Manejo de pragas em milho transgênico

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TRANSFORMAÇÃO DE MILHO COM O GENE *cry1C* DE *Bacillus thuringiensis* VISANDO O CONTROLE DA LAGARTA DO CARTUCHO, *Spodoptera frugiperda*

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A lagarta do cartucho, *Spodoptera frugiperda*, é uma das principais pragas da cultura do milho no Brasil. A bactéria *Bacillus thuringiensis* (*Bt*) é um patógeno com grande potencial inseticida pela presença de um cristal protéico, que se forma durante o processo de esporulação desta bactéria. Este cristal é codificado pelos genes *cry* (que podem ser classificados de *cry1* a *cry43*). A cepa 1644, proveniente de uma amostra de solo de Rolândia, PR, se mostrou muito eficiente no controle da lagarta do cartucho em laboratório. Através da PCR, utilizando primers específicos para o *cry1C*, ficou confirmado que esta cepa possui esse gene cuja proteína é considerado tóxica para a S. frugiperda. A banda amplificada correspondendo a este gene (aproximadamente 2,7 kb) foi isolada do gel, clonado no vetor TOPO-TA (Invitrogen) e seqüenciada conforme instruções do fabricante (BigDye 3.1 – Sequenciador 3100 - AppliedBiosystem, USA). Para o seqüenciamento foram utilizados 12 primers ao longo da fita dupla do gene. Após o seqüenciamento, este gene foi clonado no vetor pCAMBIA 3301, sob o controle do promotor Ubiquitina de milho e o terminador NOS. O plasmídio foi amplificado em E. coli, purificado em gradiente de CsCl e usado para transformar calos embriogênicos de milho tropical via biobalística (L3 - Linhagem Elite da Embrapa Milho e Sorgo). A integração do gene *cry1C* foi confirmada por PCR em 5 das plantas de milho geradas. Folhas das plantas T0 foram submetidas ao bioensaio com S. frugiperda e mostraram diferentes níveis de resistência a praga, sendo que o evento LA 12 inibiu 100% o crescimento da lagarta.

Palavras-chave: *Spodoptera frugiperda*, Milho, Transgênicos, *cry1C*

A PROTOCOL FOR ASSESSING BEHAVIOR OF *Diabrotica* LARVAE FEEDING ON TRANSGENIC MAIZE

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The corn rootworm complex is the most destructive insect complex to maize in the United States corn-belt area. Behavioral studies of *Diabrotica* larvae have become increasingly important as the era of transgenic rootworm resistant maize begins. We have conducted behavioral studies on *Diabrotica* larvae using a novel transparent media. Direct measurements of this root feeding pest are reported. Behavioral studies provide the basis for understanding the interactions that occur at the plant root–insect interface and may help identify the way in which larvae can survive on transgenic root tissues. It has been reported that some level of survivorship and delayed emergence occurs on current rootworm resistant maize as observed through adult emergence data. The mechanism for larval survival is not entirely clear, but our data clearly show that feeding behavior is involved. For example, larvae may be able to detect subtle differences in Bt expression in the root system and survive to the next instar. This research has important impacts on our basic understanding of larval feeding behavior and provides insight into risks assigned to refuge requirements for insect resistance management strategies. Our research allows us to document the larval feeding behavior through quantitative measurements as well as digital photographic microscopy.

Palavras-chave: Plantas transgênicas, Manejo de resistência, Vaquinha

THE INTERACTION BETWEEN ALTERNATE GRASSY HOSTS AND TRANSGENIC ROOTWORM RESISTANT MAIZE: POTENTIAL IMPACTS ON DAMAGE AND RESISTANCE MANAGEMENT ISSUES

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In temperate North America, the *Diabrotica* pest complex, particularly *Diabrotica virgifera virgifera* LeConte and *D. barberi* Smith and Lawrence, known as rootworms are the most damaging pest of maize, *Zea mays* L., with more than 1$ billion (US) lost in control costs and damage. Consequently, this pest complex is the newest target for Bt mediated transgenic control by several companies. While all transgenic events are highly effective against neonate *Diabrotica* larvae, they are less effective against advanced larval instars. Our research has demonstrated that *D. v. virgifera* larvae and *D. barberi* larvae can effectively develop on many alternate grassy hosts commonly found in maize field, including species such as giant foxtail, *Setaria faberi*, and large crabgrass, *Digitaria sanquinalis*. We have also demonstrated that *Diabrotica* larvae can effectively develop on and cause damage to Bt expressing maize when larvae have initially developed on an alternate weedy host in both greenhouse and field studies. For example, in a greenhouse study where 20 neonate *D. v. virgifera* larvae were infested in pots containing glyphosate resistant *Cry3Bb1* expressing maize, its glyphosate resistant isoline planted alone and with a grassy weed combination of S. faberi, and *D. sanquinalis*, where the weeds were treated with glyphosate 10 d after infestation, results indicate that the presence and subsequent removal of weeds most likely provides *D. v. virgifera* larval refuge. Results from the prior example (and additional studies to be presented) have important implications regarding both damage potential and delaying the onset of resistance to these newest tools for management of *Diabrotica* in maize not only for North America but potentially for South American maize growing regions.

Palavras-chave: *Diabrotica*, Rootworm, Resistance management, Alternate host, Weeds