Subtropical Plant Science 58:5-11 (2006).

Development of Simple Sequence Repeat Markers from Genes Related to Stress Resistance in Sugarcane

Jorge A. da Silva[1] and Nora Solís-Gracia

Texas Agricultural Experiment Station, Texas A&M University, 2415 E. Hwy. 83, Weslaco, TX, 78.596, USA

ABSTRACT

The discovery of simple sequence repeats (SSR) in expressed sequence tags (EST) provided the opportunity to develop SSR markers in a simple and direct way, through the electronic search on EST databases. In this paper we report the results obtained using this approach in two hundred and seventy one stress resistance related ESTs, of which 29 were found carrying SSR. Our data suggest that in sugarcane, the presence of SSRs within disease resistance genes could be one of the mechanisms that enable response to a new pathogen and/or insect damage. DNA rearrangements, resulting from slippage during replication and characteristic of SSR sequences, would allow the plant to generate novel resistance to match the changing pattern of pathogen virulence. Polymerase chain reaction (PCR) primers, flanking these SSRs, have been designed and tested as markers on sugarcane commercial hybrids, enabling the detection of polymorphism. The utilization of SSRs originated from ESTs may increase the chance of tagging genes controlling stress resistance.

Additional Index Words: Sugarcane, stress resistance, simple sequence repeat (SSR), fingerprinting
Abbreviations: SUCEST – Sugarcane Expressed Sequence Tag Project, Fundacão de Amparo à Pesquisa do Estado de São Paulo

Sugarcane is an important commodity crop of tropical and sub-tropical regions, contributing with 60% of raw sugar production in the world. Its massive productivity of sucrose, which can approach 20 percent by weight of the ripened stalk, is suggestive of a photosynthetic potential exceeding that of most plant species. Sugarcane has perfected the art of synthesizing, translocating, and storing sugar in such massive quantities like no other organism on earth (Alexander, 1973).

The genomic tools that became available during the last decade represent useful breeding adjuncts for complex crops. This brings new opportunities to explore complex plant biological systems, such as sugarcane. Single-pass sequencing of expressed sequence tags (ESTs) has proven to be one such tool that allows the identification of genes being expressed in certain tissues or cell types of multicellular organisms (Adams et al., 1991). Several sugarcane EST projects have recently been initiated in South Africa (Carson and Botha, 2000), Australia (Casu et al., 2001) and Brazil - http://sucest.lad.ic.unicamp.br/en (Vettore et al., 2001).

Another powerful technique being applied for the genome analysis of higher organisms is the development of simple sequence repeats (SSRs), which are composed of arrays of short DNA motifs of 1-6 nucleotide units repeated in tandem and randomly spread in eukaryotic genomes. These single-locus markers are characterized by their hyper variability, abundance, reproducibility, Mendelian inheritance and co-dominant nature. They are generated by the Polymerase Chain Reaction (PCR) amplification of the repeat, using primers designed from the regions flanking the repeat unit (Litt and Lutty, 1989). The computational analysis of SSR in rice (Temnykh et al., 2001) led to the categorization of SSRs in two groups based on the length of SSR tracts and their potential as informative genetic markers: Class I, containing perfect SSR $\geq$ 20 nucleotides in length and Class II, containing perfect SSR > 12 nucleotides and < 20 nucleotides in length. Longer perfect repeats (Class I) are highly polymorphic, as reported in humans (Weber, 1990) and rice (Temnykh et al., 2001). SSRs in Class II tend to be less variable, representing sites where SSR expansion may occasionally occur, but the probability of this is limited due to a smaller chance of slipped-strand mispairing over the shorter SSR template. SSRs shorter than 12 bp have a mutation potential that is no different than that of most unique sequences and were not considered in this study.

Applications of SSRs to sugarcane include the construction of molecular maps, accurate cultivar identification, parent evaluation, and marker assisted breeding (Cordeiro et al., 2000). The identification of SSR markers associated with stress resistance in sugarcane would facilitate the breeding and selection of resistant cultivars.

