ECOSISTEMAS Y RECURSOS AGROPECUARIOS

Introgression of transgenic events and accompanying sequences into Mexican maize varieties

Introgresión de eventos transgénicos y secuencias acompañantes en maíces mexicanos

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ABSTRACT. Representative seed samples of 215 of maize varieties from four (Jalisco, Michoacán, Oaxaca and Puebla) Mexican States were collected. The total genotypes collected in each State were 53, 96, 46 and 20 samples, respectively. Each seed collection was geo-referenced, then seed was planted and leaf tissue was used for DNA isolation, after that a PCR analysis was performed to determine the presence or absence of eight accompanying sequences and nine transgenic events in the 215 maize genotypes. Results showed introgression of accompanying sequences and transgenic events (*cry1Ab*) into some maize varieties. The major proportion of *cry1Ab* residues was observed in maize samples from Puebla (70%) followed by that displayed in the Oaxaca samples (52%), while Jalisco samples showed 15 and 7.5% of the *cry1Ab* gene and *ntpII* accompanying sequence, respectively, and Michoacán samples exhibited 44 and 17.7% for *cry1Ab* gene and the accompanying sequence, respectively. These results supported for applicate corrective measures for to prevent the genetic contamination of Mexican maize varieties and to clean up out-of-type phenotypes that could be sources of contamination of transgenic genes within maize populations contaminated with transgenes.

Key words: Mexican maize varieties, genetic contamination, transgenic events, *Cry1Ab*, *ntpII*.

RESUMEN. Se recolectaron muestras de semillas de 215 de variedades de maíz representativas de cuatro (Jalisco, Michoacán, Oaxaca y Puebla) estados de México. El total de genotipos colectados en cada Estado fue de 53, 96, 46 y 20, respectivamente. Cada colecta de semilla fue georreferenciada; posteriormente cada colecta se sembró y el tejido foliar se usó para el aislamiento de ADN, luego se realizó un análisis de PCR para determinar la presencia o ausencia de ocho secuencias acompañantes y nueve eventos transgénicos en los 215 genotipos. Los resultados mostraron la introgresión de secuencias acompañantes y eventos transgénicos (*cry1Ab*) en el genoma de algunas variedades locales de maíz. La mayor proporción de residuos de *cry1Ab* observados en las variedades locales de maíz fue la muestra de Puebla (70%), seguida por las muestras de Oaxaca donde se observó 52%, mientras que 15 y 7.5% de los maíces nativos del estado de Jalisco presentaron el gen *cry1Ab* y la secuencia acompañante *ntpII*, respectivamente. Las muestras de Michoacán mostraron 44 y 17.7% para el gen *cry1Ab* y la secuencia acompañante, respectivamente. Estos resultados deben respaldar la aplicación de medidas correctivas para prevenir la contaminación genética de variedades locales de maíz y limpiar fenotipos fuera de tipo que podrían ser fuentes de contaminación de genes transgénicos dentro de poblaciones de variedades locales de maíz contaminadas con estos transgenes.

Palabras clave: Variedades mexicanas de maíz, contaminación genética, eventos transgénicos, *Cry1Ab*, *ntpII*.

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INTRODUCTION

Currently, the most important transgenic crops worldwide are: soybeans, maize, cotton and canola. Soybean ranks first, followed by maize, cotton and canola with 105, 60.9, 25.7 and 10 million ha, respectively (ISAAA 2019, Voora *et al.* 2020). Because of the agronomic benefits that the use of genetically modified seeds represents, GM crops have gained acceptance in some countries in such a way that the estimated global area of transgenic crops has increased from 1.7 million ha in 1996 to 273.4 million at the present time (Brookes 2022). However, use of these transgenic organisms has generated an intense debate that goes from the scientific field to economic, social, political, ethical and moral levels (Sharma *et al.* 2022). Maize is the most important agricultural crop in Mexico, from the point of view of food, as well as industrial, political and social sector (Ibarrola-Rivas *et al.* 2020), as referred to the different archaeological, genetic, biochemical and historical studies that allow understanding the use of corn in the Mexican history, and evolutionary history of this cereal (Erenstein *et al.* 2022), that helps to understand the diversity of endemic maize varieties in this country (Orozco-Ramírez *et al.* 2016, Fonseca *et al.* 2023).

Worldwide, only 20% of maize production goes to human consumption, and 80% is used as animal feed or industrial raw material (Grote *et al.* 2021, Erenstein *et al.* 2022), however, in Mexico, 68% of its maize production is used for human consumption (Ibarrola-Rivas *et al.* 2020, Arce *et al.* 2020). Based on these antecedents and adding that Mexico has a wide diversity of natural conditions that provides numerous environments for maize growth (Vidal and Brusca 2020, Grote *et al.* 2021). In addition, these particular diverse environmental conditions have allowed to developing by selection and interaction with environment, very specific populations, which are commonly referred as maize varieties, a term used to refer to a population native of the community, region, state or country, differing from a foreign material as a hybrid or an improved variety. Local varieties are heterogeneous populations of plants that are differentiated by farmers based on their color, texture, and shape of the grain, shape of the ear, crop cycle and use. They are maize populations developed by farmers for many years, through the management and conservation of seeds and genes through a system of exchange year after year. Maize varieties (hybrid or improved varieties) is also considered to be the set of plants that are the product of a natural or artificial crossing (crossed by farmers, by breeders or both) with an improved material, respecting that this set of plants. It is constituted with 75% of the original variety's genetic material and 25% of the improved material (Salgotra and Chauhan 2023).

Maize in a large percentage is an open pollinated crop, which facilitates its crossing between varieties (Guzzon *et al.* 2021, Li *et al.* 2023) and with some of the species considered as ancestors as *Zea perennis* (Lohn *et al.* 2021), but also with commercial hybrids (Eze *et al.* 2020). This situation has led to a genetic contamination by transgenes in the natural ecosystems of Mexico, as it has been documented. Since 2001, there are isolated reports that indicate the presence of GM sequences in maize varieties in Oaxaca and some others Mexican states (Quist and Chapela 2001, Cleveland *et al.* 2005, Serratos-Hernández *et al.* 2007, Mercer and Wainwright 2008, Piñeyro *et al.* 2009, Orozco-Ramírez *et al.* 2016, Rendón-Aguilar *et al.* 2019). Based on these antecedents, and in view of the degree of contamination of these native maize with transgenic genes is unknown in other Mexican states, as well as the transgenic sequence (s) that have been introduced. The objectives of this study were to determine the level of introgression of transgenes into maize varieties of Puebla, Oaxaca, Jalisco and Michoacán States, to estimate of frequencies of maize contamination and to identify the main transgenic events transferred to the maize varieties.

MATERIALS AND METHODS

Collection of maize varieties

Samples were collected by field trips to rural community from the states of Puebla, Jalisco, Michoacán and Oaxaca. Each sample weighted 1000

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g of composite seed and was obtained from participate producer which were randomly chosen. In this study, a total of 215 maize local varieties were obtained, 53 out of 215 seed samples were collected from Jalisco, 96 samples from Michoacán, 46 samples from Oaxaca and 20 samples from Puebla State (Table 1). Each sampling site was geo-referenced in terms of altitude, latitude and longitude. Seed from each sample was placed for conservation in the maize germplasm bank of the Universidad Autonoma Agraria Antonio Narro.

DNA extraction, PCR amplification and detection of transgenes in vegetal material

Seeds from each collected variety were planted and germinated to obtain foliar tissue for GMO-DNA analyses. Polystyrene trays were filled with sterile forest soil and substrate (peat-moos) in a 6:4 ratio. After, a group of 10 seeds of each maize variety were planted. At 14 days after plantlet emergence, leaf tissue from 10 germinated seeds was cutting in mass and stored at 4 °C. For DNA isolation was used the method proposed by Graham *et al.* (1995). DNA integrity was determined by electrophoresis in 1.5% agarose gel and DNA quality was estimated in an Epoch Microplate Spectrophotometer with the Gen5 1.11 software. A Polymerase Chain Reaction (PCR) was performed with each DNA sample composite to amplify eight accompanying sequences (Table 2) and nine transgenic events (Table 3), in this case, specific primers and specific amplification temperatures for each of these sequences were used. Most of the published studies analyzed one or a few transgenic sequences and, in some cases, only a few samples of maize varieties and most of the studies were concentrated in Oaxaca (Mercer and Wainwright 2008). The PCR amplified segments were visualized using agarose gel electrophoresis (1.5%). The molecular markers of 50bp and 100bp DNA ladder (Invitrogen) were used as reference for determination of amplified segments size. In addition, the different amplified segments were sequenced to corroborate the identity of the amplified sequence.

