evaluated in Burkina Faso for resistance to smut.

The genetic maps of the two parents of the bi-parental progeny, 'R 570' and 'MQ 76/53', were constructed using a population of 198 progeny. A total of 1666 polymorphic markers were produced using 37 AFLP primer pairs combinations, 46 SSRs and 9 RFLP probes. Field trials and greenhouse trials using different inoculation methods were conducted in order to characterize the resistance

of the progeny . The distribution of disease scores observed in all those trials were highly unbalanced toward the resistant parent indicating the segregation of multiple dominant resistance factors. A QTL detection was performed using the 1666 available markers allowing the identification of a few genomic zones with small effects. In parallel, an association study performed in a population of 74 cultivars (constituted of two subpopulations, one highly susceptible to smut and the other highly resistant to smut) using 1626 polymorphic markers revealed interesting haplotypes associated with resistance. Two QTAs have been detected through both approaches. The progress obtained toward a better understanding of the genetic determinism of sugarcane resistance to smut are modest. The potential of association studies in sugarcane appeared interesting although many more markers and an extended population would have to be used to fully realize it.

Keywords: QTL mapping, sugarcane, Ustilago scitaminea, genetic diversity

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A framework for linkage disequilibrium mapping in the highly polyploid genome of sugar cane

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Linkage disequilibrium (LD) in crops, established by domestication and early breeding, can be a valuable basis for mapping the genome. We undertook an assessment of LD in sugarcane, characterized by one of the most complex crop genomes, with its high ploidy level (>=8) and chromosome number (>100) as well as its interspecific origin. Using AFLP markers, we surveyed 1537 polymorphisms among 72 modern sugarcane cultivars. We exploited information from available genetic maps in order to determine a relevant statistical threshold that discriminates marker associations due to linkage from spurious associations. LD steadily decreases within a 0-30 cM window. Many instances of linked markers cannot be recognized due to the confounding effect of polyploidy. However, LD within a sample of cultivars appears as efficient as linkage analysis within a controlled progeny in terms of assigning markers to cosegregation groups. Saturating the genome coverage remains a challenge, but applying LD-based mapping within breeding programs will considerably speed up the localisation of genes controlling important traits by making use of phenotypic information produced in the course of selection.

Keywords: Linkage disequilibrium, sugar cane, polyploid

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2. GENETIC IMPROVEMENT, MARKER ASSISTED BREEDING AND GENETIC DIVERSITY

New markers from sugar metabolism ESTs

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Limited genetic gains, obtained from breeding for sugar content in different breeding programs worldwide, suggest that a plateau has been reached for this trait. One way to overcome this obstacle would be to identify and introduce into commercial germplasm, alternative alleles controlling sugar metabolism, present in other Saccharum species that could increase the synthesis of sucrose. S. spontaneum has played an important role in the development of modern sugarcane varieties, but a limited number of clones from this species was used in the production of those varieties. The introgression of positive alleles through traditional methods, however, would be expensive and lengthy, due to "genetic drag". In this scenario, molecular markers associated with traits of interest, allowing a more efficient selection to increase genetic gains, would be extremely valuable to sugarcane breeders. We used the DNA sequence information of genes involved in sucrose metabolism, recently deposited in the Genebank, as a result of the Brazilian Sugarcane Expressed Sequence Tag (EST) project - SUCEST, to develop Target Region Amplified Polymorphism - TRAP - markers. These markers are currently being applied to a collection of 51 S. spontaneum, that has been characterized and shown to have significant variation in sugar and cane yield components. Polymorphisms detected in these genomic regions are candidate markers for gene tagging of sucrose and fiber content, and could be used in marker-assisted selection for the introgression of superior alleles. TRAP markers developed from Carbohydrate Metabolism-ESTs are also being applied to tag genes involved in biomass accumulation, aiming at energy production.