In the present study we describe the identification of SSRs within sugarcane stress related-ESTs and their utilization as markers in a group of modern sugarcane commercial-type genotypes.

MATERIAL AND METHODS

Electronic Search of SSRs. The presence of SSRs was investigated within stress-related genes identified in the SUCEST project (Falco et al., 2001; Rossi et al., 2003) using the software - SSRIT - Simple Sequence Repeat Identification Tool, which finds all perfect SSRs in a given sequence and is available at http://www.gramene.org/gramene/searches/ssrtool (Temnykh et al., 2001). We also searched for SSRs within a set of 3,338 ESTs

[1] Corresponding author, e-mail: jadasilva@ag.tamu.edu.

generated by the International Consortium of Sugarcane Biotechnology (ICSB) (Anderson Paterson, unpublished). Only SSRs with repeat units > 12 nucleotides were considered. PCR primer pairs were designed using the Primer3 program (Whitehead Institute for Biological Research (http://www.genome.wi.mit.edu/).

SSR Analysis. To assess the utility of SSR primers derived from stress resistance ESTs for polymorphism detection, we utilized 24 sugarcane commercial-type genotypes (Table 1). Plant DNA was extracted using the CTAB method (Brasileiro et al., 1998). PCR reactions were performed in a PTC-100 thermocycler (MJ Research, Inc. Waltham, MA). Each 20 μL of PCR reaction consisted of 1X PCR buffer, 0.4 mM dNTPs, 1.2 mM MgCl2, 0.2 units of Taq DNA Polymerase, 4 pmol of each primer and 25 ng of DNA. The amplification conditions were: 95°C for five minutes, followed by 35 cycles of 94°C for one minute, primer melting temperature for 45 seconds, 72°C for 30 seconds followed by 72°C for 30 minutes. The polymorphism level detected with SSR primers derived from stress resistance ESTs, was evaluated by the band profile generated by the PCR reaction resolved on a 6% polyacrylamide gel, stained with SYBR Gold (Molecular Probes, Eugene, OR) and visualized in ultraviolet light. Those primer pairs that successfully amplified PCR products were re-synthesized with an Infrared modification (either Irdye700 or Irdye800) for visualization of their PCR products in a NEN DNA analyzer 4300S (Li-Cor, Inc. Lincoln, NE), according with manufacturer's instructions.

SSR markers obtained from ESTs will be referred to as EST-SSRs.

Table 1. Genotypes used for assessing the polymorphism of EST-derived SSR markers.

Genotype	
CP70-321	TCP93-4245
CP72-1210	US90-18
HOCP85-845	US93-15
HOCP91-555	US93-17
HOCP93-776	US99-2
HOCP95-988	US01-39
HOCP96-540	US01-40
HOCP00-960	US02-96
L97-128	US02-96
LCP85-384	US02-97
TCP87-3388	US02-98
TCP89-3505	US02-99

RESULTS

Out of 271 stress related-ESTs investigated, 29 were positive for the presence of SSRs. Table 2 presents the EST-SSRs obtained, the ESTs' annotations and the DNA sequence of PCR primers designed to generate the SSR markers. From these, 2 presented repeat units with more than 20 base pairs, being considered hypervariable, and showed polymorphic bands when applied to the genotypes studied (Figure 1). The remaining EST-SSRs presented repeat units between 10 and 20 base pairs in length (potentially hypervariable), and failed to detect polymorphism among the commercial-type genotypes studied (Figure 2). The low level of polymorphism detected among these genotypes is in agreement with the narrow genetic base of modern sugarcane varieties (Hogarth, 1987).