Determination the level of introgression of transgenes into maize varieties

The level of introgression of transgenic event and accompanying sequence into local maize varieties which were planted as not biotech crops was determined in percent, in respect to varieties with presence of GMO into its genome vs total number of analyzed maize varieties in each Mexican State.

Statistical analysis

The PCR amplified bands of transgenic inserts were coded as absent (0) and present (1), then these values were summarized and employed for all percentage calculations. In addition, categorical analyses (tables SxR) were performed using the SAS software (9.0 version).

RESULTS

Collection of maize seed

Each collection site of maize varieties was georeferenced on a map (Figure 1). From 215 maize seed samples, 24.65% were from Jalisco, 44.65% from Michoacán, 21.39% from Oaxaca and 9.3% from Puebla.

Detection of transgenic sequences in plant tissue

In this study, it was only detected the presence of the *cry1Ab* transgenic event in all the maize varieties collected in the Mexican Central Region, and the *ntpII* accompanying sequence only was detected in Michoacan and Jalisco; which suggest that local maize varieties from these Mexican States are contaminated with transgene insert.

Estimate of maize contamination frequencies

Only the number of varieties positive for the transgenes *cry1Ab* and *ntpII* presence were included in the statistical analysis. In this analysis about frequency of these transgenic sequences present in the maize varieties were detected highly significant differences (P < = 0.01) (Table 4), the frequency rate for the *cry1Ab* gene was 41%, while the frequency rate for the *ntpII* sequence was 9.7%, in this analysis Q value was 56.2661 which was strongly significant

Table 1. Localities and maize genotypes collected from Jalisco (J), Michoacán (M), Oaxaca (O) and Puebla (P) states.

				Coordinates		
No	Key	Genotype	Locality	Latitude (N)	Longitude (W)	Altitude (masl)
1	J1	Celaya 2010 (C-2)	Jamay	20° 17' 17.60"	102° 41' 39.46"	1530
2	J2	Celaya 2010 (3)	Jamay	20° 17' 18.44"	102° 40' 35.91"	1528
3	J3	Celaya 2010 (4)	La Barca	20° 18' 14.07"	102° 31' 57.01"	1535
4	J4	Celaya 2010 (5)	La Barca	20° 17' 17.89"	102° 31' 45.67"	1538
5	J5	Celaya 2010 (6)	La Barca	20° 17' 20.72"	102° 33' 14.11"	1533
6	J6	Celaya 2010 (7)	Jesus Maria	20° 36' 33.29"	102° 12' 57.00"	2121
7	J7	Celaya 2010 (8)	Arandas	20° 41' 54.89"	102° 20' 0.67"	2045
8	J8	Celaya 2010 (10)	Arandas	20° 41' 47.11"	102° 21' 39.20"	2054
9	J9	Celaya 2010 (11)	Arandas	20° 41' 10.49"	102° 20' 23.92"	2063
10	J10	Celaya 2010 (12)	San Juan de los Lagos	21° 14' 34.26"	102° 21' 40.89"	1870
11	J11	Celaya 2010 (15)	San Juan de los Lagos	21° 16' 1.50"	102° 21' 2.47"	1750
12	J12	Celaya 2010 (16)	San Juan de los Lagos	21° 15' 22.04"	102° 18' 52.99"	1784
13	J13	Celaya 2010 (17)	Lagos de Moreno	21° 21' 4.92"	101° 54' 54.31"	1875
14	J14	Celaya 2010 (18)	Lagos de Moreno	21° 20' 25.71"	101° 56' 23.89"	1868
15	J15	Celaya 2010 (19)	Jesus Maria	20° 36' 44.99"	102° 13' 35.45"	2047
16	J16	Celaya 2010 (20)	Lagos de Moreno	21° 21' 3.65"	101° 54' 19.99"	1883
17	J17	Celaya 2010 (22)	San Diego	20° 9' 38.30"	103° 3' 25.44"	1541
18	J18	Celaya 2010 (23)	Mazamitla	19° 54' 55.78"	103° 0' 44.19"	2237
19	J19	Celaya 2010 (24)	San Diego	20° 10' 20.59"	103° 3' 43.75"	1528
20	J20	Celaya 2011 (25)	Atoyac	20° 0' 45.84"	103° 31' 15.87"	1356
21	J21	Celaya 2010 (26)	Mazamitla	19° 54' 53.76"	103° 1' 41.44"	2197
22	J22	Celaya 2010 (27)	Tala	20° 38' 54.70"	103° 42' 56.39"	1317
23	J23	Celaya 2010 (28)	Tala	20° 39' 0.69"	103° 43' 24.04"	1310
24	J24	Celaya 2010 (29)	Tala	20° 39' 39.62"	103° 43' 16.07"	1312
25	J25	Celaya 2010 (30)	Tapalpa	19° 56' 25.18"	103° 45' 33.94"	2039
26	J26	Celaya 2010 (32)	Tapalapa	19° 56' 37.41"	103° 46' 7.16"	2038
27	J27	Celaya 2010 (34)	Atoyac	20° 0' 35.07"	103° 31' 25.77"	1353
28	J28	Celaya 2010 (35)	Sayula	19° 52' 22.70"	103° 34' 57.37"	1365
29	J29	Celaya 2010 (37)	Sayula	19° 53' 45.17"	103° 35' 49.82"	1359
30	J30	Celaya 2010 (38)	Gomez Farias	19° 47' 56.00"	103° 28' 55.90"	1516
31	J31	Celaya 2010 (39)	Zapotiltic	19° 37' 32.73"	103° 26' 9.10"	1370
32	J32	Celaya 2010 (40)	Zapotiltic	19° 37' 10.96"	103° 25' 34.27"	1335
33	J33	Celaya 2010 (42)	Tuxpan	19° 32' 48.94"	103° 22' 8.36"	1130
34	J34	Celaya 2010 (43)	Tuxpan	19° 32' 48.28"	103° 23' 0.04"	1124
35	J35	Celaya 2010 (45)	Techaluta	20° 4' 26.72"	103° 33' 25.90"	1470
36	J36	Celaya 2010 (46)	Tamazula	20° 0' 58.77"	104° 3' 3.71"	929
37	J37	Celaya 2010 (47)	Techaluta	20° 4' 40.19"	103° 33' 28.47"	1486
38	J38	Celaya 2010 (48)	Tamazula	20° 1' 13.20"	104° 3' 8.47"	938
39	J39	Celaya 2010 (49)	Zapotitlan	19° 32' 58.41"	103° 49' 13.04"	1098
40	J40	Ancho de color	Atengo	20° 16' 16.23"	104° 14' 40.43"	1425
41	J41	Ancho blanco	Atengo	20° 15' 58.93"	104° 14' 25.38"	1406
42	J42	Elotes occidentales	Atengo	20° 16' 13.88"	104° 14' 29.07"	1432
43	J43	Amarillo Varieties	Tapalpa	19° 57' 11.55"	103° 45' 50.05"	2114
44	J44	Dulce de jalisco	Navidad, Mascota	20° 31' 36.12"	104° 46' 29.61"	1236
45	J45	Amarillo	Atengo	20° 43' 3.65"	103° 7' 12.37"	1700
46	J46	Coamilero	Atengo	20° 16' 18.50"	104° 14' 39.88"	1426
47	J47	Tabloncillo perla	Luis A. Arciga, Atengo	20° 16' 36.77"	104° 13' 53.33"	1449
48	J48	Bofo	Atengo	20° 16' 30.78"	104° 13' 55.47"	1440
49	J49	Tabloncillo pequeño	Alista, San Gabriel	19° 37' 53.66"	103° 47' 12.89"	1344
50	J50	Maíz dulce de Jalisco	Alista, San Gabriel	19° 37' 55.96"	103° 47' 34.75"	1338
51	J51	Maíz dulce de Jalisco	Alista, San Gabriel	19° 38' 7.92"	103° 46' 55.58"	1369
52	J52	Maíz dulce de Jalisco	Alista, San Gabriel,	19° 37' 46.56"	103° 47' 35.42"	1317
53	J53	Amarillo de ocho	Plan de las Flores	19° 44' 58.48"	103° 45' 35.22"	1293
54	M1	Argentino	Buenavista	19° 11' 55.24"	102° 35' 16.77"	432
55	M2	Argentino	Periban	19° 30' 45.19"	102° 25' 22.86"	1686
56	M3	Argentino	Cotija	19° 47' 50.68"	102° 41' 27.85"	1649
57	M4	Argentino	Villamar	20° 1' 17.44"	102° 35' 24.18"	1571
58	M5	Argentino	Jocotepec	20° 16' 42.93"	103° 25' 37.81"	1537

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Table 1. Continuación.