Keywords: sugarcane, EST, sucrose metabolism, TRAP markers

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Genotyping of sugar cane using molecular markers

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The use of microsatellite marker systems in the genotyping of crop plants has now become a well-established practice across most molecular laboratories worldwide. This technology has been applied to study the genetic diversity of the noble canes, Saccharum officinarum. A collection of 125 noble canes, two wild varieties (S. spontaneum and S. robustum) and one clone of Erianthus sp. were screened with twenty radioactively labeled primers. A total of 215 polymorphic markers were scored, data was analyzed using the Numerical Taxonomy and Multivariate Analysis System (NTSYS-pc v 1.8) and a genetic distance dendogram was constructed using the Unweighted Pair Group Mean Arithmetic (UPGMA) clustering method. A high level of variability was observed among the noble canes with a coefficient of similarity ranging from 0.29 to 0.92. Clustering was also observed among varieties with similar geographical distribution. For example, the NG (New Guinea) varieties and the Tanna varieties were clustered into distinct groups with a high level of similarities across varieties. However, varieties from Mauritius (M) and Barbados (B) had a broader genetic base

with a lower coefficient of similarity. Outgroups S. spontaneum, S. robustum and Erianthus sp. were considerably distinct from the noble canes. These results confirmed broad genetic base of sugar cane from which valuable resources can still be tapped and also provided a more targeted approach to the selection of parents for future breeding programs.

Primers SMC119CG, mSSCIR31, SMC31CUQ and SMC213MS, selected for their even distribution of bands and high polymorphism, were used to further screen the collection in order to create a genetic database of noble canes. Amplification was carried out using fluorescently labeled primers and profiling carried out using the ABI 310 Genetic Analyzer. Prominent peaks were categorized and logged into an Excel Sheet. A macro was eventually devised which allows search for unknown varieties in a single step. The profiling of an unknown noble cane with primer SMC119CG and SMC31CUQ followed by a search in the database is usually sufficient to identify the variety. Further profiling with primers mSSCIR32 and SMC213MS can be used to confirm the results. This powerful method of variety identification is being extended to locally bred sugar cane varieties.

Two novel gene based marker systems have also been adapted to sugar cane; Nucleotide Binding Site (NBS) profiling and Sequence-Specific Amplification Polymorphism (SSAP). NBS profiling is an RGA-based marker system, which involves amplification of sequences targeted towards restriction sites and RGAs. Preliminary results following genotyping of commercial varieties show that up to seven polymorphisms could be detected with some primer combinations. SSAP is comparable to AFLP with the substitution of a restriction site targeted primer by a retrotransposon directed primer. This technique has been found to reveal on average, 5-6 polymorphisms per primer combination, from our mapping population. The added advantage of these marker systems is their commoniality across species making them markers of choice for comparative genetic studies.

Keywords: noble canes, microsatellite, marker, genetic diversity, NBS, RGA, SSAP

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Biodiversity of endophytic diazotrophs in sugar cane

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Endophytes are microorganisms that spend most of their life cycle inside plants. Most of these organisms do not cause disease to the host, but, on the contrary, can promote plant growth by producting and secretion of plant growth regulators, antagonistic activity against phytopathogens and supply biologically fixed nitrogen. The diversity of endophytes has been evaluated in many plants, but in sugarcane the studies have focused on isolating and characterizing diazotrophic bacteria. In the present study, the bacterial endophytic community associated with sugarcane was determined. Aerial parts (leaves and stems) of sugar cane plant were surface sterilized with 70% ethanol and macerated in sterile water. The endophyte-containing extracts were plated on potato dextrose agar medium and incubated at 30ºC for several days. Forty-four different colonies were isolated and further characterized. The partial sequences of the 16S rDNA genes of the isolates were determined using primers Y1 and Y2. Two main groups of gama-Proteobacteria were identified: one belonging to the Enterobacteriaceae family, which included genera Enterobacter, Citrobacter, Pantoea, Kluyvera and Klebsiella, and the other was of the Pseudomonaceae family. Members of genera Brevibacillus, Staphyloccocus and Curtobacterium were also identified. Four of these isolates belonging to genera Enterobacter (3 strains) and Pseudomonas (1 strain) were capable of fixing nitrogen in N-free medium.

