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Genética de poblaciones de lepidóptera (noctuidae) colectados sobre maíz transgénico y no transgénico en México

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Abstract: Allele frequency of the resistance of the gene Onb3GalT5 to Cry proteins was analyzed in insect populations (*Helicoverpa zea* and *Heliothis virescens*), collected from maize transgenic and non-transgenic plants. The genetic parameters estimated in these populations were allele and genotype frequency, genetic balance and Wright statistics (Fis, Fit and Fst). Statistical analyses were performed using the package *Genepop* (4.0.10). Results showed that from the DNA of *H. virescens*, collected on non-transgenic maize, a higher number of bands were amplified in a range from 250 bp to 600 bp. In contrast, the bands amplified from insects collected on transgenic maize were in the range of 250 bp-500 bp, being the 400 bp band in both cases the most frequent, while in the DNA of *Helicoverpa zea*, collected on transgenic maize, three bands (350 bp, 400 bp and 450 bp) were amplified, and in *H. zea*, collected on non-transgenic maize, only two bands (350 bp and 400 bp) were observed. Genetic diversity in insect populations was higher than within these same populations.

Keywords: Wright statistics, β-1-3 galactosyltransferases.

Resumen: En este estudio se analizó la frecuencia de alelos del gen Onb3GalT5 de resistencia a las proteínas Cry en poblaciones de insectos de *Helicoverpa zea* y *Heliothis virescens* que se colectaron de plantas transgénicas y no transgénicas de maíz. Los parámetros genéticos estimados en estas poblaciones fueron alelo y frecuencia de genotipo, balance genético y estadísticos de Wright (Fis, Fit y Fst). Los análisis estadísticos se realizaron utilizando el paquete *Genepop* (4.0.10). Los resultados mostraron que del DNA de *H. virescens*, colectados en maíz no transgénico, se tuvieron un mayor número de bandas amplificadas, con un rango de 250 bp a 600 bp. En contraste, las bandas amplificadas de los insectos obtenidos de maíz transgénico fueron en el rango de 250 bp a 500 bp, siendo la banda más frecuente en ambos casos la de 400 bp, mientras que en *Helicoverpa zea*, colectado sobre maíces transgénicos, se observaron tres bandas (350 bp, 400 bp y 450 bp), y en *H. zea*, colectado sobre maíces no transgénicos, se observaron solo dos bandas (350 bp y 400 bp). La diversidad genética dentro de las poblaciones de insectos de ambas especies fue mayor que entre estas mismas poblaciones.

Palabras clave: estadísticos de Wright β-1-3 galactosiltransferasas.

INTRODUCTION

In the past 19 years, genetically modified (GM) crops have been accepted by farmers, and the globally cultivated area using GM crops has increased from 1.7 million ha in 1996 to 185.5 million ha in 2014 (James, 2014; International Service for the Acquisition of Agri-biotech Applications [ISAAA], 2010; Pellegrino, Bedini, Nuti & Ercoli, 2018). This is an alternative technology to the use of synthetic chemical insecticides (SCI) for pest control. The use of SCI in global agriculture has imposed a significant selection pressure, leading to a rapid evolution of insecticide resistance by pests (Pélissié, Crossley, Cohen & Schoville, 2018; Tomasetto, Tylianakis, Reale, Wratten & Goldson, 2017). Some GM crops are now used worldwide for pest control; these crops express the *Bacillus thuringiensis* (Bt) Cry toxins (Bravo *et al*., 2013; James, 2014; Pardo, Soberón & Bravo, 2013). However, the development of pest resistance to transgenic crops with insecticidal toxins from Bt Berliner has been observed, and this is a major threat to their sustainable use in agriculture (Ibrahim & Shawer, 2014; Song, Kain, Cassidy & Wang, 2015; Tabashnik, Brévault & Carrière, 2013; Venugopal & Dively, 2017). Associated to the spore formation process of Bt, parasporal bodies as crystals (called Cry proteins) originate, which have an insecticidal effect (Palma, Muñoz, Berry, Murillo & Caballero, 2014). In the insect gut, the Cry protein breaks down and releases a toxin called delta endotoxin. This toxin binds to the insect gut walls and creates pores in it, resulting in an ion imbalance and paralysis of the digestive system; after a few days, the insect dies (Costa & Lopes, 2014). The Cry toxin binding to the insect intestinal wall has been associated to different mechanisms, such as Cry toxins, which can bind to extracellular domains of cadherin (Pardo *et al*., 2013; Zhang, Tiewsiri, Kain, Huang & Wang, 2012), aminopeptidase N APN (Zhao *et al*., 2017), or alkaline phosphatase receptors (Likitvivatanavong, Chen, Bravo, Soberón & Gill, 2011). Furthermore, carbohydrate modifications to peptides receptors have shown increased receptor interactions (Nizet, Varki & Aebi, 2017), suggesting that glycosylation may be common among receptors of insect midgut (Shao *et al*., 2018). Also, it has been reported that *Caenorhabditis elegans* Bt mutants (BRE) evaded pore formation in their intestine when exposed to Cry5B and Cry14A toxins (Iatsenko, Boichenko & Sommer, 2014). A b3GalT5 tentative gene from the β-1,3-galactosyltransferase family has been correlated with the bre5 mutant (Sarker & Mahbub, 2012). This suggests that glycosylation pathways, which are able to modify midgut peptide receptors, may be a type of resistance to Cry toxins (Coates, Sumerford & Lewis, 2008).