No	Key	Genotype	Locality	Coordinates		Altitude (masl)
				Latitude (N)	Longitude (W)	
59	M6	Argentino	Vista Hermosa	19° 45’ 20.62"	102° 37’ 35.35"	1625
60	M7	Argentino	Pajacuaran	20° 7’ 5.54"	102° 33’ 2.75"	1527
61	M8	Argentino	Venustiano Carranza	20° 7’ 6.49"	102° 39’ 36.33"	1530
62	M9	Argentino	Tanganmandapio	19° 57’ 8.47"	102° 26’ 38.89"	1713
63	M10	Argentino	Cotija	19° 47’ 57.64"	102° 42’ 3.65"	1717
64	M11	Celaya	Cotija	19° 48’ 0.99"	102° 42’ 11.61"	1717
65	M12	Celaya	Huaniqueo	19° 17’ 59.68"	101° 40’ 42.18"	2273
66	M13	Celaya	Huaniqueo	19° 18’ 2.46"	101° 41’ 12.31"	2276
67	M14	Chalqueño	Epitacio Huerta	20° 8’ 10.63"	100° 17’ 4.45"	2473
68	M15	Chalqueño	Angangueo	19° 36’ 33.89"	100° 17’ 33.98"	2604
69	M16	Chalqueño	Ocampo	19° 35’ 3.43"	100° 20’ 39.46"	2296
70	M17	Conico	Morelos	18° 40’ 46.67"	99° 6’ 4.78"	951
71	M18	Conico	Morelos	19° 13’ 10.75"	101° 42’ 24.42"	1960
72	M19	Conico	Morelos	19° 13’ 11.96"	101° 42’ 9.11"	1959
73	M20	Conico	Huiramba	19° 32’ 30.98"	101° 26’ 7.10"	2173
74	M21	Conico	Patzcuaro	19° 30’ 19.67"	101° 37’ 47.10"	2194
75	M22	Conico	Patzcuaro	19° 31’ 15.74"	101° 37’ 21.50"	2097
76	M23	Elotes occidentales	Caurio	19° 53’ 33.42"	101° 49’ 28.86"	2164
77	M24	Elotes occidentales	Caurio	19° 53’ 49.57"	101° 49’ 59.41"	2126
78	M25	Elotes occidentales	Caurio	19° 53’ 36.49"	101° 49’ 47.83"	2142
79	M26	Elotes occidentales	Caurio	19° 53’ 27.89"	101° 49’ 28.56"	2161
80	M27	Elotes occidentales	Caurio	19° 54’ 16.98"	101° 48’ 48.50"	2250
81	M28	Ancho	Charapan	19° 38’ 43.64"	102° 14’ 37.76"	2273
82	M29	Ancho	Tlajomulco	20° 28’ 28.02"	103° 26’ 18.97"	1590
83	M30	Ancho	Jungampeo	19° 29’ 33.93"	100° 29’ 24.99"	1581
84	M31	Ancho	Jungampeo	19° 29’ 37.21"	100° 29’ 17.80"	1603
85	M32	Mushito	Madero	19° 26’ 44.58"	100° 19’ 4.16"	2163
86	M33	Mushito	Ario	20° 1’ 40.22"	102° 20’ 19.06"	1564
87	M34	Mushito	Tacambaro	19° 13’ 32.38"	101° 27’ 19.27"	1576
88	M35	Mushito	Salvador	20° 18’ 15.04"	103° 10’ 47.80"	1538
89	M36	Mushito	Chilchota	19° 50’ 33.78"	102° 6’ 33.43"	1796
90	M37	Ratón tamaulipas	Tzizio	19° 35’ 3.08"	100° 55’ 13.99"	1592
91	M38	Ratón tamaulipas	Arteaga	18° 21’ 40.01"	102° 16’ 55.00"	909
92	M39	Ratón tamaulipas	Tepalcatepec	19° 11’ 6.01"	102° 49’ 45.57"	366
93	M40	Ratón tamaulipas	Huetamo	18° 38’ 5.56"	100° 54’ 44.23"	303
94	M41	Ratón tamaulipas	Tuzantla	19° 13’ 14.28"	100° 34’ 16.00"	603
95	M42	Ratón tamaulipas	Ziracuaretiro	19° 24’ 41.60"	101° 54’ 19.58"	1366
96	M43	Ratón tamaulipas	Benito Juarez	19° 18’ 49.73"	100° 25’ 46.97"	1341
97	M44	Ratón tamaulipas	Chinicuila	18° 2’ 1.05"	102° 12’ 50.64"	55
98	M45	Ratón tamaulipas	Tumbiscatio	18° 31’ 7.86"	102° 22’ 56.99"	955
99	M46	Ratón tamaulipas	Coalcoman	18° 45’ 56.63"	103° 8’ 42.43"	1260
100	M47	Ratón tamaulipas	Huacana	18° 58’ 6.94"	101° 48’ 38.59"	509
101	M48	Tuxpeño	Churumuco	19° 3’ 48.96"	102° 21’ 14.05"	302
102	M49	Tuxpeño	Aguililla	18° 43’ 53.78"	102° 46’ 51.64"	912
103	M50	Tuxpeño	Coalcoman	18° 46’ 2.68"	103° 8’ 39.19"	1257
104	M51	Tuxpeño	Tiquicheo	18° 54’ 3.98"	100° 44’ 39.38"	404
105	M52	Tuxpeño	Tepalcatepec	19° 10’ 15.83"	102° 51’ 10.16"	373
106	M53	Tuxpeño	Tepalcatepec	19° 10’ 4.51"	102° 50’ 39.39"	357
107	M54	Vandeño	Turicaro	19° 34’ 10.17"	101° 56’ 4.08"	2370
108	M55	Vandeño	San Lucas	18° 34’ 33.51"	100° 47’ 14.59"	298
109	M56	Vandeño	San Lucas	18° 34’ 45.81"	100° 47’ 1.00"	317
110	M57	Zamorano amarillo	Penjamillo	20° 4’ 26.01"	101° 56’ 16.43"	1795
111	M58	Zamorano amarillo	Penjamillo	20° 4’ 25.64"	101° 56’ 18.32"	1800
112	M59	Zamorano amarillo	Penjamillo	20° 4’ 22.27"	101° 56’ 19.08"	1792
113	M60	Zamorano amarillo	Penjamillo	20° 4’ 25.92"	101° 56’ 21.25"	1806
114	M61	Tabloncillo	San Juanito	19° 40’ 28.27"	101° 15’ 5.32"	1895
115	M62	Tabloncillo	Tumbiscatio	18° 31’ 7.35"	102° 22’ 28.58"	919
116	M63	Tabloncillo	Chinicuila	18° 1’ 53.26"	102° 12’ 49.82"	34

Table 1. Continuación.