In rice, EST-SSRs markers presenting repeat units with less than 20 base pairs in length have been found to be less variable than those with repeat units with more than 20 base pairs in length, due to a smaller chance of slipped-strand mispairing over the shorter SSR template (Temnykh et al., 2001). The low level of polymorphism between sugarcane cultivars detected with Class II markers suggests that the same occurs in sugarcane. This lack of polymorphism may be explained, in part, by the narrow genetic basis present in modern sugarcane varieties (Ming et al., 2001). For this reason, we expect that these primers will find polymorphism among genotypes of *S. officinarum* and *S. spontaneum*, the two species used to create modern sugarcane varieties. In this case, these markers will be useful for genome analysis of clones generated by breeding programs that use wild genotypes in hybridization crosses with elite genotypes, aiming at broadening the genetic base (Miller et al., 2005).

Six ESTs, presented repeat units in two different locations (EST-SSR30, EST-SSR31, EST-SSR38, EST-SSR41, EST-SSR43, EST-SSR54). In these cases 2 primer sets, each flanking the repeat in one location, were designed.

The search for SSRs within a group of 3,338 ESTs obtained by the ICSB showed 82 (2.5%) positive hits. To obtain their annotations, we used their sequences on a search with the BLAST program (Altschu et al., 1994) on the SUCEST database. From the 82 positive hits, only two ESTs (z0178 and z0579) were involved in stress response. The remaining was found not related to stress resistance.

SSRs were also found in four other ESTs for signal transduction components (Table 3), which may be involved in stress resistance mechanisms (Lawton et al., 1996).

Association with Insect damage. One example of association between a disease resistance EST-SSR marker and the reaction to a biotic stress resulted from our work with a stem borer (da Silva, 2005). In this study, one marker generated by an EST-SSR presented a significant association with the number of *deadhearts* - plants that have a dead apical meristem resulting from stem borer attacks (Browning et al. 1989). This marker was obtained from the primers flanking the $[CGGA]_5$ repeat unit present in the EST cluster SCSBBRZ3121G06.g, obtained by the SUCEST project. It has homology ($3.0\ e^{-47}$) to a rice (*Oryza sativa*) gene coding for a pathogenesis-related protein (accession number CA16 4678).

Fingerprinting. Sugarcane SSR markers, including the EST-SSRs reported in this paper, can be used for variety identification, also known as "fingerprinting analysis" (Cordeiro et al., 2000). Questions regarding variety identity on a particular commercial field block often arise among growers (Steve Bearden, personal communication). Before molecular markers were available for sugar cane, these questions had to be settled by visual examination of morphological traits. This criterion has limited reliability due to the possibility of morphological traits being modified by environmental conditions under which the plant was

Table 2 – SSR markers found in stress related-ESTs, their sequence similarities obtained by comparison with the NCBI database, and the DNA sequence of PCR primers flanking the repeat unit.