In Mexico, and especially in the Laguna region, for more than 12 years, transgenic cotton varieties have been planted in the three quarters of commercial batches; this circumstance certainly involve evolution of insect populations and affect the genetic diversity of crops, such as cotton. Native varieties planted by farmers constitute a reservoir of genes of global importance, while these varieties often have not a spectacular production; they retain valuable genetic information for resistance to pests and adverse environmental conditions (Montenegro de Wit, 2016; Santos & Bezerra, 2017). If GM crops with traits that increase their fitness are sowing freely, and are crossed with native varieties, it is possible that native breeds will be in risk of disappearance (Carpenter, 2011; Dobbs, 2017). Under this perspective, studies to understand the dynamics of insect resistance development to these transgenic plants are of vital importance. Maize is the most important crop in Mexico and is attacked by many insects, including Lepidoptera, Hemiptera, Thysanoptera, Orthoptera, Coleoptera, Diptera and mite's species. Some of these insects also affect cotton, such as the tobacco budworm (*Heliothis virescens*) and the maize earworm (*Helicoverpa zea* [Lepidoptera: *Noctuidae*]). Damage has been reported these moths in virtually all areas where maize is planted. This study provides an analysis of the frequency of resistance of the gene Onb3GalT5 insect to Cry proteins in populations of *Helicoverpa zea* and *Heliothis virescens*, collected in cultivated maize (GM and non-GM) in some producing regions in Mexico, in order to understand the dynamics and population genetics of insects associated to Bt crops.

MATERIALS AND METHODS

Sampling sites

From April 2011 to May 2012, different maize commercial lots, planted with GM and non-transgenic, were localized and classified in four regions: 1) Coahuila State, composed by the municipalities of Torreon, San Pedro de las Colonias, and Saltillo; 2) San Luis Potosi, integrated by the municipality Graciano Sánchez; 3) Chiapas, composed by the municipalities of Uixtla-Mazatan and Zuchate; and 4) Tamaulipas, integrated by the municipality Rio Bravo. In all maize lots (table 1), lepidopteran larvae of *Heliothis* and *Helicoverpa* from different larval instar, and inhabiting the maize cob, were collected by random sampling of plants within plots. Some larvae were preserved in plastic bottles containing ethyl alcohol (70%), and other larvae were allowed to continue their life cycle to corroborate their identification at the species level.

Table 1
Locations where Lepidopteran larvae were collected on different type of maize (transgenic and non-transgenic)

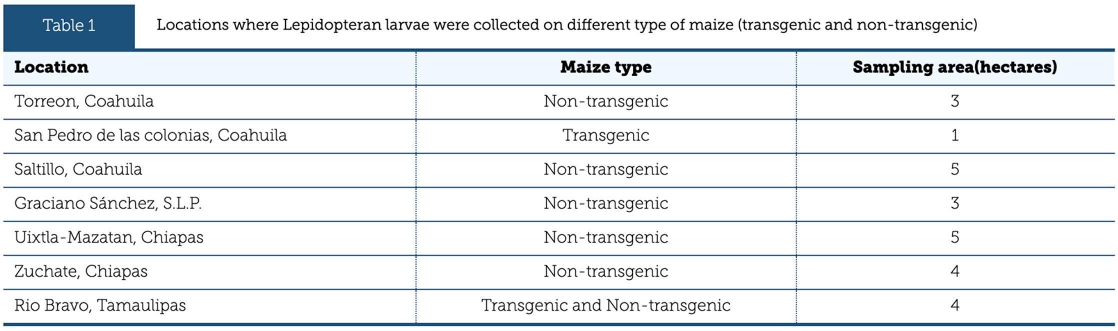

Table 1 Locations where Lepidopteran larvae were collected on different type of maize (transgenic and non-transgenic)

Location	Maize type	Sampling area(hectares)
Torreon, Coahuila	Non-transgenic	3
San Pedro de las colonias, Coahuila	Transgenic	1
Saltillo, Coahuila	Non-transgenic	5
Graciano Sánchez, S.L.P.	Non-transgenic	3
Uixtla-Mazatan, Chiapas	Non-transgenic	5
Zuchate, Chiapas	Non-transgenic	4
Rio Bravo, Tamaulipas	Transgenic and Non-transgenic	4

Source: Author's own elaboration

Taxonomic identification of insects

Lepidoptera larvae were identified using the dichotomous key reported by Stehr (2005). The larvae, collected and maintained in alcohol, were divided into two groups, according to their morphological characteristics and corresponding to one of the identified species. Then, these larvae were used for DNA isolation. On the other hand, the subgroups allowed to continue their life cycle were placed in plastic containers with moist soil and, thus, promoted its metamorphosis to complete the pupal stage and then to adult under laboratory conditions for taxonomic species level corroboration.

