A MOLECULAR APPROACH TO BREEDING FOR STEMBORESTER RESISTANCE IN SUGARCANE

By

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Abstract

LEPIDOPTERAN stemborers, such as the Mexican rice borer (MRB) \textit{Eoreuma loftini} and sugarcane borer (SCB) \textit{Diatraea saccharalis}, are important insect pests of sugarcane (\textit{Saccharum} spp.) worldwide. Alternative control strategies are needed for both species due to the high costs associated with insecticides and resulting environmental concerns. One of the most promising control strategies is host plant resistance. However, few breeding programs actively breed for insect resistance because of the absence of effective selection procedures. Recent advances in sugarcane molecular biology, such as the development of molecular markers, offer new opportunities for selection and breeding for stemborer resistance. Damage levels of both the MRB and SCB were quantified by the number of emerging shoots killed (deadheart) and percentage of internodes damaged by larvae from a diverse population of 24 sugarcane clones. These clones represented cultivars and elite clones of known and unknown reaction to both borers. The evaluation was conducted under natural infestations in a randomised complete block replicated five times, where genotypes with extreme reactions were identified. Evaluation indicated L97-128 as the most susceptible cultivar with a mean of 15 deadhearts and 7\% damaged internodes, while several clones averaged less than one deadheart and less than 1\% internode damage. Twenty-nine microsatellite (SSR) fragments were evaluated for their association with resistance to both borers. Twenty-three SSR fragments were obtained from disease and insect resistance genes identified by the Sugar Cane Expresssed Sequence Tag Project (SUCEST) and six SSR fragments were from the Sugar Cane Microsatellite Consortium (SMC). Microsatellite analysis identified informative markers developed from sugarcane disease and insect resistance genes. One of these markers showed a possible association with stemborer susceptibility and is being evaluated further.

Introduction

Stemborers are among the most damaging insect pests of sugarcane worldwide. The sugarcane borer (SCB), \textit{Diatraea saccharalis} (F.) (Lepidoptera: Crambidae) is the most important species in the Americas. In 1980, a new stemborer, the Mexican rice borer (MRB), \textit{Eoreuma loftini} (Dyar) (Lepidoptera: Crambidae) was found infesting sugarcane fields in the Lower Rio Grande Valley of Texas.

By 1991, the MRB had replaced SCB as the main insect pest of sugarcane in Texas and now poses a threat to sugarcane and rice production throughout the southern USA. Economic losses of MRB in Texas have been estimated to be over US$1181/ha (Legaspi \textit{et al.}, 1999).

Control of MRB with insecticides has been inconsistent and generally unsatisfactory (Johnson, 1985). Results from studies with different insecticides rarely showed an increase in cane yield or commercially recoverable sugar yields.

Furthermore, insecticide efficacy did not produce an economic return on the insecticide investment (Meagher \textit{et al.}, 1994). Insecticide control of the SCB has been successful in Louisiana. However,
economic and environmental concerns place increasing pressure on the use of this control tactic. Therefore, the need for alternative control strategies, such as host plant resistance, has become crucial.

Resistant cultivars can allow for a more permanent control of pest populations (Smith, 1989) and is compatible with other control strategies (Kogan, 1994). Plant resistance has been a major component of the integrated pest management (IPM) system to control SCB in Louisiana (Bessin and Reagan, 1990). Recent studies have shown SCB resistance to be a highly heritable trait (White et al., 2001), and selection indices have been determined (Milligan et al., 2003). However, few sugarcane breeding programs actively breed for insect resistance, given the labour intensive selection procedures required.

Recent advances in sugarcane molecular biology, such as the development of microsatellite markers, offer new opportunities for selection and breeding for stemborer resistance. Microsatellite markers from sugarcane have been isolated from both expressed sequence tag – EST (da Silva, 2001) and genomic libraries (Cordeiro and Henry, 2001).

A comparison between these two sources of SSRs, EST derived (EST-SSR) or obtained from genomic libraries (genomic-SSR), revealed that genomic-SSRs had a higher degree of polymorphism among sugarcane clones as compared to EST-SSRs (Cordeiro and Henry, 2001). However, for applied molecular breeding for stress resistance, such as to stemborer resistance, EST-SSRs are more attractive.