Marker	SSR	Primers*	Est Annotation
EST-SSR29	(CGGA)5	F: CGACTGCTGCTTCGACTACA R: GACCGATCCACCGAATCTC	pathogenesis-related protein (*Oryza sativa*)
EST-SSR30-1	(GA)5	F: ACCATCAAGCCGAATCAATC R: CCTTTGAGGGATCAACCGTA	cellulose synthase
EST-SSR30-2	(GCC)6	F: AGCTAGCAAgCgtgtcCCT R: CTCGCCCTACCAGATCTCC	cellulose synthase
EST-SSR31-1	(CGA)5	F: AAGTGGAAGACCAAGCAGGA R: GTGATCCGGAACTTGAGGAA	cellulose synthase
EST-SSR31-2	(TGC)6	F: CGTCGTCTTCTTCGACATCA R: TTGTCtTTCTTGCTCCGCTT	cellulose synthase
EST-SSR32	(TG)5	F: TCAACAACGGCGTGTACAAT R: AGGCAGTCAACTAGCGAAGC	Polygalacturonase
EST-SSR33	(GC)5	F: CTAGGTCGACGACAGGGATG R: CACAACACGGTTCCTCCTG	flavanone 3-hydroxylase-like protein -
EST-SSR34	(CGA)5	F: CGAGTCGACAAAGAACACCA R: CTCGGTGACTTCAGAGCCTT	4-coumarate--CoA ligase 4CL3 [Lolium perenne]; [SC.13] Secondary metabolism
EST-SSR35	(CG)5	F: GTACTCGATGTGCGGGTAGG R: GGCCTGCACTTCATCAACTC	anthranilate N-benzoyltransferase -
EST-SSR36	(CG)5	F: GTACTCGATGTGCGGGTAGG R: GGCCTGCACTTCATCAACTC	Sorbi small protein inhibitor of insect alpha-amylase2.1
EST-SSR37	(CGC)6	F: GCTTCTACCGCGACTTCGT R: CGATATGATGATTGGGATGG	Caffeoyl CoA O-methyltransferase
EST-SSR38-1	(CA)5	F: GCATTTTATTACACAAAACATCACAA R: CGTTCCTCACCCTTGACG	type-1 pathogenesis-related protein -
EST-SSR38-2	(GC)5	F: GTAGTCCTGCGCGTACTTGG R: TAGCAAACATGGCGTTTCTG	type-1 pathogenesis-related protein -
EST-SSR39	(AT)5	F: ACTGATTGTGCCTGTGATCG R: GCCGGGCCTGGACTACTAT	PRMS_Maize pathogenesis-related protein.-precursor
EST-SSR40	(GAG)6	F: TCCTCGGTCCTCCTCTCTCT R: AGACGGCGGACAAGGAAG	(AC006931) putative MAP kinase; Cellular communication/Signal transduction
EST-SSR41-1	(TG)5	F: CTGGTCAGTTGAAGGGAGGA R: GTACGTGGTGAACGCACTTG	LP1_Lyces Osmotin-like protein precursor; Stress response; Disease, virulence and defense
EST-SSR41-2	(CGC)6	F: CGAGCTCAAGGTCGTCTTCT R: ATTACGCAGCGCACTAATCA	LP1_Lyces Osmotin-like protein precursor; Stress response; Disease, virulence and defense
EST-SSR42	(TCG)5	F: CACGCATGCATCTGTGTTACT R: CAGGATCTACGACGAGACGA	disease resistance response protein~gene_id:MDH9.2; Stress response; Disease, virulence and defense
EST-SSR43-1	(GT)5	F: TTGAATTCCCTCCTTGATGG R: TTACGGTGTGGTTTGCTCTG	MAP kinase; Cellular communication/Signal transduction
EST-SSR43-2	(TGT)6	F: TGGTTTGAAATGGTTTGCTG R: AGGTGATGCTGAAAGGATGG	MAP kinase; Cellular communication/Signal transduction