DNA isolation

From each larva, a tissue sample (0.1 g) was collected for DNA isolation. Subsequently, the larval fragment was placed in liquid nitrogen for 12 h. Insect tissue was macerated using a cell disruptor MP (6 m/s by 60 sec) and the MPbio matrix D. DNA isolation was performed using the AxyPrep Blood Genomic DNA Miniprep Kit procedure. DNA integrity was determined by gel electrophoresis in 1% agarose. DNA quantification was carried out in a plate reader Epoch ™ Microplate Spectrophotometer BioTek brand with a Take3 ™ attachment plate, 8 X 2 model, with the program DNA quantification Gen5 1.11.

DNA amplification

Polymerase chain reaction (PCR) was performed to amplify the OnBreGalt5 (#-1, 3-Galactosyiltransferase) gene, using the forward Onb3GalT5-F1 (5'CGTGACAATGATGTCGTTCAA3') and reverse Onb3GalT5-R1 (5'TGCTGCGGCACTAAGCCCAC3') primer, which were previously reported by Coates *et al.* (2008). The PCR was performed with a final volume of 23 µl, integrated as follow: 14.5 ml of sterile deionized water, 2.5 ml of 10X buffer, 1 µl of MgCl2 (50 mM), 0.5 ml of dNTP's (10 mM), 2 ml of each primer (10 pM), 0.5 ml of Taq polymerase (5 U/µl) and 2 ml of the DNA sample (100 ng/µl). The PCR amplification program was: denaturation at 95 °C for 2.30 min, annealing at 69.7 °C for 0.30 min and polymerization at 72 °C for 1 min for 40 cycles using a thermocycler Px2 Thermal cycle. The amplified products were visualized using gel electrophoresis 1.5% agarose. The Marker 100 bp DNA Ladder (Invitrogen ™) was used as reference.

Statistical analysis

The amplified bands in each case were codified as presence (1) and absence (0). The following parameters were estimated with sample data codified with this binary code: allele frequencies, genotype frequencies, genetic balance of the population, and statistical Wright (F_{IS}, F_{IT} and F_{ST}). Then, genetic parameters were used to compare intra-species and inter-species diversity of the insect populations collected on transgenic and non-transgenic maize. All analyses were performed using the Statistical Package *Genepop* (4.0.10).

RESULTS

Lepidoptera insect species on transgenic and non-transgenic maize

Taxonomical identification of the Lepidoptera population associated to GM and Non-GM maize from different Mexican areas showed that *H. zea*, and *H. virescens* were the species damaging the maize ear (table 2). The larva of *Heliothis virescens* (Fabricius) was characterized by having a variable body coloration (yellow to green in dark tone), 30 mm to 45 mm long, microspines in the body pinacula. The pinacula are generally large and conical and more pronounced in the abdominal segments I, II and VIII; in addition, in the I and II segments the presence of thin microspines was observed. The mandible had retinaculum. The adult body is light brown with three dark diagonal stripes on the forewings. *H. zea* was characterized by having a mandible without retinaculum and lack of microspines on the body pinacula. The adult is a straw-yellow moth with a dark spot, although sometimes it is not very well defined. Posterior wings were clearer than the previous ones and with a dark band at the apical end.

Table 2

Number of Lepidoptera larvae collected on transgenic and non-transgenic maize

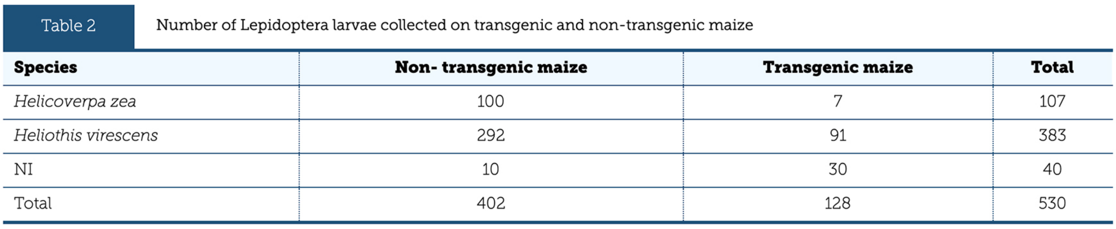

Table 2 Number of Lepidoptera larvae collected on transgenic and non-transgenic maize

Species	Non- transgenic maize	Transgenic maize	Total
Helicoverpa zea	100	7	107
Heliothis virescens	292	91	383
NI	10	30	40
Total	402	128	530

NI= no identified specimens.
Source: Author’s own elaboration.