This is explained by the higher chance they offer of tagging important genes controlling the trait (da Silva, 2001), given that polymorphism can be detected among elite genotypes. The objective of the present study was, firstly, to identify sugarcane genotypes with extreme reaction to stemborer (MRB and SCB) and, secondly, to identify polymorphic microsatellite markers derived from sugarcane ESTs, with emphasis on those related to disease and insect resistance, to be used for molecular breeding of resistant cultivars.

**Materials and methods**

**Field trial**

The sugarcane clones used in this study (Table 1) were selected because they have shown different levels of stemborer damage under SCB or MRB infestations in previous studies (Reay-Jones et al., 2003; White et al., 1993; White et al., 1998). All clones were planted on 3 September 2003 near Progresso, Texas, in 6.1 m long and 1.8 m wide one-row plots, in a randomised block design, with five replications.

The trial was conducted under normal management practices and plots were irrigated every two weeks as recommended for commercial production. Surveys for deadhearts started on 3 February 2004 when infestations of stemborders were first detected. Three surveys were made with approximately 15 days between surveys. Fifteen stalks were randomly collected from each plot for stembor damage assessment on 16 August 2004. Numbers of bored internodes were determined and positions of damage on each stalk were verified by mechanically splitting the stalks.

Percent damaged internodes were measured by the ratio of bored internodes to non-bored internodes expressed as a percentage. Arc sin transformation [Arc sin (square root (%)) * 57.3] was applied before the performance of analysis of variance. The number of deadhearts and the transformed percentage of internodes damaged, for each variety, were subjected to analysis of variance using PROC GLM.

**Microsatellite analysis**

Polymerase chain reactions (PCR) for microsatellite analysis were performed according to the procedures reported by da Silva (2001). Primers were synthsed with an infrared modification (either IRDye700 or IRDye800) for visualisation of PCR amplification in a DNA analyser.

Associations between markers and stemborer damage were analysed using ANOVA with a single factor model. Simple linear regression analysis was performed utilising the genotype information generated by each marker as an indicator variable, which received the value 1 or 0 for presence or absence.

**Results and discussion**

**Field trial**

Results of the deadheart counts are presented in Table 1, and of damaged internodes, in Table 2. In this study, we did not differentiate between deadhearts or internode damage caused by MRB and SCB, as both species were present. The recently released cultivar L97-128, considered susceptible to sugarcane borer, had the highest numbers of deadhearts, followed by US02-95 and US02-97, two genotypes considered resistant to SCB. This apparent contradiction could be attributed to differences in genotype reaction to MRB or possibly an expression of a different mechanism of resistance to deadhearts, although White and Dunckelment (1989) reported a relationship between deadhearts and percent internodes bored in the field. However, these two genotypes presented less stalk damage (Table 2).
Stalk damage results in the present study indicate that the stemborer incidence was low, particularly for MRB as sugarcane was grown in a non-stress condition (absence of drought). The regression analysis of deadhearts on stalk damage shows a poor association between these two variables (regression coefficient = 1.64 \( ^{\text{a}} \)), with a low correlation (\( r^2 = 0.38 \)).