Keywords: biological nitrogen fixation, sugar cane, 16S rDNA, Enterobacter, gama proteobacteria, endophytes

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POSTER
Evaluation of genetic diversity among collection of S. Spontaneum and related species using AFLP markers

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Modern sugarcane cultivars are genetically vulnerable because the gene pool from which they were derived was comprised of a few interspecific hybrids between Saccharum officinarum and its wild relative S. spontaneum. A basic breeding program was established by the USDA at Houma, Louisiana, with two main objectives: 1.) to broaden the genetic base of sugarcane and 2.) to identify and introgress useful genes from wild relatives into the cultivated background. S. spontaneum continues to be the most important source of wild germplasm for Louisiana because it is generally better adapted to the Louisiana environment. Unfortunately, S. spontaneum is considered a noxious weed in the USA and can not be evaluated in the field. The objective of this study was to characterize the level and pattern of genetic diversity among a collection of S. spontaneum and related species using AFLP markers. A better understanding of the genetic diversity available in a germplasm collection and the interrelationship between different species in the collection will facilitate exploitation of the germplasm resource in breeding programs.

Six enzyme-primer pair combinations of EcoRI and MseI with three (instead of the commonly used two) selective nucleotides revealed a total of 607 unambiguous fragments of which 595 (98.1%) were polymorphic. Cluster analysis based on the Dice similarity coefficient and the Unweighted Pair Group Method with Arithmetic mean following the Sequential Agglomerative

Hierarchical Nested Cluster Analysis (SAHN) module of NTYSYS revealed a high amount of genetic diversity among the collection of S. spontaneum clones. Clustering of the other species on the tree corresponded to known taxonomic classifications. Similar results were obtained using the multi-dimensional scaling analysis technique.

The proportion of bands monomorphic to either S. spontaneum or S. officinarum present in cultivars was analysed as a crude measure of the ancestral genome content in cultivated sugarcane. Cultivars shared a higher proportion of the monomorphic bands from S. officinarum (30 %) compared to S. spontaneum (15%). Among the more recently derived species hybrids, the proportion was 44% from S. officinarum and 17 % from S. spontaneum. This supports the idea that modern sugarcane was derived from both species through interspecific crosses and repeated backcrossing to S. officinarum.

Keywords: genetic diversity, AFLP, Saccharum spontaneum

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3. SUGAR CANE GENOMICS

Complexities of chromosome landing in a highly polyploid, aneuploid, interspecific genome : towards map-based cloning of a resistance gene (Bru1) in sugar cane (2n=ca 115)

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Modern sugarcane cultivars have a particularly complex genome, highly polyploid (100-130 chromosomes), aneuploid and of interspecific origin. A major gene conferring resistance to brown rust has been identified in the cultivar R570. It is the first major resistance gene identified in sugarcane. We have been working for several years to characterize this locus and to clone this gene following a map-based approach. Exploiting the good syntenic relations between sugarcane, sorghum and rice, we have built a dense genetic map around the gene and have initiated a physical map of the target region. Probes derived from the physical map have been used to complete the genetic map. The target haplotype map encompasses 15 markers that cosegregate with Bru1 and markers at 0,14 and 0.28 cM on each side of the gene. The physical map of the region encompasses 16 BAC clones that could be assigned to 7 different homologous haplotypes. Two BAC corresponding to two homologous haplotypes cover completely the target area. However, the contig corresponding to the target haplotype comprised 7 BAC clones that only partially overlap, with two gaps still not covered. Moreover several probes derived from the BAC contig of the target haplotype do not hybridize with the homologous contig revealing the presence of an insertion in the target haplotype. Several BAC clones representing the different homologous haplotypes are currently being sequenced. Their sequences will be used to continue the characterization of this resistance locus and the complete map-based cloning of the gene.