The distribution of the collected larvae depending on the maize condition and location is showed in table 3. As expected, a higher proportion of larvae attacking maize was found in the non-transgenic maize as opposed to the transgenic maize (4:1 times). In both locations, where transgenic maize with Bt genes were planted, Lepidoptera insects attacking ears were observed, which suggests that this insect species had acquiring resistance to Cry toxins from Bt, because some of these larvae surpass the five-instar step. Insect resistance to Bt plants has been reported previously (Monnerat *et al*., 2015; Tabashnik *et al*., 2013), which could reduce the use of these varieties to long-term (Carrière, Crowder & Tabashnik, 2010; Gatehouse, Ferry, Edwards & Bell, 2011). The main mechanisms by which resistance to insect based on Cry toxins is overcome are mutations in any of four genes encoding glycosyltransferases, such as glycoside specific arthropod glycolipids which only occur in these organisms but not in vertebrates (Deist, Raush, Fernandez-Luna, Adang & Bonning, 2014; Peterson, Bezuidenhout & Van den Berg, 2017). Post-transduction glycosylation of medium stomach epithelial proteins and lipids receptors in insects may be required prior the binding of activated Bt Cry toxins (Coates, Sumerford, Hellmich & Lewis, 2007). The insect resistance by loss of carbohydrate modification is relevant in resistance to multiple Bt toxins (Tay *et al*., 2015).

Table 3
Insect species from Lepidoptera order associated to GM and non-GM from maize planting in some regions of Mexico

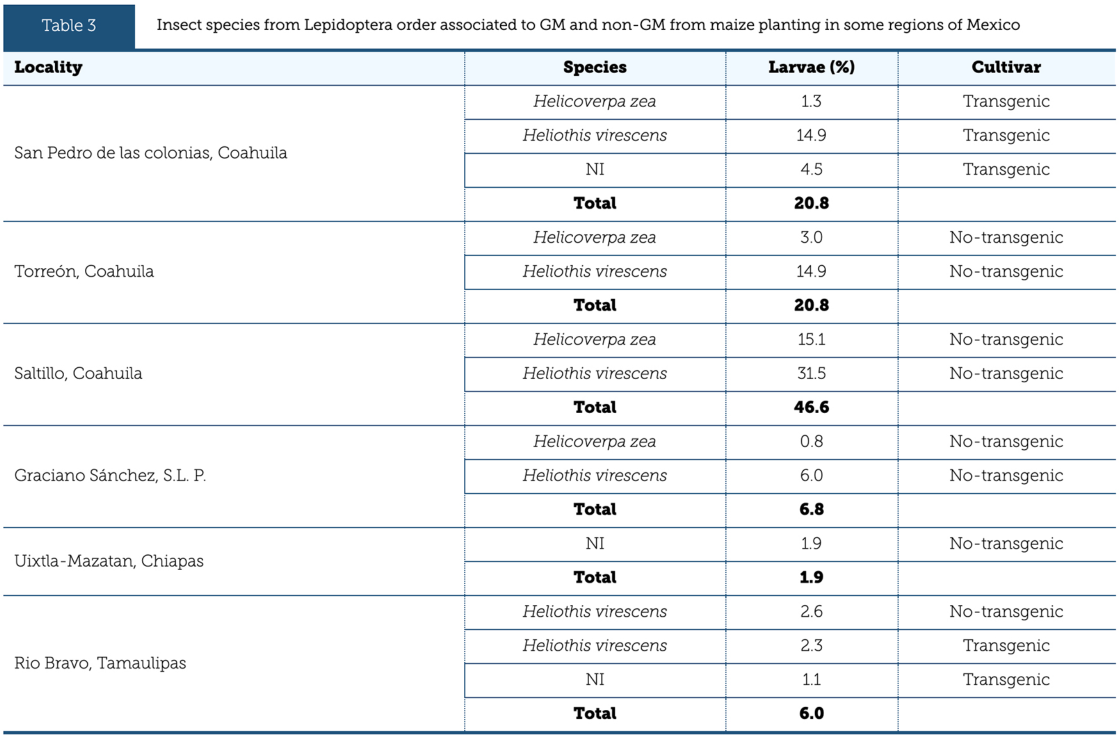

Table 3 Insect species from Lepidoptera order associated to GM and non-GM from maize planting in some regions of Mexico

Locality	**Species**	**Larvae (%)**	**Cultivar**
San Pedro de las colonias, Coahuila	*Helicoverpa zea*	1.3	Transgenic
	Heliothis virescens	14.9	Transgenic
	NI	4.5	Transgenic
	Total	**20.8**	
Torreón, Coahuila	*Helicoverpa zea*	3.0	No-transgenic
	Heliothis virescens	14.9	No-transgenic
	Total	**20.8**	
Saltillo, Coahuila	*Helicoverpa zea*	15.1	No-transgenic
	Heliothis virescens	31.5	No-transgenic
	Total	**46.6**	
Graciano Sánchez, S.L. P.	*Helicoverpa zea*	0.8	No-transgenic
	Heliothis virescens	6.0	No-transgenic
	Total	**6.8**	
Uixtla-Mazatan, Chiapas	NI	1.9	No-transgenic
	Total	**1.9**	
Rio Bravo, Tamaulipas	*Heliothis virescens*	2.6	No-transgenic
	Heliothis virescens	2.3	Transgenic
	NI	1.1	Transgenic
	Total	**6.0**	

