Crop production

Cry 1F and Cry 2Ab2 proteins survey in maize landraces and teosinte cultivated in Alto Jequitinhonha region

Laize Cristina Rossini[1], *Ronnie Von dos Santos Veloso*[1*], *Josimar Rodrigues Oliveira*[2], *Marcus Alvarenga Soares*[1], *Márcia Regina da Costa*[1], *Ricardo Siqueira da Silva*[1]

10.1590/0034-737X2024710008

ABSTRACT

Maize landraces diversity is important for many smallholder and indigenous populations. Gene flow constitutes a threat to the genetic conservation of several locally adapted maize landraces and, risk management depends on monitoring transgenic occurrence in non-GM maize. This study evaluated the occurrence of exogenous proteins from GM-maize in maize landraces and teosinte cultivated in the Alto Jequitinhonha region, State of Minas Gerais. Gene flow from GM maize to non-transgenic varieties was evaluated in eight maize groups and one teosinte variety. A transgenic maize variety was used as a positive control. Proteins Cry1F and Cry2Ab2 were monitored using the Protein Detection Assay kit (Envirologix QuickStix® kit protocols for detecting Cry1F and Cry2Ab2 proteins). Transgenic proteins were not detected in the leaves of landraces and teosinte cultivated in the Alto Jequitinhonha region.

Keywords: transgenic; non-transgenic; gene flow; *Zea mays*; smallholder.

INTRODUCTION

The Brazilian territory has one of the largest areas cultivated with Bt maize in the world. This technology was first grown commercially in 2008 with Bt maize varieties expressing Cry1F and Cry1Ab proteins (Parentoni *et al.*, 2013). More than one decade after their introduction, maize expressing Cry1F and Cry1Ab have been widely cultivated in the Brazilian agroecosystem (Marques *et al.*, 2019). However, adverse impacts are still not entirely known, particularly about the flow of these proteins to the maize landraces cultivated by small farmers.

Maize landraces include several locally adapted phenotypes and constitute an important component of the genetic diversity within the genus *Zea*, particularly in domesticated subspecies *Zea mays* ssp. *mays*. Plants in this group are open-pollinated with no restriction to gene flow via pollen, contributing to genetic variability and phenotypic adaptation under different edaphoclimatic conditions (Arteaga *et al.*, 2016). Adverse effects on morpho-agronomic traits, reduced fitness, and potential extinction are the main issues of scientific debate related to the risks of unintended gene flow from GM maize to non-genetically modified maize (non-GM maize) and its wild relatives (Agapito-Tenfen & Wickson, 2018; Bauer-Panskus *et al.*, 2020). Pollen flow between maize plants is the main form of gene flow in this species (Zhang *et al.*, 2020). A spatial or temporal separation distance between GM maize and maize landraces can reduce the risks of pollen-mediated gene flow (Baltazar *et al.*, 2015). On the other hand, these strategies are ineffective if the contamination of landraces seeds by transgenic seeds occurs during the social practices of seed exchange, which are common among traditional family farmers (Bellon & Berthaud, 2006).

Submitted on April 13th, 2022 and accepted on July 20th, 2023.
[1] Universidade Federal dos Vales do Jequitinhonha e Mucuri, Departamento de Agronomia, Diamantina, MG, Brazil. laize_rossini_@hotmail.com; ronniesvelso@gmail.com; agronojc@gmail.com; marcus.alvarenga@ufvjm.edu.br; marcia.costa@ufvjm.edu.br; ricardo.ufvjm@gmail.com
[2] Universidade Federal dos Vales do Jequitinhonha e Mucuri, Divisão de Fazendas Diamantina, MG, Brazil. josimar.oliveira@ufvjm.edu.br
*Corresponding author e-mail: ronniesvelso@gmail.com

The Alto Jequitinhonha is a geographic region of the State of Minas Gerais, where agricultural systems are predominantly composed of small-scale food producers in family farming systems. This region contributes with less than 1% of the total area occupied by maize crops in the State of Minas Gerais (907,347.00 ha), according to the Agricultural Census carried out in 2017 (IBGE, 2017; Parentoni *et al.*, 2013). In situ/on-farm conservation strategies can substantially contribute to maize landraces conservation in secondary centers of diversity such as Brazil (Guzzon *et al.*, 2021). Additionally, genetic diversity conservation of maize landraces is an important issue for food security. In this context, the present work evaluated if GM maize's Cry1F and Cry2Ab2, the two most common proteins, occur in teosinte and maize landraces cultivated in the Alto Jequitinhonha region, State of Minas Gerais.