Table 1—Mean number of deadhearts caused by stemborers in sugarcane clones, under natural field infestation, Runn Farm, Progresso, TX, 2003.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Deadhearts</th>
<th>Clone</th>
<th>Deadhearts</th>
</tr>
</thead>
<tbody>
<tr>
<td>L97-128</td>
<td>15.0 a</td>
<td>US01-40</td>
<td>1.6 defg</td>
</tr>
<tr>
<td>US02-95</td>
<td>8.0 b</td>
<td>US93-17</td>
<td>1.4 efg</td>
</tr>
<tr>
<td>US02-97</td>
<td>7.8 b</td>
<td>Ho95-988</td>
<td>1.0 fg</td>
</tr>
<tr>
<td>US02-99</td>
<td>6.2 bc</td>
<td>US01-39</td>
<td>0.8 fg</td>
</tr>
<tr>
<td>HoCP85-845</td>
<td>4.6 cd</td>
<td>CP72-1210</td>
<td>0.8 fg</td>
</tr>
<tr>
<td>TCP67-3388</td>
<td>4.2 dce</td>
<td>HoCP96-540</td>
<td>0.6 fg</td>
</tr>
<tr>
<td>US02-96</td>
<td>4.2 cde</td>
<td>TCP93-3605</td>
<td>0.6 fg</td>
</tr>
<tr>
<td>US90-18</td>
<td>3.4 cdef</td>
<td>HoCP00-960</td>
<td>0.6 fg</td>
</tr>
<tr>
<td>LCP85-384</td>
<td>2.4 defg</td>
<td>CP70-321</td>
<td>0.6 fg</td>
</tr>
<tr>
<td>TCP93-4245</td>
<td>2.0 defg</td>
<td>US99-2</td>
<td>0.4 fg</td>
</tr>
<tr>
<td>HoCP91-555</td>
<td>2.0 defg</td>
<td>HoCP93-776</td>
<td>0.2 g</td>
</tr>
<tr>
<td>US02-98</td>
<td>1.8 defg</td>
<td>US93-15</td>
<td>0.2 g</td>
</tr>
</tbody>
</table>

1 Means within the same column followed by the same letter are not significantly different (\( P \leq 0.05 \), t-test)

Table 2—Internode damage expressed as number of internodes injured divided by total internodes examined.

<table>
<thead>
<tr>
<th>Clone</th>
<th>Damage</th>
<th>Clone</th>
<th>Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>L97-128</td>
<td>0.07</td>
<td>US02-97</td>
<td>0.03</td>
</tr>
<tr>
<td>HoCP91-555</td>
<td>0.06</td>
<td>CP72-1210</td>
<td>0.03</td>
</tr>
<tr>
<td>TCP93-4245</td>
<td>0.06</td>
<td>HoCP96-540</td>
<td>0.03</td>
</tr>
<tr>
<td>US02-99</td>
<td>0.05</td>
<td>US02-95</td>
<td>0.03</td>
</tr>
<tr>
<td>LCP85-384</td>
<td>0.04</td>
<td>US93-17</td>
<td>0.02</td>
</tr>
<tr>
<td>HoCP00-960</td>
<td>0.04</td>
<td>US02-98</td>
<td>0.01</td>
</tr>
<tr>
<td>US99-2</td>
<td>0.04</td>
<td>US93-15</td>
<td>0.01</td>
</tr>
<tr>
<td>CP70-321</td>
<td>0.04</td>
<td>US01-40</td>
<td>0.01</td>
</tr>
<tr>
<td>HoCP95-988</td>
<td>0.04</td>
<td>HoCP93-776</td>
<td>0.01</td>
</tr>
<tr>
<td>HoCP85-845</td>
<td>0.04</td>
<td>US01-39</td>
<td>0.01</td>
</tr>
<tr>
<td>TCP87-3388</td>
<td>0.03</td>
<td>US02-96</td>
<td>0.01</td>
</tr>
<tr>
<td>US90-18</td>
<td>0.03</td>
<td>TCP89-3505</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Microsatellite markers

Six microsatellite primer pairs were tested on 24 sugarcane clones and produced a total of 29 markers. Five of these primer pairs were developed from stress response genes (da Silva and Solis-Gracia, 2003), and one from genomic DNA (Cordeiro and Henry, 2001). The underlying assumption of using marker loci to detect polygenes is that linkage disequilibrium (LD) exists between alleles at the marker locus and alleles of the linked polygene(s) ( Tanksley, 1993).

To analyse the genetic variation of stemborer reaction, we followed the LD approach proposed by Lu et al. (1994), which utilises a population of modern clones rather than progeny of a cross, and does not require a molecular marker linkage map. To identify candidate markers for further genetic analysis in a segregating progeny, we utilised the point analysis approach, which uses one marker at a time to analyse the data (Edwards et al., 1987). To search for potential associations between these markers and stemborer resistance, we compared marker occurrence (presence/absence) with the stemborer reaction of the elite.
genotypes studied. From the 29 SSRs screened, this preliminary assessment identified one marker (EST-SSR29iii), present in 9 of the 24 clones studied, with a significant association to stemborer susceptibility as measured by deadhearts (Table 3). EST-SSR29iii is derived from a disease resistance gene (EST SCSBRZ3121 G06.g) that has homology (3.0 e-47) to a pathogenesis-related gene in rice.