H. virescens was the most frequently observed insect species on maize ears, independently of the transgenic condition (table 3). The *H. virescens* frequency on non-transgenic maize was up to 8-fold than *H. zea* frequency and, in transgenic maize, this frequency deceased up to 2-fold. These differences may be because the transgenic varieties expressing Cry protein are less effective against *H. virescens* or this insect had acquiring Cry toxin resistance. *H. virescens* commonly attacks cotton (Blanco, Terán-Vargas, López & Abel, 2009), however, in this study, this species was identified as the most frequent insect pest associated to transgenic and non-transgenic maize ears. In Mexico, transgenic cotton has been cultivated at commercial levels since 1996, whereas experimental transgenic maize plantations were authorized only six years ago. This may explain that *H. virescens* has been evolving resistance to Bt genes, because it has been exposed to BT toxins for a longer time, although *H. zea* is the most associated insect on maize ear (Zuñiga, Angulo, Rebolledo & Navarro, 2011). However, in the present study, on non-transgenic maize, *H. virescens* was found in a higher frequency than *H. zea*, which suggested that H. virescens, being exposed to transgenic cotton, migrated to maize, expanding its hosts range.

Allelic diversity in Heliothis virescens and Helicoverpa zea

By using the polymerase chain reaction was possible to amplify different bands for genes encoding galactosyltransferases in both *H. virescens* and *H. zea*, collected from transgenic and non-transgenic maize. The variation found in the number of amplified bands in both types of maize (table 4) could be due to mutations that arose with and without selection pressure of the Bt transgenic maize. Moreover, it is interesting that a band of 500 bp was amplified only from DNA isolated from insects collected from GM maize; besides, the frequency of the 250 bp band was higher in these insects. Moreover, bands 300 bp and 600 bp were only obtained from DNA of insects collected in non-GM maize. The main mechanism for resistance to Bt toxins in *C. elegans* involves a loss of glycolipid carbohydrates. In this case, Bt toxins bind directly and specifically to glycolipids, and this binding is carbohydrate-dependent and relevant to toxin in vivo action (Schünemann, Knaak & Fiuza, 2014). Glycolipids loss has been associated with the loss of at least two genes coding for glycosyltransferases. These enzymes work in the intestine conferring susceptibility to the toxin, given that they are required for interaction of the active toxin with intestinal cells, suggesting that they make an oligosaccharide receptor for toxin (Xu, Bi-Cheng, Yu & Sun, 2014).

Table 4
Bands amplified by PCR from genes encoding galactosyltransferases present in *Heliothis virescens* larvae from non-transgenic and transgenic maize

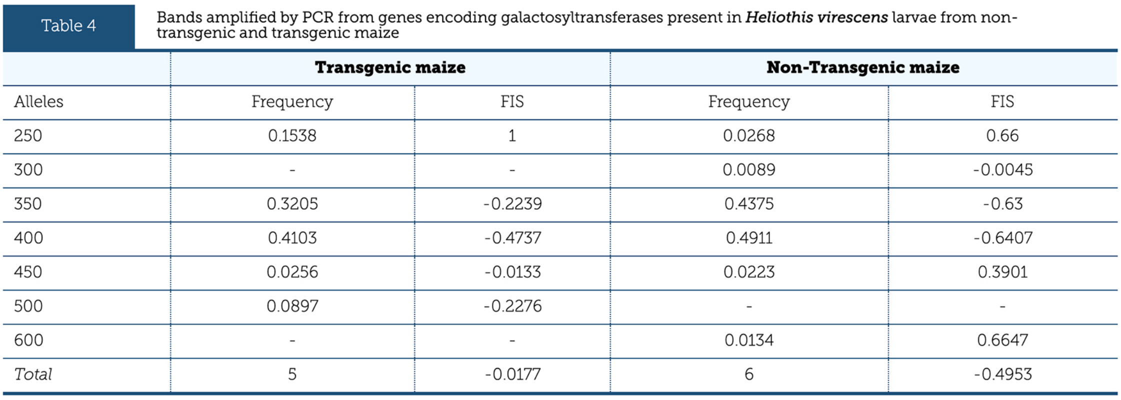

Table 4 Bands amplified by PCR from genes encoding galactosyltransferases present in ***Heliothis virescens*** larvae from non-transgenic and transgenic maize

	Transgenic maize		**Non-Transgenic maize**	
Alleles	Frequency	FIS	Frequency	FIS
250	0.1538	1	0.0268	0.66
300	-	-	0.0089	-0.0045
350	0.3205	-0.2239	0.4375	-0.63
400	0.4103	-0.4737	0.4911	-0.6407
450	0.0256	-0.0133	0.0223	0.3901
500	0.0897	-0.2276	-	-
600	-	-	0.0134	0.6647
Total	5	-0.0177	6	-0.4953

Source: Author’s own elaboration

In *H. zea* specimens less bands were amplified (table 5), But in this case, from DNA of insects collected from GM maize, a different band (450 bp) was amplified, while, from the DNA of *H. virescens*, collected on non-transgenic maize, some bands (300 bp and 600 bp) were different to those amplified from insects collected on non-transgenic maize. It is important to continue studying the evolution of these bands. All insects were collected feeding on maize ears, so those collected from transgenic maize must have resistance to Cry toxins.