MATERIAL AND METHODS

Maize landraces used in this study were: Amarelo Bateias, Avermelhado Amendoim and Vicente Avermelhado from the local communities of Fazenda Bateias, Água Espalhada and Abóboras, respectively. All seeds were donated by family farmers from these communities, all located in Couto de Magalhães de Minas, State of Minas Gerais. These family farmers have cultivated these seeds for over 30 years on their rural properties (Figure 1). The variety known as Crioulo Branco was obtained in the municipality of Carbonita and the variety Pipoca Preto obtained in São Gonçalo do Rio Preto, both regions belong to the Alto Jequitinhonha region (Figure 1). The cultivation history of these varieties is currently unknown. The landraces Rajado and Roxo, were obtained from a Landraces Seed Exchange Fair in the city of Viçosa, State of Minas Gerais (Figure 1). The UFVM 200 variety, registered under number 12,379 in the National Cultivar Registry of the Ministry of Agriculture, Livestock, and Supply (MAPA-Brazil), was developed from genetic improvement Federal University of Viçosa (Brasil, 2002). The ancestor teosinte was obtained from the municipality of Venâncio Aires - State of Rio Grande do Sul and, it was not possible to determine how long this seed has been cultivated in that region (Fig. 1). Seeds of maize landraces (Rajado and Roxo) were planted for more than five years in the municipality of Couto de Magalhães de Minas. Maize and teosinte seeds were obtained directly from family farmers and grown only once in a greenhouse in the experimental field (Fazenda Rio Manso) of the Universidade Federal dos Vales do Jequitinhonha and Mucuri - UFVJM.