No association was found between SSR markers and internode damage. The overall low internode damage observed (1%–7%) may have precluded the detection of a statistical association. Stem borer damage will be evaluated on the same trial in the first ratoon crop, when field practices will be applied to increase stem borer incidence. These practices will include the control of ants, important predators of stemborers, and reduced irrigation. Since only 24 clones were studied, this marker will be applied to other clones, in order to confirm its association with stem borer reaction. These new genotypes will be planted on the same site in September 2004, to have their stemborer reaction assessed in September 2005. If confirmed, the marker will be applied in the progeny of a cross involving parents with contrasting reactions to stemborer for marker assisted selection.

Table 3—Results of simple linear regression analysis of marker EST-SSR29iii on the mean number of deadhearts obtained from sugarcane genotypes under natural stemborer infestation.

<table>
<thead>
<tr>
<th>Source</th>
<th>Coefficient</th>
<th>Std. Error</th>
<th>t Stat</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>6</td>
<td>3.19</td>
<td>1.88</td>
<td>0.07</td>
</tr>
<tr>
<td>EST-SSR29iii</td>
<td>12.3</td>
<td>5.21</td>
<td>2.37</td>
<td>0.027</td>
</tr>
</tbody>
</table>

Simple coefficient of determination, $r = 0.45$; $r^2 = 0.20$.

DNA repeats found within sugarcane resistance genes may provide a mechanism to generate novel resistance specificities and the microsatellite markers derived from them may represent a powerful tool for resistance gene tagging and subsequently more efficient stemborer selection programs. By targeting sugarcane genes coding for defence proteins, we expect to speed up the process of finding suitable sources of pest resistance. The putative association of a marker developed from a SSR found within a resistant gene supports the hypothesis proposed by da Silva and Solis-Gracia (2003), explaining the presence of SSR within stress resistance genes, as a mechanism to generate genetic variation, creating new resistant alleles.

Conclusions

Our preliminary results allowed the identification of informative (polymorphic) markers derived from sugarcane stress resistance genes with one of these being potentially associated with susceptibility to deadhearts. Other more traditional methods of determining stemborer resistance also will be evaluated. Among these are laboratory bioassays to determine larval growth on diets incorporating freeze-dried plant tissue. Different mechanisms of resistance will provide a greater inference base for identifying additional markers useful in selecting for stemborer resistance.

Different levels of resistance to MRB are currently present in sugarcane cultivars and elite genotypes. This information will be used to cross genotypes with contrasting reactions (resistance/susceptibility) for segregation studies. Microsatellite markers derived from sugarcane stress resistance ESTs may represent a powerful tool for tagging stemborer resistance genes in sugarcane. These informative markers will be applied in conjunction with other kinds of markers such as AFLP and cDNA-AFLP (Butterfield et al., 2004) to the progeny of crosses involving contrasting parents, with the goal of genetic mapping of stemborer resistance.

Acknowledgement

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REFERENCES


APPROCHE MOLÉCULAIRE DANS LE PROGRAMME DE SÉLECTION POUR LA RÉSISTANCE AU FOREUR DE LA CANNE À SUCRE

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MOTS CLES: Foréurs des Tiges, Eoreuma Loftini, Diatraea Saccharalis, Sélection Assistée par Marqueurs, Microsatellites.