Table 5
PCR amplified bands of genes encoding galactosyltransferases present in larvae of Helicoverpa zea in transgenic and non-transgenic maize

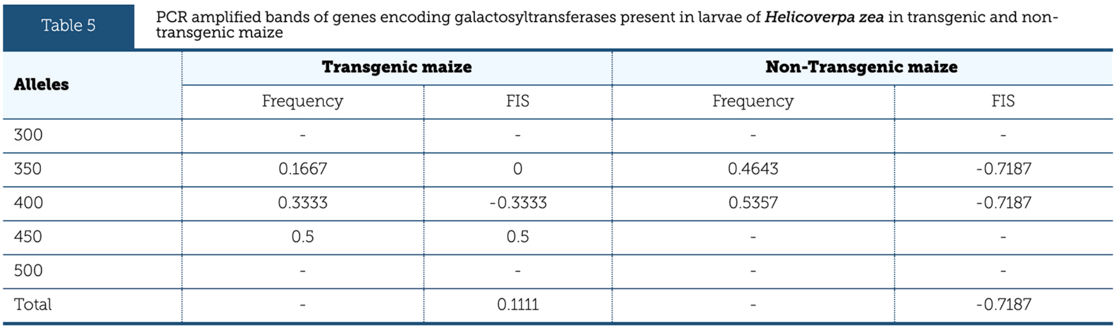

Table 5 PCR amplified bands of genes encoding galactosyltransferases present in larvae of ***Helicoverpa zea*** in transgenic and non-transgenic maize

Alleles	**Transgenic maize**		**Non-Transgenic maize**	
	Frequency	FIS	Frequency	FIS
300	-	-	-	-
350	0.1667	0	0.4643	-0.7187
400	0.3333	-0.3333	0.5357	-0.7187
450	0.5	0.5	-	-
500	-	-	-	-
Total	-	0.1111	-	-0.7187

Source: Author’s own elaboration

Hardy-Weinberg equilibrium

It was interesting to observe three patterns of the insect species populations, based on Hardy-Weinberg equilibrium (table 6). Insect populations of *H. zea* and *H. virescens* collected in GM maize were found in equilibrium, since the number of homozygotes and heterozygotes observed was like the one expected. This suggests that mating is at random and that gene and genotype frequencies are constant from generation to generation, while natural selection, genetic drift, migration and mutation do not occur (Cañon, Cortes, Garcia-Atance, Tupac-Yupanqui & Dunner, 2007). Furthermore, *H. zea* and *H. viresens* populations collected on non-transgenic maize showed a tendency to have an excess of heterozygous and deficiency of homozygous. Different possible causes to this phenomenon have been proposed. One possible cause is that reproductive isolation of these populations may lead to a non-random mating or selective mating, which could cause an increase in homozygotes of the genes involved in the character under the selection for mating (and genes that are in linkage disequilibrium with them) (Otto, Servedio & Nuismer, 2008). Another possible cause is the small population size which causes a random change in genotypic frequencies, and that may be related to sampling (genetic drift) and can be measured by the FIS statistics (Cañon *et al.*, 2007). In addition, the subdivision of the local population into isolated and differentiated reproductive units (Wahlund effect) and the non-random sampling of members from a limited number of families may be other possible causes.

Table 6

Homozygotes and heterozygotes frequency observed (O) and expected (E) under Hardy-Weinberg model from *Heliothis virescens* and *Helicoverpa zea* collected on transgenic and non-transgenic maize