Keywords: map-based cloning, chromosome landing, brown rust, sugar cane

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The SUCEST-FUN Project: searching for sugarcane genes with a biotechnological prospect

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The cultivated sweet cane, is a hybrid of Saccharum officinarum and Saccharum spontaneum where a gene can be represented by 10-14 copies. The unraveling of gene functions in such a complex crop is a formidable task and important in order to understand the characteristics of sugar cane. To expedite sugarcane genome research the SUCEST Project sequenced 238 000 expressed sequence tags (ESTs). The SUCEST-FUN project aims to associate function to sugarcane's genes and to identify putative targets for variety improvement (www.sucest-fun.org). Using cDNA microarrays, we identified genes associated to high sucrose content, responsive to phytohormones, endophytic bacteria, insect interaction and phosphate defficiency, that may have a role in the control of metabolic, developmental and defensive processes in plants. Expression data was validated by real-time PCR and gene promoters were cloned to drive the efficient expression of target genes in transgenic plants. Sugarcane's harvested biomass makes it the world's largest crop but despite its economical importance very few studies describe the molecular signaling responses to the environment and the role of phytohormones in this process. The data presented can be useful in assigning function for the sugarcane genes and reveal potential targets for genetic improvement of sugarcane varieties.
[(This work was supported by FAPESP, Cane Technonolgy Center and Centralcool)]

Keywords: Phytohormones, endophytic bacteria, microarray, sucrose content, Saccharum

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POSTER
Changes in leaf gene expression patterns associated with source-sink perturbation

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The well-documented decline in gross photosynthesis during stalk maturation in sugarcane may parallel the phenomenon described for other carbohydrate-accumulating crops in which the rate of photosynthesis in the source is determined by the capacity of the sink

to accommodate recently fixed photosynthate. We have previously examined this relationship in sugarcane by monitoring photosynthesis, photosynthate allocation and leaf and internode sugar levels in plants (Saccharum spp hybrid cv. N19) in which the source:sink ratio had been decreased through the shading of all but one source leaf over a period of 14 days. That work clearly demonstrated that source leaf photosynthetic capacity is significantly up-regulated when internode sucrose levels decline and further indicated that the effect may be mediated by hexoses (glucose or fructose). The current study was initiated to examine the molecular basis of the observed correlations. To this end, variations in the expression of 131 genes with known involvement in photosynthesis, leaf carbon metabolism and sugar transport were examined in leaves subsequent to the imposition of a decreased source:sink ratio. Amongst the genes displaying altered expression, variations in transcript levels of those encoding the enzymes phosphoenolpyruvate carboxylase (PEPc), NADP+-malate dehydrogenase (NADP-MDH) and hexokinase (HXK) were significantly correlated to rising leaf hexose levels and increased photosynthetic rates, which were in turn correlated to a decrease in internode sucrose concentrations over time. Sugarcane possesses the NADP+-malic enzyme C4 photosynthetic pathway, in which PEPc and NADP-MDH facilitate the fixation of CO_2 in the mesophyll cells for subsequent malate transport into the bundle sheath cells, while HXK has been postulated to play a role in the differential regulation of plant carbohydrate metabolism genes, a role that may be mediated by metabolic hexose delivery. Expression of the PEPc gene was positively correlated with carboxylation efficiency (Vrubisco) and CO_2 saturated photosynthetic rate (Jmax), while negatively correlated with leaf glucose and fructose levels. Similarly, the abundance of transcripts encoding NADP-MDH were positively correlated to Vrubisco and Jmax, and negatively correlated to glucose and fructose. In contrast, the expression of the HXK gene was negatively correlated to photosynthesis Vrubisco and Jmax, and positively correlated to glucose concentrations. The results of this study have confirmed that decreasing sucrose levels in the internode result in increased photosynthetic activity in the leaf and demonstrated that the effect may be exerted through the transcriptional regulation of photosynthetic carbon metabolism of the mesophyll cells. In addition, the potential role of hexoses as effectors of this relationship is further supported by the strong negative correlation observed between HXK gene transcript levels and photosynthetic activity.