*F = Forward; R = Reverse

Table 2 –CONTINUED

Marker	SSR	Primers*	Est Annotation
EST-SSR44	(CA)6	F: GTACGTGGTGAACGCACTTG R: CGAGCTCAAGGTCGTCTTCT	Olp1_Lyces Osmotin-like protein precursos; Stress response; Disease, virulence and defense
EST-SSR45	(GGC)5	F: AGCCTCCCTCTCCTTCTCTG R: GCTCACGTCGTAGAAGTCCA	thaumatin-like protein precursor; Stress response; Disease, virulence and defense
EST-SSR46	(GCA)5	F: GAGGATCCAGATGGATGAGG R: CTCACGAATGGCGTGTCA	(AB015855) transcription factor TEIL; Cellular communication/Signal transduction
EST-SSR47	(GCC)5	F: AACGGCTCCATGGTCTACCT R: TGGCTGATATGGACGACAAA	pathogenesis-related protein 19K4.140; Stress response*; Disease, virulence and defense
EST-SSR48	(TGG)6; (GCG)6	F: GCCAATCTCGGACCTACAAC R: CGGCTTCCTTAGTGGAGAGA	hypothetical protein; MAL5, MUC1 glucoamylase s1/s2 precursor (glucan 1,4-alpha-glucosidase) (1,4- alpha-D-glucan glucohydrolase) [EC:3.2.1.3]
EST-SSR49	(CGA)5	F: CAGTCCACGTCGTACCAGTC R: ATTCCCACACCTGCTACTCG	polygalacturonase inhibitor; Plant growth and development*
EST-SSR50	(GAG)5	F: CTGCTGCTGTGTGCTGTAGG R: CAACTTTTCGCCCTCCAAT	probable ethylene-response protein; Cellular communication/Signal transduction
EST-SSR51	(GGC)7	F: GCGTAGGCCGTACCAAAG R: TAGTAGCCCTCGAGGCAGAG	pir||T05314 hypothetical protein F26P21.180; Protein metabolism; Protein folding and stabilization
EST-SSR52	(TA)5	F: TGCACACGGACGTGTCTATAA R: TGGTACAACTACGCCACCAA	PRMS_maize pathogenesis related protein PRMS precursor; Stress response; Disease, virulence and defense
EST-SSR53	(CGT)5	F: ACGTCGTCGtTACCCCAGAT R: GTATCTAGCGACCGCTGCTC	AP2 domain transcription factor-like*; Cellular communication/Signal transduction
EST-SSR54-1	(CA)5	F: CTCAGACAGTATGCCCCACA R: ACGCTAGTTCAGGGTGATGg	AB018117) contains similarity to EREBP-4~gene_id:MQL5.17; [Putative protein]
EST-SSR54-2	(CA)5	F: CCGCTATGGAAGTGTGGACT R: CCTCTACTGGTTCGGACTCG	AB018117) contains similarity to EREBP-4~gene_id:MQL5.17; [Putative protein]
EST-SSR56	(AT)5	F: AATCAAAGAGTGACACCTTACTTTC R: TCCAGCATATAGCTCCTTCATC	receptor-protein kinase-like protein; Stress response; Disease, virulence and defense
EST-SSR57	(CAC)6	F: GCGTCTCTGACACGTGAAAC R: CAGGCGAGAGGTTGTAGAGG	CITRATE CYCLE
EST-SSR80	(CGC)5	F: GTTCCCACCGCTGTCATC R: TACGAGCACGTGTCCAACTC	cysteine protease component of protease-inhibitor complex [Zea mays]; Stress response; Cell rescue activities ; Insect Resistance

*F = Forward; R = Reverse

grown (Moore, 1987). Our results indicate that the hypervariable EST-SSR markers presented in this paper will be useful for fingerprinting analysis.

DISCUSSION

SSR sequences were first identified and analyzed in human DNA, where the mapping of different hereditary disease genes led to the discovery of tri-nucleotides SSR sequences, either within or proximal to these sites. Although tri- and tetra-nucleotide SSRs may be less abundant, they may prove to be more useful than any of the di-nucleotide SSRs due to the absence of the stutter bands produced by the Taq Polymerase enzyme (Brown et al., 1996). In fact, tri-nucleotide repeats were found to be more abundant than di-nucleotide repeats in the stress-related genes investigated. In addition, the EST-SSR marker associated with stem borer reaction (da Silva, 2005) results from a tetra-nucleotide repeat. The EST cluster (SCSBRZ3121G06.g) where this repeat unit is found has been annotated in the Stress Response, Disease,