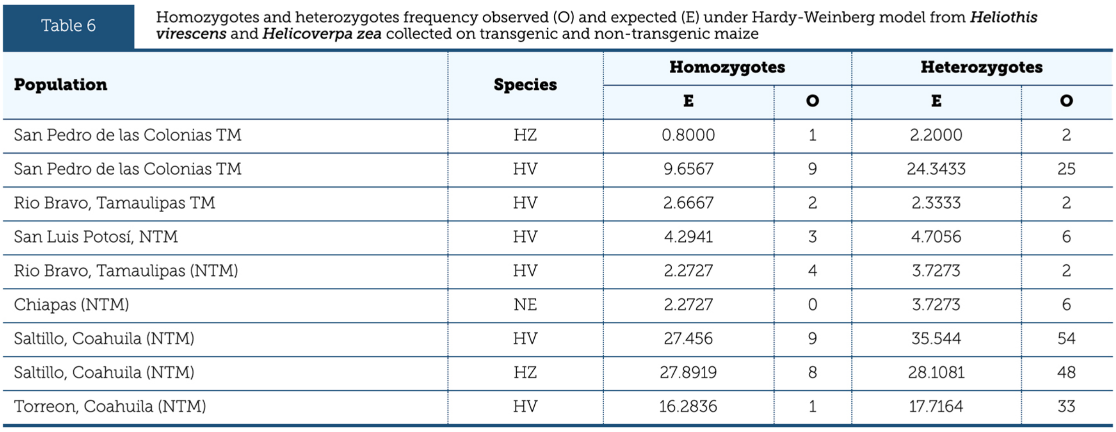

Table 6 Homozygotes and heterozygotes frequency observed (O) and expected (E) under Hardy-Weinberg model from ***Heliothis virescens*** and ***Helicoverpa zea*** collected on transgenic and non-transgenic maize

Population	**Species**	**Homozygotes**		**Heterozygotes**	
		E	**O**	**E**	**O**
San Pedro de las Colonias TM	HZ	0.8000	1	2.2000	2
San Pedro de las Colonias TM	HV	9.6567	9	24.3433	25
Rio Bravo, Tamaulipas TM	HV	2.6667	2	2.3333	2
San Luis Potosí, NTM	HV	4.2941	3	4.7056	6
Rio Bravo, Tamaulipas (NTM)	HV	2.2727	4	3.7273	2
Chiapas (NTM)	NE	2.2727	0	3.7273	6
Saltillo, Coahuila (NTM)	HV	27.456	9	35.544	54
Saltillo, Coahuila (NTM)	HZ	27.8919	8	28.1081	48
Torreon, Coahuila (NTM)	HV	16.2836	1	17.7164	33

TM = GM maize, NTM = non-GM maize, HV = *Heliothis virescens*, HZ = *Helicoverpa zea*, E=expected, O=observed
Source: Author's own elaboration

Genetic diversity

Genetic diversity within and between populations of the two species collected on transgenic and non-transgenic maize is shown in table 7. It was observed that in most of the cases, the diversity within the population was higher than that observed among populations which may be attributed to the different combinations of alleles of individuals of each species. Only in the collected insect attacking the non-transgenic maize in Tamaulipas was an opposite effect observed, where inter-population diversity was higher than that of intra-population, this can be attributed to preference mating among individuals of the same populations. Detection of polymorphism among and within populations can provide information about the genome evolution, the origin of species, and the current state of genetic diversity (Maggert, 2012). Moreover, by determining the intra-population diversity, it is possible to obtain information on the species' behavior in a specific environment to persist under adverse conditions such as, feeding on a host with Cry toxins (Schünemann *et al*., 2014).

Table 7

Genetic diversity between and within *Helicoverpa zea* and *Heliothis virescens* populations collected on transgenic (TM) and non-transgenic (NTM) maize

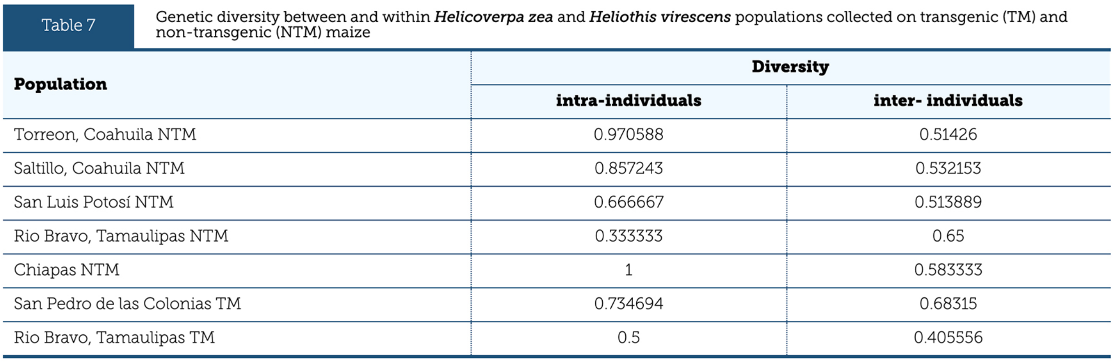

Table 7 Genetic diversity between and within **Helicoverpa zea** and **Heliothis virescens** populations collected on transgenic (TM) and non-transgenic (NTM) maize

Population	**Diversity**	
	intra-individuals	**inter- individuals**
Torreon, Coahuila NTM	0.970588	0.51426
Saltillo, Coahuila NTM	0.857243	0.532153
San Luis Potosí NTM	0.666667	0.513889
Rio Bravo, Tamaulipas NTM	0.333333	0.65
Chiapas NTM	1	0.583333
San Pedro de las Colonias TM	0.734694	0.68315
Rio Bravo, Tamaulipas TM	0.5	0.405556