Keywords: Saccharum, phytosynthesis, C4 plants, carbohydrate-accumulation, phosphoenolpyruvate, carboxylase

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SUGAR CANE TRANSFORMATION

Sugarcane proteins interacting with HC-Pro, a suppressor of RNA silencing from Sorghum mosaic virus

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HC-Pro is a well known viral protein that suppresses RNA silencing in plants. We confirmed that Sorghum mosaic virus (SrMV) infection in a transgenic sugarcane line showing post-transcriptional gene silencing, induced the expression of the silenced transgene (GUS). Also, the introduction of SrMV P1/HC-Pro into another transgenic line which is post-transcriptionally silenced for SrMV coat protein (CP) resulted in the accumulation of SrMV CP RNA. These data confirmed that SrMV P1/HC-Pro acts as a suppressor of RNA silencing in sugarcane, one of the most economically important crops worldwide. In order to investigate cellular component(s) involved in RNA silencing and its suppression in sugarcane, HC-Pro was used as bait in a yeast-two-hybrid assay to screen a cDNA expression library constructed from a transgenic line of the sugarcane hybrid cultivar CP65-357 showing RNA silencing for the SrMV CP gene. Yeast-two-hybrid screening identified several cellular proteins as interactors with HC-Pro. One of them is a ca. 22KD protein that preferentially binds to RNA. In vitro binding assays such as pull down and far western assays further confirmed that SrMV HC-Pro interacts with the 22KD protein. Yeast-two-hybrid screening of the same cDNA expression library with the 22KD protein identified a ca. 33KD protein as an interactor which shows high identity with 14-3-3 proteins. In vivo interactions between HC-Pro, the 22KD and the 33KD protein were investigated by fusion with various fluorescent proteins. The possible involvement of the sugarcane proteins in RNA silencing and its suppression is under investigation.

Keywords: RNA silencing, HC-Pro, transgenic sugar cane, Sorghum mosaic virus

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Genetic manipulation of sugar cane

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Flower formation depends on the transition of a vegetative meristem to an inflorescence meristem and subsequently to a floral meristem. This transition requires the action of a number of genes, including LEAFY, a gene that is expressed very early and considered to be very important in the process. In order to study flowering in sugarcane, we have analyzed the SUCEST database (http://sucest.lad.ic.unicamp.br/en/) and found a DNA expressed sequence tag (EST) with significant similarity to the Arabidopsis LEAFY sequence. The corresponding cDNA was completely sequenced. It contains 1531 bp encoding a polypeptide with two regions highly conserved in the LEAFY/FLORICAULA family of transcription factors. In order to perform a functional characterization of the putative LEAFY gene from sugarcane we have constructed a plant expression vector containing the sugarcane cDNA in the antisense orientation under the control of a constitutive promoter. For sugarcane transformation, this vector, containing the maize ubiquitin promoter plus an enhancer and the NOS terminator was used. The selective marker was the nptII gene that confers kanamycin resistance to transformed cells. Embryogenic callus of sugarcane variety SP87-432 was transformed by particle bombardment and a number of sugarcane plants transformed with the LEAFY antisense construct were taken to the field. Observations made on their flowering behavior have allowed the conclusion that this gene truly plays an important role in sugarcane flower development.

Keywords: SUCEST database, flowering, sugar cane, particle, bombardment

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Progress towards the generation of drought tolerant transgenic sugar cane containing the CBF4 transcriptional factor from Arabidopsis

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CBF4 is one of a group of transcriptional factors whose expression is induced by drought or cold and which in turn activate many cold/drought responsive genes containing C-repeat/dehydration-responsive elements in their promoter. Activation of this set of genes in turn allows many plants to increase their tolerance to drought and cold. We are currently attempting to improve drought tolerance in sugar cane by introducing the Arabidopsis CBF4 gene into sugar cane under the control of the maize Ubiquitin promoter and the nos terminator. Three known orthologues from maize are also being tested in like manner. Selection of a suitable Mauritian sugar cane cultivar with efficient transformation and regeneration characteristics has proven difficult, however a recent change to the Australian cultivar Q117 has yielded promising results. At this stage, embryogenic callus co-transformed with CBF4, and the marker genes nptII and GFP, are showing stable expression of GFP while undergoing regeneration in the light. Transgenic lines containing the CBF4 gene will be indentified by PCR. The CBF4 transformants will be multiplied in tissue culture and then tested for their response to various environmental stresses including drought, cold and salinity. To achieve this end, a number of assays from the published literature have been adapted to sugar cane. It has been determined that most of the initial testing can be done while the plants are still in tissue culture which will greatly reduce the time taken to identify the most likely drought-tolerant candidates. These candidates will then be assessed in glasshouse and field trials for drought tolerance. Real-time PCR will be employed to assess the impact that overexpression of the CBF4 transcriptional factor in sugar cane has on key downstream water-stress-responsive genes, including putative sugar cane orthologues of Cor47 and Kin1 as identified by tblastn searches of the NCBI sugar cane EST database.