No	Key	Genotype	Locality	Coordinates		Altitude (masl)
				Latitude (N)	Longitude (W)	
117	M64	Tsiri charapiti	Patzcuaro	19° 30’ 42.45"	101° 35’ 26.32"	2285
118	M65	Tsiri charapiti	Tingambato	19° 29’ 59.91"	101° 51’ 33.40"	1945
119	M66	Elotes conicos	Tingambato	19° 30’ 19.65"	101° 51’ 38.13"	1955
120	M67	Elotes conicos	Tingambato	19° 30’ 6.30"	101° 51’ 36.04"	1949
121	M68	Elotes conicos	Tingambato	19° 29’ 33.01"	101° 50’ 58.55"	1968
122	M69	Elotero sinaloa	Buenavista	19° 12’ 11.08"	102° 35’ 51.87"	453
123	M70	Elotero sinaloa	Aguililla	18° 44’ 9.44"	102° 46’ 24.09"	909
124	M71	Elotero sinaloa	Arteaga	18° 21’ 17.52"	102° 16’ 43.37"	856
125	M72	Elotero sinaloa	Tacambaro	19° 13’ 25.94"	101° 27’ 20.50"	1569
126	M73	Elotero sinaloa	Aquila	18° 36’ 3.48"	103° 29’ 48.02"	273
127	M74	Elotero sinaloa	Coahayana	18° 51’ 1.77"	103° 37’ 12.58"	46
128	M75	Elotero sinaloa	Chinicuila	18° 2’ 6.06"	102° 13’ 4.91"	63
129	M76	Elotero sinaloa	Tamazula	19° 34’ 51.97"	102° 27’ 6.88"	1367
130	M77	Elotero sinaloa	Tecatitlan	19° 21’ 48.78"	103° 1’ 6.03"	801
131	M78	Elotero sinaloa	Jilotlan	19° 22’ 15.71"	103° 0’ 44.48"	754
132	M79	Elotero sinaloa	Paramo	19° 22’ 43.12"	102° 1’ 38.35"	1589
133	M80	Reventador	Aquila	18° 36’ 8.32"	103° 30’ 18.21"	197
134	M81	Reventador	Aquila	18° 35’ 50.92"	103° 30’ 28.31"	233
135	M82	Reventador	Aquila	18° 35’ 33.14"	103° 30’ 29.07"	190
136	M83	Reventador	Aquila	18° 35’ 38.66"	103° 30’ 0.93"	242
137	M84	Reventador	Aquila	18° 36’ 14.80"	103° 29’ 59.85"	276
138	M85	Arrocillo	Aporo	19° 39’ 53.63"	100° 24’ 33.59"	2292
139	M86	Arrocillo	Angangueo	19° 36’ 35.63"	100° 17’ 30.43"	2616
140	M87	Arrocillo	Zitacuaro	19° 26’ 55.95"	100° 19’ 59.13"	2132
141	M88	Arrocillo	Ocampo	19° 35’ 38.35"	100° 20’ 26.80"	2320
142	M89	Arrocillo	Ocampo	19° 35’ 31.45"	100° 20’ 48.17"	2304
143	M90	Perepecha	Ocampo	19° 35’ 14.77"	100° 20’ 43.80"	2300
144	M91	Perepecha	Ocampo	19° 34’ 59.68"	100° 20’ 47.52"	2287
145	M92	Dulce	Ocampo	19° 34’ 48.96"	100° 20’ 58.47"	2275
146	M93	Dulce	Ocampo	19° 34’ 34.88"	100° 21’ 5.35"	2278
147	M94	Dulce	Ocampo	19° 34’ 36.47"	100° 20’ 43.99"	2292
148	M95	Dulce	Ocampo	19° 34’ 31.59"	100° 20’ 28.88"	2326
149	M96	Dulce	Ocampo	19° 34’ 25.16"	100° 20’ 19.39"	2340
150	O1	tuxpen	San Jose Chiltepec	17° 57’ 17.68"	96° 9’ 18.15"	51
151	O1	tuxpen…olotil	San Jose Chiltepec	17° 57’ 14.59"	96° 9’ 30.96"	53
152	O3	tuxpen…tepeci	Valle Nacional	17° 45’ 44.92"	96° 13’ 12.70"	61
153	O4	tepeci…dzitba	Valle Nacional	17° 48’ 37.49"	96° 13’ 26.59"	50
154	O5	tepeci	San Juan Lalana	17° 27’ 10.45"	95° 45’ 59.76"	249
155	O6	olotil	San Juan Lalana	17° 27’ 0.35"	95° 46’ 2.01"	208
156	O7	hibrido tuxpen	Santiago Yaveo	17° 19’ 49.25"	95° 41’ 57.85"	339
157	O8	olotil 8	Santiago Yaveo	17° 19’ 46.67"	95° 42’ 55.07"	218
158	O9	tepeci…zapagr	San Juan Guichicovi	16° 59’ 22.77"	95° 1’ 15.84"	108
159	O10	zapagr…olotil	San Juan Guichicovi	16° 58’ 40.64"	95° 1’ 5.03"	121
160	O11	tepeci…tuxpen	San Juan Cotzocon	17° 9’ 36.23"	95° 46’ 40.41"	1408
161	O12	tepeci	Santiago Yaveo	17° 19’ 47.81"	95° 40’ 9.59"	339
162	O13	olotil…zapagr	Santiago Yaveo	17° 20’ 8.96"	95° 40’ 41.55"	346
163	O14	chalou…bolita	Nochistlan	17° 28’ 12.90"	97° 16’ 23.45"	2052
164	O15	chalou	Nochistlan	17° 27’ 40.46"	97° 16’ 2.89"	2046
165	O16	bolita	Nochistlan	17° 26’ 55.85"	97° 14’ 51.77"	2055
166	O17	bolita conico	Nochistlan	17° 31’ 19.09"	97° 16’ 44.99"	2090
167	O18	connor…pepiti	Nochistlan	17° 30’ 32.38"	97° 16’ 46.06"	2082
168	O19	bolita	San Andres Andua	17° 26’ 52.71"	97° 18’ 11.45"	2055
169	O20	bolita	San Andres Andua	17° 26’ 9.44"	97° 17’ 36.60"	2048
170	O21	bolita	San Andres Andua	17° 27’ 10.80"	97° 16’ 2.49"	2039
171	O22	chalou	La Paz	17° 16’ 51.35"	97° 20’ 25.23"	2221
172	O23	chalou	La Paz	17° 17’ 0.92"	97° 20’ 14.93"	2178
173	O24	chalou…bolita	San Juan Diuxi	17° 17’ 6.84"	97° 22’ 31.82"	2316
174	O25	chalou 9	San Juan Diuxi	17° 17’ 0.14"	97° 22’ 49.39"	2472

Table 1. Continuación.

No	Key	Genotype	Locality	Coordinates		Altitude (masl)
				Latitude (N)	Longitude (W)	
175	O26	bolita	San Pedro Topiltepec	16°39' 46.52"	96°17' 16.90"	922
176	O27	chalou	Santo Domingo Yanhuitlan	17°31' 20.43"	97°20' 13.34"	2143
177	O28	bolita. . .chalou	Santo Domingo Yanhuitlan	17°32' 16.36"	97°20' 53.48"	2182
178	O29	tepeci. . .tuxpen	Pinotepa de Don Luis	16°25' 23.51"	97°59' 11.91"	435
179	O30	tepeci. . .olitill	San Juan Colorado	16°27' 22.17"	97°57' 29.96"	447
180	O31	tuxpen. . .olotill	Pinotepa de Don Luis	16°25' 37.07"	97°57' 43.71"	427
181	O32	olotil 9	San Pedro Jicayan	16°26' 51.50"	98°1' 26.79"	336
182	O33	tepeci. . .olotill	San Pedro Jicayan	16°27' 15.94"	98°1' 19.89"	348
183	O34	tepeci. . .tuxpen	San Pedro Jicayan	16°26' 36.04"	98°1' 8.91"	287
184	O35	olotil 7	San Pedro Jicayan	16°27' 40.02"	98°0' 45.03"	394
185	O36	olotil 8	San Miguel Tlacamama	16°25' 10.33"	98°3' 36.81"	276
186	O37	tuxpen	San Miguel Tlacamama	16°24' 38.92"	98°3' 35.07"	306
187	O38	tuxpen. . .fasciado	Santiago Pinotepa Nacional	16°11' 1.35"	97°58' 6.80"	16
188	O39	tuxpen. . .olotill	Santa Maria Tonameca	15°44' 38.05"	96°33' 9.85"	31
189	O40	olotill 7	Santa Maria Tonameca	15°44' 30.90"	96°33' 1.71"	28
190	O41	tuxpen. . .tepeci	Santa Maria Tonameca	15°44' 58.27"	96°32' 27.13"	32
191	O42	tuxpen. . .olotill	San Pedro Mixtepec	15°59' 37.02"	97°5' 30.37"	310
192	O43	olotil 9	Pinotepa de Don Luis	16°25' 11.27"	97°58' 31.62"	442
193	O44	tuxpeño	San Meteo Sindihui	16°59' 58.07"	97°20' 55.73"	1484
194	O45	tuxpeño	San Meteo Sindihui	17°0' 26.56"	97°21' 8.24"	1461
195	O46	tuxpeño	San Meteo Sindihui	17°0' 1.51"	97°21' 9.48"	1471
196	P1	Cacahuacintle	Chignahuapan	19°50' 44.87"	98°1' 25.64"	2268
197	P2	Conico Amarillo	Zacatlan	19°56' 9.52"	97°56' 39.16"	2032
198	P3	Arrocillo	San Nicolas Buenos Aires	18°29' 43.52"	97°25' 42.18"	1687
199	P4	Conico Amarillo	San Martin Texmelucan	19°17' 42.29"	98°26' 24.18"	2263
200	P5	Palomero Blanco	Tetela de Ocampo	19°48' 46.58"	97°48' 16.33"	1759
201	P6	Chalqueño	Aljojuca	19°5' 55.26"	97°32' 20.83"	2444
202	P7	Elotes Conicos	Tlachichuca	19°6' 46.89"	97°24' 39.80"	2639
203	P8	Conico Blanco	San Juan Tianguismanalco	18°56' 5.44"	98°27' 38.26"	1951
204	P9	Chalqueño	Tepatlaxco	19°4' 16.79"	97°58' 11.54"	2370
205	P10	Elotes Conicos	Chalchicomula de Sesma	18°57' 2.17"	98°12' 44.96"	2071
206	P11	Cacahuacintle	Tlachichuca	19°7' 35.00"	97°25' 18.39"	2588
207	P12	Olotillo	Xicotepec	18°56' 53.77"	98°15' 36.60"	2098
208	P13	Tuxpeño	Francisco Z. Mena	18°32' 9.70"	98°30' 10.43"	1210
209	P14	Amilaceo	Tetela de Ocampo	19°49' 24.79"	97°48' 41.60"	1698
210	P15	Conico Amarillo	Tepatlaxco	19°4' 49.67"	97°57' 38.96"	2390
211	P16	Pepitilla Morado	Atlixco	18°54' 4.80"	98°25' 49.97"	1826
212	P17	Ancho	Atlixco	18°53' 58.65"	98°27' 6.57"	1862
213	P18	Ancho	Cohuecan	18°46' 56.70"	98°42' 58.81"	1706
214	P19	Vandeño	Albino Zertuche	18°1' 3.30"	98°32' 3.45"	1313
215	P20	Pepitilla Blanco	Tochimilco	18°53' 7.39"	98°34' 34.34"	2075