Source: Author's own elaboration

The population genetic structure of the two species was inferred by Wright statistics (F_{IT}, F_{ST} and F_{IS}) (Wright, 1978). The FIS values in the insect populations, collected in both transgenic and non-transgenic, were negatives; the only exception was the insect population collected in non-trangenic maize in Tamaulipas. FIS estimates the heterozygote deficiency because of the reproductive isolation, which leads to a non-random mating of individuals; in other words, this parameter is a measurement of endogamy coefficient and its values could be between 0 and 1 (Wray & Visscher, 2008). Thus, the data suggest that both insect populations collected in non-transgenic maize from Tamaulipas are non-random mating, indicating a genetic differentiation between these two populations. Negative values found in most of the insect populations indicated that the observed heterozygotes number was higher than the expected, which may be a consequence of the Wahlund effect if the analyzed insects of those populations are the product of recent crossing between animals belonging to genetic different lines, or it could be the result of negative associative mating (Cañon *et al*., 2007).

The F_{ST} value, by definition, is the degree of genetic differentiation among populations as a function of allele frequencies (Balzarini, Bruno, Peña, Teich & Rienzo, 2010), and it indicates the proportion of genetic variation in the sub-population relative to the total variation. The observed FST values were very low, and this gives an idea of the differences that exist among populations as a result of low genetic drift (Jakobsson, Edge & Rosenberg, 2013).

The FIT value can be interpreted as a deficiency (1) or excess of heterozygotes (-1) in a population (Balzarini *et al*., 2010). In this study, it was observed an excess of heterozygotes in the insects' populations collected from non-transgenic maize at Saltillo, and insects' populations collected from transgenic maize at Tamaulipas and San Pedro de las Colonias (table 8).

Table 8

Wright statistics for *Helicoverpa zea* and *Heliothis virescens* populations collected on transgenic (TM) and non-transgenic (NTM) maize

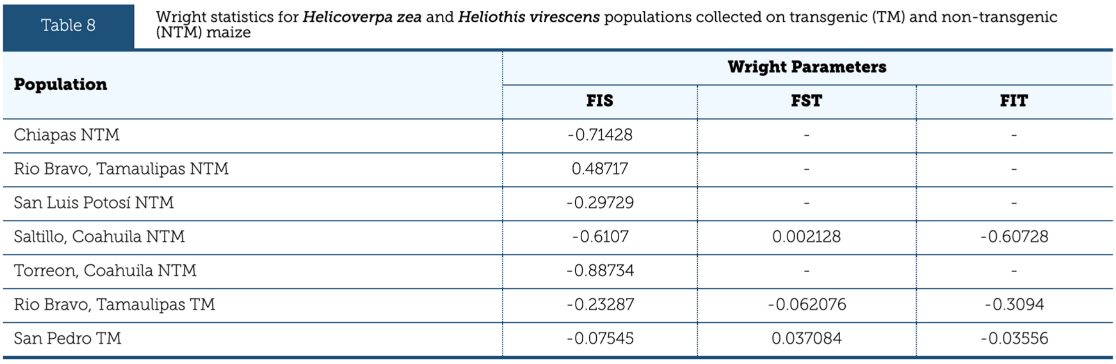

Table 8 Wright statistics for ***Helicoverpa zea*** and ***Heliothis virescens*** populations collected on transgenic (TM) and non-transgenic (NTM) maize

Population	Wright Parameters		
	FIS	FST	FIT
Chiapas NTM	-0.71428	-	-
Rio Bravo, Tamaulipas NTM	0.48717	-	-
San Luis Potosí NTM	-0.29729	-	-
Saltillo, Coahuila NTM	-0.6107	0.002128	-0.60728
Torreon, Coahuila NTM	-0.88734	-	-
Rio Bravo, Tamaulipas TM	-0.23287	-0.062076	-0.3094
San Pedro TM	-0.07545	0.037084	-0.03556

TM = transgenic maize, NTM = non-transgenic maize

Source: Author's own elaboration

It has been reported the insect resistance to Bt endotoxins by different mechanisms, which is a challenger to the major bio-pesticide used in traditional formulations and to its genes used in transgenic crops (Coates *et al*., 2008). Planting of transgenic maize varieties definitively has a high impact on the pest population regulation. A crucial requirement for development of an effective strategy for insect resistance is the knowledge of nature and inheritance mechanisms of this resistance.

CONCLUSIONS

Lepidopteran insects were detected on transgenic and non-transgenic maize, the identified species were *Helicoverpa zea* and *Heliothis virescens*. The prevalence of pest insect was higher on the non-transgenic maize crop (76.4 %). The presence of *Helicoverpa zea* and *Heliothis virescens* on transformed Bt plants indicated that these insect species had acquiring resistance to Cry toxins because some larvae surpassed the five instar. A key for the development of a successful strategy of resistance management requirement understood the nature and mechanisms of inheritance of this resistance.

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