Résumé

Les foréurs lépidoptères, tels le foreur mexicain du riz, Eoreuma loftini et le foreur de la canne à sucre, Diatraea saccharalis, sont des ravageurs importants de la canne à sucre (espèces Saccharum) à travers le monde. En raison des coûts élevés des insecticides et des problèmes liés à l'environnement, des stratégies...
diferentes son necesarias para lutter contre ces deux espèces. La résistance de la plante hôte à l'insecte constitue une des méthodes de lutte les plus prometteuses. Cependant, peu de programmes de sélection sont axés sur le développement de la résistance aux ravageurs en raison de l'absence de méthodes de sélection efficaces. Les progrès récents en biologie moléculaire tels que les marqueurs offrent de nouvelles possibilités de sélection pour la résistance au foreur. Les dégâts causés par ces deux foreurs ont été quantifiés à travers le nombre de rejets morts (tiges mortes) et le pourcentage d'entre-œufs endommagés par les larves chez une population diverse de 24 variétés de canne, comprenant des cultivars et des clones prometteurs dont la réaction aux foreurs n'était connue que dans certains cas. L'évaluation a été menée dans des conditions d'infestations naturelles, suivant un dispositif de bloc complètement aléatoire avec cinq répétitions et où les génotypes montrant des réactions extrêmes ont été identifiés. La variété L97-128 s'est avérée la plus sensible, avec un moyen de 15 tiges mortes et 7% d'entre-œufs endommagés, alors que bon nombre de clones montraient en moyenne moins d'une tige morte et des dégâts aux entre-œufs inférieurs à 1%. Vingt-neuf marqueurs microsatellites (SSR) ont été évalués par rapport à leur association à la résistance aux deux foreurs. Vingt-trois d'entre eux ont été obtenus à partir des gènes de résistance aux maladies et aux ravageurs identifiés par le Sugar Cane Expressed Sequence Tag Project (SUCEST) et les six autres provenaient du Sugar Cane Microsatellite Consortium (SMC). L'analyse des microsatellites a permis l'identification des marqueurs importants à partir des gènes de résistance aux maladies et aux ravageurs. Un de ces marqueurs, qui est probablement associé à la sensibilité au foreur, fait l'objet d'une étude approfondie.

**UNA ESTRATEGIA MOLECULAR PARA EL MEJORAMIENTO POR RESISTENCIA A LOS BARRENADORES DEL TALLO EN CAÑA DE AZÚCAR**

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**PALABRAS CLAVES:** Barrenadores, Eoreuma loftini, Diatraea saccharalis, Selección Asistida por Marcadores, Microsatélites.

**Resumen**

Los lepidópteros barrenadores del tallo, el barrenador mexicano del arroz (MRB) Eoreuma loftini y el barrenador de la caña de azúcar (SCB) Diatraea saccharalis, son plagas importantes de la caña de azúcar (Saccharum spp.) en todo el mundo. Son dos plagas que requieren de otras estrategias de control debido a los altos costos asociados a los insecticidas utilizados además de las preocupaciones ambientales que se tienen. Una de las estrategias de control que más promete es la resistencia de la planta a la plaga. Sin embargo, existen muy pocos programas de mejoramiento que busquen mejorar por resistencia al insecto debido a la ausencia de procedimientos efectivos en la selección. Los recientes avances en biología molecular de la caña de azúcar, tal como el desarrollo de marcadores moleculares, ofrecen nuevas oportunidades para la selección y mejoramiento por resistencia al barrenador del tallo. Los niveles de daño del MRB y SCB fueron cuantificados mediante el número de brotes muertos (corazón muerto) y el porcentaje de entreunos barrenados por las larvas en la población de 24 clones de caña de azúcar. Estos clones representaron cultivares y variedades seleccionadas con reacción conocida y desconocida en un experimento de bloques completos al azar con cinco repeticiones y donde se identificaron los genotipos con reacciones extremas. La evaluación mostró que L 97-128 es el cultivar más susceptible con una media de 15 corazones muertos y 7% de entreunos dañados, mientras que varios clones tuvieron menos de un corazón muerto y menos de 1% de entreunos dañados. Veintinueve fragmentos de microsatélite (SSR) fueron evaluados en su asociación con resistencia a ambos barrenadores. Veintitrés fragmentos de SSR fueron obtenidos de los genes de resistencia a plagas y enfermedades del proyecto de caña de azúcar de Expressed Sequence Tag (EST) de SUCEST y seis fragmentos de SSR fueron entregados por el consorcio de microsatélites de caña de azúcar (SMC). El análisis de los microsatélites identificó marcadores informativos desarrollados de genes de resistencia a enfermedades y plagas de la caña de azúcar. Uno de estos marcadores mostró una posible asociación con susceptibilidad al barrenador del tallo y se encuentra bajo evaluación.