and indicated that both frequencies are different. One explanation may be that during transformation of plants with the *cry1Ab* gene, different marker genes are utilized which may be different to *ntpII*.

The frequency of transgenic events presents in to the maize varieties from Puebla, Oaxaca, Jalisco and Michoacán, are shown in Table 5. The present study focused on detect transgenic events and accompanying sequences into maize varieties by PCR and using seeds collected of local maize varieties from Puebla, Jalisco, Michoacán and Oaxaca States, allowed to detecting the presence of the *cry1Ab* transgenic gene and the presence of the *ntpII* accompanying sequence in the maize varieties (Table 5). The localities with presence of the *cry1Ab* transgenic event and accompanying sequence *ntpII* in the maize populations are described in Table 6.

Introgression of transgenes into maize varieties

According to the presence of *cry1Ab* into the genome of the sampled maize varieties from these four Mexican states, it was observed that maize varieties grown as non-biotech crops from Puebla State have the highest levels (70%) of contamina-

Table 2. Target sequence, primer sequence, sequence size, and annealing temperature used during PCR, for detection of accompanying transgenic sequences.

Target sequence	Primer sequence	Sequence size (bp)	Annealing temperature (℃)
ubi [25]	F-5’-gctaacttgccagtgtttctctttgg-3’ R-5’-ggctggcattatctactcgaaacaag-3’	220	55
35 s [24]	F-5’-gctcctacaaatgccatca-3’ R-5’-actgcgtgttagggtgatag-3’	238	60
bar [26]	F-5’-gcacagggcttcaagagcgtggtc-3’ R-5’-gggcggtaccggcaggctgaa-3’	177	55
ntpII [26]	F-5’-gaggctattcggctatgact-3’ R-5’-aaggtgagatgacaggagat-3’	271	64
uidA [27]	F-5’-ggtgggaaagcgcgttacaag-3’ R-5’-accgccttcgttgcgcatttg-3’	150	55
luc [28]	F-5’-cgccaaaaacataaagaaaggc-3’ R-5’-tgtccctatcgaaggactctgg-3’	450	64
ocs [27]	F-5’-ctcgagctgctttaatgagatatgcg-3’ R-5’-tctagactgctgagcctcgacatgttg-3’	120	55
nos [26]	F-5’-gaatcctgttgccggtcttg-3’ R-5’-gcgggactctaatcataaaaacc-3’	125	57

Table 3. Target gene, primer sequence, sequence size, and annealing temperature used during PCR for detection of transgenic events.

Gene	Primer sequence	Sequence size (bp)	Annealing temperature (℃)
cry 3a	F-5’-acatgcatgcattaactagaaagtaaagaagtag-3’ R-5’-acatgcatgcaagcttacagagaaatacacgaggg-3’	479	62.6
cry2a	F-5’-gctctagaataggaggaaaagattttatgctaaaa-3’ R-5’-acgcgtcgacaaatatctagttttatattaa-3’	850	62.6
cry 1e	F-5’-ggatcccatatggagatagtg-3’ R-5’-cgcggatcctatctagaatcgtaatt-3’	756	64
ec	F-5’-ccagtctgttgacctggttgt-3’ R-5’-tttctgcagatgtcaacgtattctatacc-3’	239	64.7
cry11a	F-5’-acatgcatgcagtcatgttagcacaagagga-3’ R-5’-acatgcatgctttaggtctttaaaaattaga-3’	850	62.6
Accasa	F-5’taggactggtaccgtaaagcagagtaacacaaggtcag-3’ R-taggactctcgagagtctttcggaacctcacaccataagg-3’	514	64.7
Als	F-5’-gggttacgcacgcgccaccgg-3’ R-5’-ggctgatcccagtcaggtatc-3’	397	64
cry 1ab	F-5’-accatcaacagccgctacaacgacc-3’ R-5’-tggggaacaggctcacgatgtccag-3’	184	70
Epsps	F-5’-tggcgcccaaagcttgcatggc-3’ R-5’-ccccaagttcctaaatcttcaagt-3’	356	62

tion o introgression of transgenes inserts into their genomes, followed by maize variety samples from Oaxaca with 52.17%, Michoacán maize samples with 44.79% and Jalisco maize samples with 15.09%, respectively. While, the presence of accompanying sequence *ntpII* was only detected in the Michoacán and Jalisco maize samples in 89.47 and 10.52%, respectively (Figure 2).

DISCUSSION

Collection of maize seed

Although some studies have confirmed the contamination of native maize by transgenic events, these studies considered a low number of samples, for example, De-Ita (2012) indicates the use of samples from 22 localities, while The National Commission for the Knowledge and Use of Biodiversity (Conabio) confirmed the presence of transgenes from 3 to 10% in 15 locations. Another study for the determination of transgenes carried out by Serratos-Hernández *et al.* 2007 is based on a sample of 25 communities. The present study considered a greater number of localities (215) from 4 Mexican States, which allows to have a better perspective of the contamination of native maize by transgenic segments.

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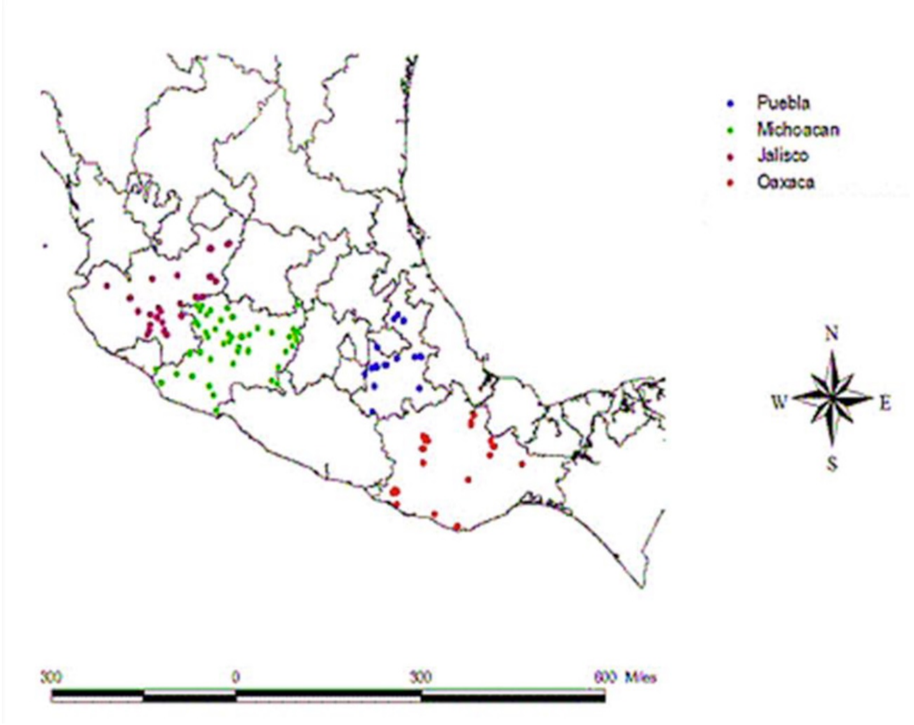

Figure 1. Geo-referential distribution for the collection sites of maize varieties in the Jalisco, Michoacán, Oaxaca and Puebla States.