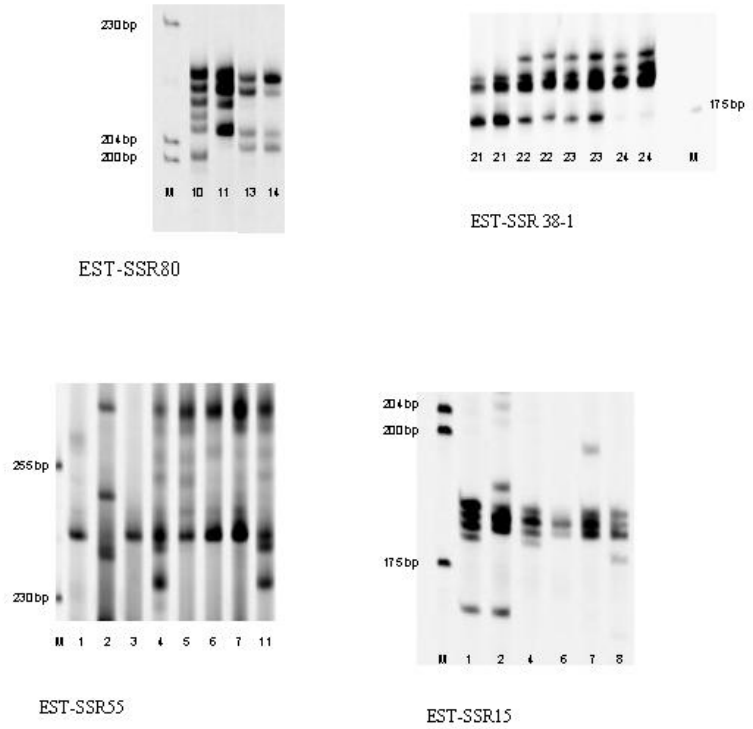

Figure 1 – SSR markers resulting from amplification with four different EST-SSR markers, visualized in the Li-Cor 4300 DNA analyzer; according with sample number and genotype name, respectively: 1)US99-2; 2)US93-15; 3)CP70-321; 4)HOCP93-776; 5)HOCP00-960; 6)HOCP96-540; 7)US02-98; 8)HOCP91-555; 9)LCP85-384; 10)US01-39;11)HOCP95-988;12)US02-99;13)L97-128;14)US02-96;15)US93-17;16)US01-40;17)US90-18;18)US02-95;19)HOCP85-845; 20)US02-97; 21)CP72-1210; 22)TCP87-3388; 23)TCP89-3505; 24)TCP93-4245.

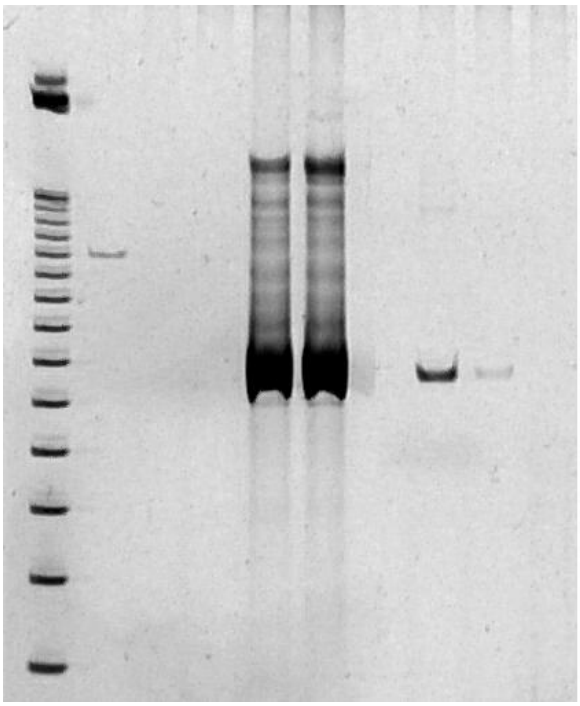

Figure 2 - PCR products visualized in 7% poliacrylamide gel, stained with SYBR gold and observed under UV light.

Virulence and Defense categories. The cluster contains ESTs obtained from different cDNA libraries, such as calli, leaf roll, apical meristem, stem, root to shoot zone, root apex, flower, and seeds. This indicates that this gene is expressed not only on the tissues where the pest attacks (leaf and inter-node tissue), but also in other tissues.