Keywords: CBF4, transcriptional factors, drought resistance, sugar cane

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SCIENTIFIC AND TECHNICAL ADVANCES

An alternative approach for micropropagation of sugar cane using leaf discs as explants

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Micropropagation of sugar cane has mostly been effected using buds or shoot tips as explants. Use of these explants, however, can result in severe oxidation or contamination with saprophytic microorganisms especially when initiated from mother plants that have been in the field for a number of years. An alternative method for the production of in vitro plantlets of sugar cane from such mother plants, using leaf discs as explants, was therefore investigated. Young leaf discs from three commercial sugar cane varieties, M 3035/66, S 17 and R 570, were dissected from surface-sterilized tops and cultured on callus-induction medium containing 3 mg/L 2,4-D for a period of two weeks. They were then cultured on regeneration medium containing 2 mg/L Kinetin and 5 mg/L NAA for four to six weeks before being transferred onto hormone-free solid medium. When shoots were about two to three cm high, they were transferred to liquid medium. Subculture onto fresh medium was effected every two to three weeks and shoot development was monitored over a period of 22 to 24 weeks. Six cane tops were used for each variety and twenty leaf discs per cane top were cultured. Prolific shoot regeneration occurred in all three varieties. Contamination and oxidation were minimal and more than 70% of the leaf discs cultured formed shoots in all varieties. About 400 and 280 shoot clumps were formed in varieties M 3035/66 and S 17 respectively whilst for variety R 570, some 250 clumps were regenerated. This study, therefore, demonstrates the potential of using leaf disc culture for micropropagation of sugar cane, especially for recalcitrant varieties that cannot be initiated from buds or shoot tips as a result of oxidation or contamination.

Keywords: micropropagation, leaf discs, cane tops, shoot regeneration

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POSTER

Increased embryogenic competence and transformation efficiency of sugarcane explants containing pre-emergent inflorescences

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Improvements to sugarcane transformation techniques are continually being made in an attempt to overcome limitations such as the labour-intensive component of developing and maintaining callus cultures, the length of time required to produce a transgenic plant and the possible negative effects of somaclonal variation due to prolonged exposure to 2,4-D. Recently, microprojectile bombardment of leaf roll discs followed by direct somatic embryogenesis has led to lower levels of exposure to 2,4-D, reduced time in culture and more cost effective production of transgenic plantlets. In this study, further refinements to the direct embryogenic transformation protocol were made by establishing the developmental phases most appropriate for efficient transformation. Length of time in culture between tissue excision and DNA delivery was investigated. In addition, the embryogenic capacities of immature leaf roll explants with and without pre-emergent inflorescences were compared. Geneticin resistance was used as the selection system and herbicide resistance as the reporter system in the study. It was shown that several days of leaf roll disc culture, correlated with initiation of embryo development, were required prior to bombardment for effective transformation to occur. In an experiment using cultivar 88H0019 and a Murashige and Skoog (1962) medium containing sucrose (30 g/l), casein (0.5 g/l), 2,4-D (0.6 mg/l) and agargel (5 g/l), pH 5.8, leaf discs containing floral initials bombarded seven days after excision yielded the highest number of transgenic plants. Subsequently, the responses of other cultivars were evaluated. Results showed that inflorescent leaf discs with pre-emergent inflorescence are invariably more competent than their non-inflorescence counterparts. Although 'escapes' occurred at a significant frequency, it was shown that combining the use of tissues undergoing floral morphogenesis with direct embryogenic regeneration has the potential to improve the speed and efficiency of transgenesis in sugarcane.

Keywords: pre-emergent inflorescences, microprojectile bombardment, leaf roll discs, embryogenic regeneration