Table 4. Statistical values of the categorical analysis in SxR tables for the presence of transgenic sequences in maize varieties in Mexico.

Statistic	df	Value	Probability
Chi-square	1	56.3966	<0.0001
Likelihood Ratio chi-square	1	59.7029	<0.0001
Continuity Adj. chi-square	1	54.7501	<0.0001
Mantel-Haenszel Chi-square	1	56.2661	<0.0001
Phi Coefficient		-0.3613	
Contingency Coefficient		0.3398	
Cramer's V		-0.3613	

Table 5. Frequency (%) of transgenic events transferred to the maize varieties collected in Puebla, Oaxaca, Jalisco and Michoacán States.

Transgenic sequence	non- introgressed Genotypes	Percent	Introgressed Genotypes	Percent	Total
cry1Ab	127	58.80	89	41.20	216
ntpII	195	90.28	21	9.72	216

Detection of transgenic sequences in plant tissue

These results confirm the introgression of transgenes into maize varieties as indicated by (Quist and Chapela 2001, Cleveland *et al.* 2005, Serratos-Hernández *et al.* 2007, Mercer and Wainwright 2008, Piñeyro *et al.* 2009, Orozco-Ramírez *et al.* 2016, Rendón-Aguilar *et al* 2019) who reported the presence of transgenic DNA in native varieties of maize grown in remote mountains of Oaxaca, this place is part of the center of origin and diversification of the crop in Mexico.

Estimate of maize contamination frequencies

The high detection rate of the *cry1Ab* transgenic event in the maize varieties populations implies that protective measures must be taken in the

Table 6. Localities with presence transgenic event (*cry1Ab*) and accompanying sequence (*ntpII*) in maize varieties collected from Jalisco, Michoacán, Oaxaca and Puebla States.

Varieties corn					Varieties corn				
Key	Name	Locality	*Cry1Ab*	*ntpII*	Key	Name	Locality	*Cry1Ab*	*ntpII*
J11	Celaya 2010(15)	San Juan de los Lagos	1	0	M91	Perepecha	Ocampo	1	0
J13	Celaya 2010(17)	Lagos de Moreno	1	1	M92	Dulce	Ocampo	1	0
J14	Celaya 2010(18)	Lagos de Moreno	1	1	M93	Dulce	Ocampo	1	0
J21	Celaya 2010(26)	Mazamitla	1	0	M94	Dulce	Ocampo	1	0
J24	Celaya 2010(29)	Tala	1	0	M95	Dulce	Ocampo	1	0
J28	Celaya 2010(35)	Sayula	1	0	M96	Dulce	Ocampo	1	0
J29	Celaya 2010(37)	Sayula	1	0	O1	Tuxpen	San Jose Chitepec	1	0
J30	Celaya 2010(38)	Gomez Farias	1	0	O10	zapagro-olotillo	San Juan Guichicovi	1	0
M13	Celaya	Huaniqueo	1	1	O11	tepeci..tuxpen	San Juan Cotzocon	1	0
M14	Chalqueño	Epitacio Huerta	1	1	O12	tepeci	Santiago Yaveo	1	0
M15	Chalqueño	Angangueo	1	1	O13	olotil…zapagr	Santiago Yaveo	1	0
M16	Chalqueño	Ocampo	1	1	O15	Chalou	Nochistlan	1	0
M17	Conico	Morelos	1	1	O16	Bolita	Nochistlan	1	0
M18	Conico	Morelos	1	1	O17	Bolita conico	Nochistlan	1	0
M19	Conico	Morelos	1	0	O18	connor…pepiti	Nochistlan	1	0
M21	Conico	Patzcuaro	1	1	O19	Bolita	San Andres Andua	1	0
M40	Ratontamaulipas	Huetamo	1	1	O2	Tuxpen…olotil	San Juan Chitepec	1	0
M48	Tuxpeño	Churumuco	1	0	O20	Bolita	San Juan Andua	1	0
M49	Tuxpeño	Aguililla	1	0	O26	Bolita	San Pedro Topiltepec	1	0
M51	Tuxpeño	Tiquicheo	1	0	O27	Chalou	Santo Domingo Yanhuitlan	1	0
M52	Tuxpeño1	0Tepalcatepec	1	0	O29	tepeci…tuxpen	Pinotepa de Don Luis	1	0
M53	Tuxpeño	Tepalcatepec	1	0	O4	tepeci…dzitba	Valle Nacional	1	0
M66	Elotes Conicos	Tingambato	1	1	O41	Tuxpen…tepeci	Santa maria Tonameca	1	0
M67	Elotes Conicos	Tingambato	1	0	O42	Tuxpen…olotil	San Pedro Mixtepec	1	0
M68	Elotes Conicos	Tingambato	1	1	O43	Olotil9	Pinotepa de Don Luis	1	0
M69	Elotero Sinaloa	Buenavista	1	1	O45	Tuxpeño	San Meteo Sindihui	1	0
M70	Elotero Sinaloa	Agulilla	1	0	O46	Tuxpeño	San Meteo Sindihui	1	0
M72	Elotero Sinaloa	Tacambaro	1	0	O7	Hibrid Tuxpen	Santiago Yaveo	1	0
M74	Elotero Sinaloa	Coahayana	1	0	O8	Olotil 8	Santiago Yaveo	1	0
M75	Elotero Sinaloa	Chinicuila	1	0	O9	Tepeci…zapagr	San Juan Guichicovi	1	0
M76	Elotero Sinaloa	Tamazula	1	1	P1	Cacahuacintle	Chingnahuapan	1	0
M77	Elotero Sinaloa	Tecatitlan	1	1	P2	Conico Amarillo	Zacatla	1	0
M78	Elotero Sinaloa	Jilotlan	1	1	P3	Arrocillo	San Nicolas Buenos Aires	1	0
M79	Elotero Sinaloa	Paramo	1	1	P4	Conico Amarillo	San Martin Texxmelucan	1	0
M80	Reventador	Aquila	1	1	P6	Chalqueño	Aljojuca	1	0
M81	Reventador	Aquila	1	0	P7	Elotes Conicos	Tlachichuca	1	0
M82	Reventador	Aquila	1	0	P8	Conico Blanco	San Juan Tiangusmanalco	1	0
M83	Reventador	Aquila	1	0	P10	Elotes Conicos	Chalchicomula de Sesma	1	0
M84	Reventador	Aquila	1	0	P12	Olotillo	Xicotepec	1	0
M85	Arrocillo	Aporo	1	0	P14	Amilaceo	Tetela de Ocampo	1	0
M86	Arrocillo	Zitacuaro	1	0	P18	Ancho	Cohuecan	1	0
M88	Arrocillo	Ocampo	1	0	P19	Vandeño	Albino Zertuche	1	0
M89	Arrocillo	Ocampo	1	0	P20	Pepetilla Blanco	Tochimilco	1	0
M90	Perepecha	Ocampo	1	1					

J, M, O, P = Initials of the Jalisco, Michoacán, Oaxaca and Puebla states, respectively including the varieties number; 1 = Positive, 0 = Negative for the transgenic event or accompanying sequence respectively.

short term to produce maize varieties seeds free of these transgenic segments. This detection is controversial because in Mexico, it is not allowed to grow commercially maize transgenic cultivars, only at an experimental level (García and Toscana 2016, Ortega-Villegas *et al*. 2018, Lopez-Hernandez 2020), precisely, for preservation of biodiversity of the native maize, because Mexico is considered a center of origin and diversity of this commodity (Lohn *et al*. 2021).