A review on the molecular genetic analysis of resistance genes shows that the predominant strategy which plants utilize in the defense against pathogens is the hypersensitive response (HR), characterized by a rapid, localized cell death at the point of pathogen attack/recognition (Rossi et al., 2003). The gene-for-gene model proposed by Flor (1956) to explain HR states that a dominant gene from the host interacts with a corresponding avirulence dominant gene from the pathogen. This interaction elicits HR thus providing resistance. The current hypothesis explaining this mechanism is supported by information obtained with genomic tools and includes the interaction of a ligand produced by the pathogen with a corresponding plant receptor thereby triggering the activation of a defense response. In this scenario, those genes involved in such interaction, both from the pathogen and the plant, are subjected to different evolutionary forces. Under this hypothesis, since virulence is recessive, a simple loss-of-function mutation in the avirulence gene of the pathogen allows it to become virulent on the host. The plant needs a mechanism flexible enough to ensure response to a new pathogen (Richard and Ronald, 2000).

Table 3- ESTs for signal transduction components carrying SSR.

Sequence	Annotation
SCRFRT3058H03.g	omega-3 fatty acid desaturase (EC 1.14.99.-) FAD3 – wheat; Lipid, fatty-acid and isoprenoid metabolism
SCCCCL4013D09.g	12-oxophytodienoate reductase 3 [Lycopersicon esculentum] - Cellular communication/Signal transduction ; Secondary metabolism.
SCSBHR1052D02.g	12-oxophytodienoate reductase OPR1 [Arabidopsis thaliana] Stress response; Secondary metabolism
SCSGLR1025A04.g	probable giberellin 3beta-dioxygenase (EC 1.14.11.15) – wheat; Secondary metabolism; Cellular communication/Signal transduction

DNA rearrangements play a key role in the evolution of these genes, thus allowing plants to generate novel resistance to match the changing pattern of pathogen virulence (Pryor, 1987). Intra-genic recombination provides a mechanism to generate novel resistance specificities whereas repeated DNA flanking disease resistance genes, may enhance subsequent duplication through unequal crossing-over events (Ellis et al., 1995). Our results suggest that the DNA repeats found within sugarcane resistance genes may contribute to the generation of novel resistance specificities. DNA rearrangements resulting from slippage during replication, which is characteristic of SSR sequences, could generate novel resistance to match the changing pattern of pathogen virulence.

ACKNOWLEDGEMENT

Seed cane from Louisiana varieties was kindly provided by William White. Funds for this research were provided by the Rio Grande Valley Sugar Growers Inc. and the American Society of Sugar Cane Technologists, Louisiana Division.

REFERENCES

Adams, M.D., J.M. Kelley, J.D. Gocayne, M. Dubnick, M.H. Polymeropoulos, H. Xiao, C.R. Merril, A. Wu, B. Olde, and R.F. Moreno. 1991. Complementary DNA sequencing: Expressed sequence tags and human genome project. Science 252 (5013): 1651-1656.

Alexander, A.G. 1973. Sugarcane Physiology, 752 pp. Elsevier, Amsterdam.

Brasileiro, A.C.M., V.T.C. Carneiro. 1998. Manual de Transformação Genética de Plantas., Embrapa-SPI/Embrapa-Cenargen 309 pp., Brasília.

Brown, S.M., A.K. Szewc-McFadden and S. Kresovich.1996. Development and Application of Simple Sequence Repeat (SSR) Loci for Plant Genome Analysis. In: Methods of Genome Analysis in Plant. ed. Prem Jauhar 386 pp. CRC Press, Inc. Boca Raton, Florida, USA.

Browning, H.W., M.O. Way and R.M. Drees. 1989. Managing the Mexican rice borer in Texas . Texas Agric. Ext. Serv. Bull 1620.

Carson, D.L. and F.C. Botha. 2000. Preliminary Analysis of Expressed Sequence Tags for Sugarcane. Crp Sci. 40: 1769-1779.