Introgression of transgenes into maize varieties

This level of introgression of transgenes into the tested maize varieties could be considered very high, as it is determined in a specific way, what was found for each state (Figure 2), and assuming that the states with the lowest experimental seeding (Puebla

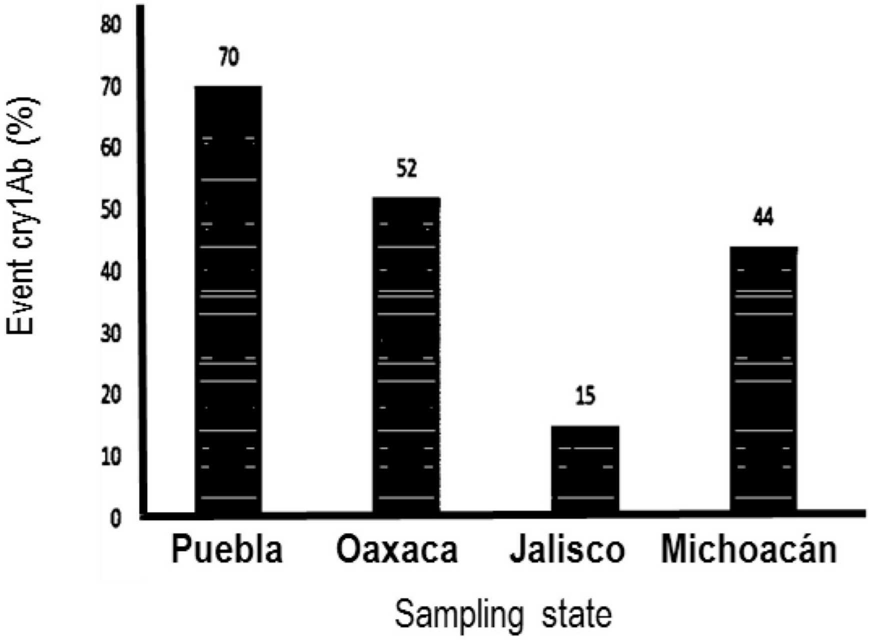

Figure 2. Level of introgression (Percentage) of the transgene event *cry1Ab* in Mexican maize varieties from Puebla, Oaxaca, Jalisco and Michoacán states.

and Oaxaca) are those with the greater genetic contamination, having thus that 70% of the varieties collected in Puebla state presented residues of the *cry1Ab* gene; while, 52% of the maize collected in Oaxaca were positive for the *cry1Ab* gene. On the other hand, in these states, not varieties were detected contaminated with accompanying sequences.

The *cry1Ab* gene was detected in 15% of the maize varieties collected in Jalisco, and 4 out of 53 samples collected were found to be introgressed with the accompanying *ntpII* sequence. While 44% of the varieties collected in Michoacán were observed to have the *cry1Ab* gene, and 17.7% of the samples collected in this state showed the accompanying *ntpII* sequence. The presence of transgenic events in maize varieties could have an cultural, economic, and social impact, on continuity of indigenous and peasant peoples as refer (Sánchez and Romero 2018, Ibarrola-Rivas *et al.* 2020), who point out the importance of this area on maize conservation, it is in this territory where the milpa takes place, and at the same time, the milpa is part of the territory; and with the cultural and natural characteristics of the territory, particular milpa are configured. Therefore, the contamination of maize or its substitution by transgenic varieties is not limited to changes in maize as a source of food, but also implies negative repercussions for farmers in the milpa and in the territory, increasing the risk to their cultural continuity. Milpa is defined as agroecosystem conformed by polyculture, which is a dynamic space of genetic resources (Ortiz *et al.* 2014, González and Reyes 2014, Fonteyne *et al.* 2023). CCA (2004) mentioned that in Mexico, maize is not only a commercial commodity, it is the base of Mexican diet and constitutes an integral expression of the relation between nature and culture, from this relation depends the subsistence of a great part of the Mexican rural population, and through this relation, the social tissue and people of these communities and diversity conservation are fortified.

Milpa is not only the space where maize, and other plant species are sown, but it is also a space for linguistic, cultural, symbolic, spiritual, social, and economical activities and food (Agapito-Tenfen *et al.* 2017, Ibarrola-Rivas *et al.* 2020), is more than a farming technique, it is an agroecosystem in which farmers of Mesoamerican origin cultivate dozens of comestible and medicinal herbs, along with fruit and timber trees. For these communities, the most important thing is that the diversity of the maize germplasm can be lost. Year after year, the farmers keep the best seeds for planting in the following season, passing the knowledge of selection of them, the preparation of the land and the accumulation of information on the weather, among other elements, to the next generation (Sánchez and Romero 2018). Maize varieties genetic diversity offers specialized genotypes which have demonstrated capacity of adaptation to different environments, pest and disease resistance, and satisfy divers demands for culinary, artisanal or industrial uses; this diversity is a key factor for the Mexican food security (WHO 2015).

It is considered that maize is a continuous process of domestication, since it depends completely on the farmer, who through selection has favored the survival, and reproduction of phenotypes that have advantageous characteristics to be used mainly as food for humans, and to date represent a total of 64 native maize races reported for Mexico (Santillán-Fernández *et al.* 2021). It is also convenient to clarify that natural selection operates during the process of domestication. The domestication process results in Mexico being a center of maize genetic diversity (Santillán-Fernández *et al.* 2021), and is the Mexicans' responsibility to preserve this great maize genetic diversity, in addition to conducting selection programs to gather more, and better characters in the plants that will serve as progenitors to the next generation. Agricultural and cultural practices have been very important during the whole process of maize domestication, since each variety have adapted to the specific environment that includes the cultivation method (Guzzon *et al.* 2021).

Maize is currently important in the diet of Mexicans and for many of them, it is their main source of protein and energy, which can be considered as cheap diet (Erenstein *et al.* 2022); with an average consumption of 350 g per capita per day in 600 different presentations of maize. Therefore, it has been pointed out that maize requires a special protection regime for genetically modified organisms that can negatively modify the nutritional qualities of this cereal (Brookes and Barfoot, 2016). The genetic contamination by transgenes of the natural ecosystems of Mexico has been documented. Since 2001, there have been isolated reports that indicate the unintentional presence of GM maize (Quist and Chapela 2001, Serratos-Hernández *et al.* 2007, Mercer and Wainwright 2008, Piñeyro *et al.* 2009) and there is only low work in Mexico in which the absence of GMOs was reported in maize (Cleveland *et al.* 2005, Rendón-Aguilar *et al* 2019). Most of the published studies analyzed one or a few transgenic sequences and, in some cases, only a few samples of maize varieties and most of the studies were concentrated in Oaxaca (Mercer and Wainwright 2008). On the other hand, there are reports that maize shipments imported for consumption have a large percentage of transgenic residues, in addition, the grain is viable. From 29 imported shipments of maize for consumption were analyzed for presence of transgenic residues, all the samples presented at least one transgenic sequence, either an event or an accompanying sequence (Carvajal *et al.* 2017). So, there is a risk that grains fall during transport, germinate on the sides of roads or railroads, or that farmers take these grains as seed and when they are planted in regions with high genetic diversity of maize, pollen can transfer transgenic genes to the native maize. It is proposed that the information obtained in this work be integrated into the National Program for the Monitoring of Genetically Modified Organisms, which will allow a diagnosis of the current situation in Mexico on the introgression of transgenes to the native maize and will serve as the basis for taking the actions necessary in matters of Biosafety.

CONCLUSIONS

The present study reports presence of transgenic events (*cry1Ab*), and sequences associated with the gene (*ntpII*) into maize varieties from Puebla, Michoacán, Oaxaca and Jalisco states in about 45% of the sampled localities. Corrective measures must be taken to prevent the contamination of Mexican maize varieties, and to train the varieties producers to clean the out-of-type phenotypes that could be sources of contamination of transgenic genes within the contaminated maize varieties with transgenes.

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LITERATURE CITED

Agapito-Tenfen S, Lopez FR, Mallah N, Abou-Slemayne G, Trtikova M, Nodari RO, Wickson F (2017) Transgene flow in Mexican maize revisited: Socio-biological analysis across two contrasting farmer communities and seed management systems. Ecology and Evolution 7: 9461-9472.

Arce Romero A, Monterroso Rivas AI, Gómez Díaz JD, Palacios Mendoza M, Navarro Salas EN, López Blanco JC, Álvarez AC (2020) Crop yield simulations in Mexican agriculture for climate change adaptation. Atmósfera 33: 215-231.

Brookes G, Barfoot P (2016) GM crops: Global socio-economic and environmental impacts 1996- 2016. PG Economics Ltd. UK. 198p.

Brookes G (2022) GM crops: Global socio-economic and environmental impacts 1996-2020. PG Economiscs Ltd. UK. 215p.

Carvajal P, Ureña H, Umaña J, Sancho C, Solano F, Arleo M, Martínez C, Umaña R (2017) Detección molecular de secuencias de ADN transgénico en alimentos de consumo humano y animal en Costa Rica. Agronomía Costarricense 41: 53-68.

CCA (2004) Maíz y biodiversidad: Efectos del maíz transgénico en México. Comisión para la Cooperación Ambiental. Edicion en español. Canada. 51p.

Cleveland DA, Soleri D, Aragón Cuevas F, crossa J, Gepts P (2005) Detecting (trans)gene flow to landraces in centers of crop origin: lessons from the case of maize in Mexico. Environmental Biosafety Research 4: 197-208.

De-Ita A (2012) La defensa internacional del maíz contra la contaminación transgénica en su centro de origen. El Cotidiano 73: 57-65.

Erenstein O, Jaleta M, Sonder K, Mottaleb K, Prasanna BM (2022) Global maize production, consumption and trade: trends and R&D implications. Food Security 14: 1295-1319.

Eze CE, Akinwale RO, Michel S, Burstmayr H (2020) Grain yield and stability of tropical maize hybrids developed from elite cultivars in contrasting environments under a rainforest agro-ecology. Euphytica 216(89): 1: 13. DOI: 10.1007/s10681-020-02620-y.

Fonseca FMA, Guevara HF, García GA, Márquez RC, Parra VMR (2023) Diversity of local maize in ejidos of the Frailesca region of Chiapas, Mexico. Tropical and Subtropical Agroecosystems 26: 017. DOI: 10.56369/tsaes.4131.

Fonteyne S, Castillo CJB, Lopez-Ridaura S, Van Loon J, Espidio BJ, Osorio AL, Martínez HF, Odjo S, Verhulst N (2023) Review of agronomic research on the milpa, the traditional polyculture system of Mesoamerica. Frontiers in Agronomy 5: 1115490. DOI: 10.3389/fagro.2023.1115490.

García JA, Toscana AA (2016) Presencia de maíz transgénico en la Sierra Norte de Oaxaca. Un estudio desde la mirada de las comunidades. Sociedad y Ambiente 12: 119-144.

González JA, Reyes ML (2014) El conocimiento agrícola tradicional, la milpa y la alimentación: el caso del Valle de Ixtlahuaca, Estado de México. Revista de Geografía Agrícola 52-53: 21-42.

Graham HC, Mayers P, Henry RJ (1995) A simplified method for the preparation of fungal genomic DNA for PCR an RAPD analysis. Biotechniques 16: 48-50.

Grote U, Fasse, A Nguyen TT, Erenstein O (2021) Food security and the dynamics of wheat and maize value chains in Africa and Asia. Frontiers in Sustainable Food Systems 4: 617009. DOI: 10.3389/fsufs.2020.617009.

Guzzon F, Arandia Rios LW, Caviedes Cepeda GM, Céspedes Polo M, Chavez Cabrera A, Muriel Figueroa J, Medina Hoyos AE, Jara Calvo TW, Molnar TL, Narro León LA, Narro León TP, Mejía Kerguelén SL, Ospina Rojas JG, Vázquez G, Preciado-Ortiz RE, Zambrano JL, Palacios Rojas N, Pixley KV (2021) Conservation and use of Latin American maize diversity: Pillar of nutrition security and cultural heritage of humanity. Agronomy 11: 172. DOI: 10.3390/agronomy11010172.

Ibarrola-Rivas MJ, Castillo G, González J (2020) Social, economic and production aspects of maize systems in Mexico. Investigaciones Geográficas (102): e60009. DOI: 10.14350/rig.60009.

ISAAA (2019) Global status of commercialized Biotech/GM crops in 2019: Biotech crops drive socio economic development and sustainable environment in the new frontier. ISAAA. Brief No. 55. ISAAA: Ithaca, NY. https://www.isaaa.org/resources/publications/briefs/55/. Accessed: June 26, 2023.

Li H, Lui K, Zhang M, Zhang Y, Li S, Wang X, Zhou J, Zhao Y, Liu T, Li Ch (2023) Mixing trait-based corn (*Zea mays* L.) cultivars increases yield through pollination synchronization and increased cross-fertilization. The Crop Journal 11: 291-300.

Lohn AF, Trtikova M, Chapela I, Binimelis R, Hilbeck A (2021) Transgene behavior in genetically modified teosinte hybrid plants: transcriptome expression, insecticidal protein production and bioactivity against a target insect pest. Environmental Sciences Europe 33(67): 1-13. DOI: 10.1186/s12302-021-00506-x.

Lopez-Hernandez E (2020) GMO Corn, Mexico, and coloniality. Vanderbilt Journal of Entertainment and Technology Law 22: 725-783.

Mercer KL, Wainwright JD (2008) Gene flow from transgenic maize to varieties in Mexico: An analysis. Agriculture Ecosystems and Environment 123: 109-115.

Orozco-Ramírez Q, Ross-Ibarra J, Santacruz-Varela A, Brush S (2016) Maize diversity associated with social origin and environmental variation in Southern Mexico. Heredity 116: 477-84.

Ortega-Villegas MN, Zizumbo-Villarreal L, Monterroso-Salvatierra N, Hernández-Lara OG (2018) Seed and transgenic maize laws: Analysis from the coproduction between science and rconomic-political regimes in Mexico. Agricultura, Sociedad y Desarrollo 15: 413-442.

Ortiz TJ, Sánchez-Sánchez OM, Ramos-Prado JM (2014) Actividades productivas y manejo de la milpa en tres comunidades campesinas del municipio de Jesús Carranza Veracruz México. Polibotánica 38: 173-191.

Piñeyro N, Heerwaarden AJV, Perales HR, Serratos HJA, Rangel A, Hufford MB, Gepts P, Garay-Arroyo A, Rivera-Bustamente R, Alvarez-Buylla ER (2009) Transgenes in mexican maize: Molecular evidence and methodological considerations for GMO detection in landrace populations. Molecular Ecology 18: 750-761.

Quist D, Chapela I (2001) Transgenic DNA introgressed into traditional maize landraces in Oaxaca Mexico. Nature 44: 541-543.

E. ISSN: 2007-901X

Rendón-Aguilar B, Bernal-Ramírez LA, Bravo-Avilez D, Rocha-Munive MG (2019) Temporal dynamics of detected transgenes in maize landraces in their center of origin. Revista mexicana de biodiversidad 90: e902653. DOI: 10.22201/ib.20078706e.2019.90.2653.

Salgotra RK, Chauhan BS (2023) Genetic diversity, conservation, and utilization of plant genetic resources. Genes 148(1): 174. DOI: 10.3390/genes14010174.

Sánchez Morales P, Romero Arenas O (2018) Evaluación de la sustentabilidad del sistema milpa en el estado de Tlaxcala México. Revista de El Colegio de San Luis 8: 107-134.

Santillán-Fernández A, Salinas-Moreno Y, Valdez-Lazalde JR, Bautista-Ortega J, Pereira-Lorenzo S (2021) Spatial Delimitation of Genetic Diversity of Native Maize and Its Relationship with Ethnic Groups in Mexico. Agronomy 11(4): 672. DOI: 10.3390/agronomy11040672.

Serratos-Hernández JA, Gómez-Olivares JL, Salinas-Arreortua N, Buendía-Rodríguez E, Islas-Gutiérrez F, de-Ita A (2007) Transgenic proteins in maize in the soil conservation area of Federal District Mexico. Frontiers in Ecology and the Environment 5: 247-252.

Sharma P, Singh SP, Iqbal HMN, Parra-Saldivar R, Varjani S, Tong YW (2022) Genetic modifications associated with sustainability aspects for sustainable developments. Bioengineered 13: 9508-9520.

Vidal O, Brusca R (2020) Mexico's biocultural diversity in peril. Revista de Biología Tropical 68: 669-691.

Voora V, Larrea C, Bermudez S (2020) Global market report: Soybeans. International Institute for Sustainable Development. https://www.jstor.org/stable/pdf/resrep26554.pdf. Accessed: May 20, 2023.

WHO (2015) Connecting global priorities: Biodiversity and human health. A state of knowledge review. First edition. World Health Organization and Secretariat of the Convention on Biological Diversity. Canada. 344p.