Casu, R, C. Dimmock, M. Thomas, N. Bower, D. Knight, C. Grof, L. McIntyre, P. Jackson, D. Jordan, V. Whan, J. Drenth, Y. Tao, and J. Manners, 2001. Genetic and Expression Profiling in Sugarcane. Proc. Int. Soc. Sugarcane Technol. 24: 626-627.

Cordeiro, G.M., G.O. Taylor and R.J. Henry. 2000. Characterization of SSR Markers from Sugarcane (Saccharum spp.), a highly polyploid species. Plant Science 155: 161-168.

da Silva J. A., W.H. White, N. Solís-Gracia and M. Setamou. 2005. A Molecular Approach to Breeding Sugarcane for Borer Resistance. Proc. Int. Soc. Sugar Cane Technol.-ISSCT Silver Jubilee Congress, 487 - 492. Guatemala.

Ellis, J, J.G. Lawrence, E.J. Finnegan, P.A. Anderson 1995. Contrasting complexity of two rust resistance loci in flax. Proc. Natl. Acad. Sci. USA. 92: 4185-4188.

Falco, M.C., P.A.S. Marbach, P. Pompermayer, F.C.C. Lopes, and M.C. Silva-Filho. 2001. Mechanisms of sugarcane response to herbivory. Genetics and Molecular Biology, 24 (1-4), 113-122.

Flor, H. 1956. The complementary genetic system in flax and flax rust. Adv. Genet. 8: 29-54.

Hogarth, D. M. 1987. Genetics of sugarcane: 255-272. *In* Sugarcane improvement through breeding. DJ Heinz (ed.). Elsevier, Amsterdam.

Lawton, K., L. Friedrich, M. Hunt, K. Weymann, T.P. Delaney, H. Kessmann, T. Staub and J. Ryals. 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. Plant J.10: 71-82.

Litt, M and J. Lutty. 1989. A hypervariable SSR revealed by invitro amplification of a di-nucleotide repeat within the cardiac muscle actin gene. Am. J. Hum. Genet. 44, 397-401.

Miller, J.D., P.Y Tai, S.J. Edme, J.C. Comstock, B.S. Glaz, R.A. Gilbert. 2005. Basic Germplasm Utilization in the Sugarcane Development Program at Canal Point, Fl, Usa. International Society of Sugar Cane Technologists Proceedings. Vol. 2:532-536.

Ming, R., S-C. Liu, P.H. Moore, J.E. Irvine, and A.H. Paterson. 2001. QTL Analysis in a Complex Autopolyploid: Genetic Control of Sugar Content in Sugarcane. Genome Research 11: 2075-2084.

Moore, P. 1987. Anatomy and Morphology. In: Sugarcane Improvement Through Breeding. 85-142. Ed Don Heinz Elsevier Amsterdam 603pp.

Pryor, A.J. 1987. The origin and structure of fungal disease resistance genes in plants. Trends Genet. 3: 157-161.

Richard, T.E. and P.C. Ronald. 2000. The evolution of disease resistance genes. Plant Mol. Biol. 42: 195-204.

Rossi, M, P.G. Araujo, F. Paulet, O. Gasmeur, V. Dias, H. Chen, M-A Van Sluys, and A. d'Hont. 2003. Genonic distribution and characterization of EST-derived resistance gene analogs (RGAs) in sugarcane. Mol Gen Genomics 269: 406-419.

Temnykh, S, G. DeClerck, A. Lukashova, L. Lipovich, S. Cartinhour and S. McCouch, 2001. Computational and experimental analysis of SSRs in rice (Oryza sativa L.): Frequency, length variation, transposon association, and genetic marker potential. Genome Research 11: 1441-1452.

Vettore, A.L., F.R. da Silva, E.L. Kemper, and P. Arruda, 2001. The libraries that made SUCEST . Genetics and Molecular Biology 24 (1-4): 1-8.

Weber, J L. 1990. Informativeness of human (dC-dA)n (dG-dT)n polymorphisms. Genetics 7: 524-530.