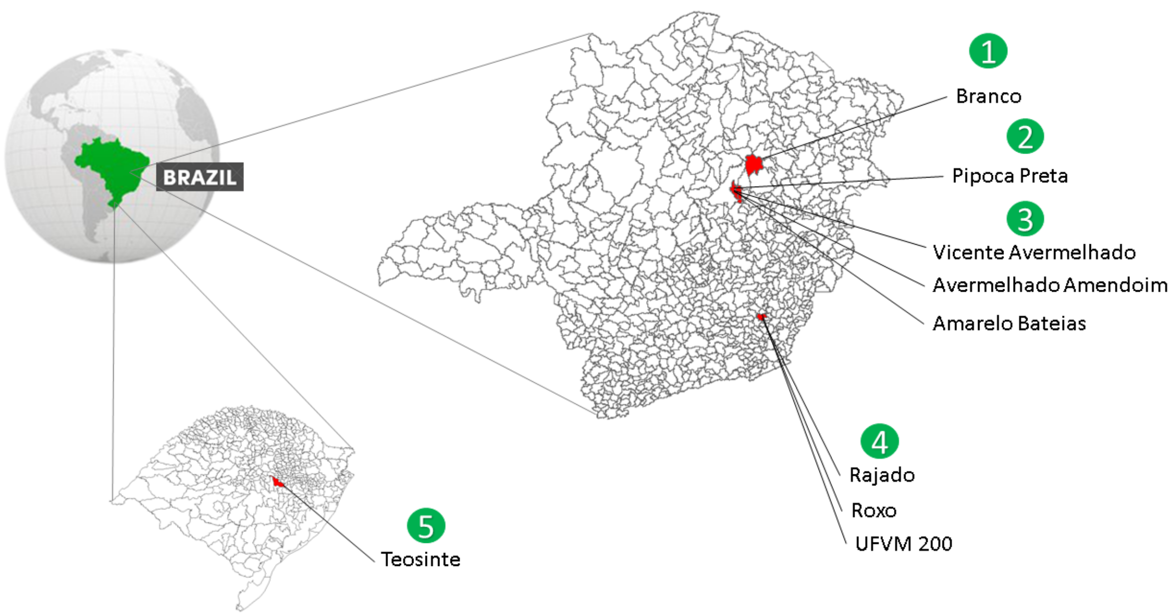

Figure 1: Maize varieties and teosinte origin. (1)The landrace Crioulo Branco was rescued in the municipality of Carbonita-MG (17°31'59.62"S e 43° 0'56.82"W); (2) Landrace Pipoca preta in São Gonçalo do Rio Preto-MG (18° 0'11.78"S e 43°22'47.96"W); (3) Landrace Vicente Avermelhado, Avermelhado Amendoim and Amarelo Bateias from the municipality of Couto de Magalhães de Minas-MG (18°10'07,2" S e 43°25'18,6" W); (4) The landrace Rajado, Roxo and the improved variety UFVM200 from municipality of Viçosa-MG (20°45'28.85"S e 42°52'56.37"W); (5) Teosinte was obtained from Venâncio Aires – RS (29°36'55.94"S e 52°11'37.61"W).

To assess the possible gene flow from GM maize to non-transgenic varieties, nine groups were evaluated: Amarelo Bateias, Avermelhado Amendoim, Crioulo Branco, Pipoca Preta, Rajado, Roxo, UFVM 200, Vicente Avermelhado and a teosinte variety. A transgenic maize variety with PowerCore® technology (pyramided transgenic maize that encodes the proteins Cry1F, Cry1A.105 and Cry2Ab2) was used as a positive control. The seeds were planted in pots with a volumetric capacity of 0.5 dm^3. Irrigation and fertilization were carried out following technical recommendations (Cruz, 2010). Plant treatments with fungicides, herbicides, and insecticides were not carried out.

Leaf samples of teosinte and maize varieties were collected 20 days after germination. All leaf samples were collected with 1.0 cm in diameter. The occurrence of Cry1F and Cry2Ab2 proteins were evaluated using the protein detection assay kit (Envirologix QuickStix® kit protocols for detection of Cry1F and Cry2Ab2 proteins). Leaf samples were harvested from a total of 60 plants. Six replicates per variety were used for the analysis of leaf proteins (each replicate corresponds to a single plant). Six leaf disks of 1.0 cm diameter were obtained from each plant and used for protein detection bioassay. The leaf samples were transferred and macerated into 1.5 mL microtubes using plastic pistils and homogenized by adding 0.5 mL of lysis buffer solution for one minute. For detection of transgenic proteins, samples were submitted to lateral flow immunochromatography. Envirologix QuickStix® lateral flow strips were incubated at room temperature for 5 minutes. Subsequently, the presence of proteins Cry1F and Cry2Ab2 was evaluated according to the Envirologix QuickStix® protocol.

RESULTS

In this study, qualitative results were obtained through the immunochromatographic detection technique for the presence or absence of Cry1F and Cry2Ab2 in maize leaves (transgenic and non-transgenic) and teosinte. Cry1F and Cry2Ab2 proteins were detected in all leaf samples of transgenic maize, used as a positive control. On the other hand, the expression of Cry1F and Cry2Ab2 was not found in all landraces from the Alto Jequitinhonha region and Rajado and Roxo varieties. These proteins were also not detected in the improved variety UFV200 (collected from Viçosa) and in the leaves of teosinte (Table 1).

DISCUSSION

The genetic composition of maize landraces cultivated in traditional or family farming is influenced mainly by two factors: pollen flow (environmental) and social factors related to seed sharing (Bøhn *et al.*, 2016). In many geographic regions where family farming predominates, contamination of local varieties with exogenous genes from transgenic crops can occur inadvertently through seed exchange among smallholders farmers (Van Heerwaarden *et al.*, 2012). Thus, maize landraces may be more exposed to hybridization and introgression when cultivated nearby areas with significant adoption of GM maize by farmers (Nascimento *et al.*, 2012; Chaparro-Giraldo *et al.*, 2015). The method applied in this study did not detect Cry1F, and Cry2Ab2 proteins in all non-GM maize evaluated. Hybridization between transgenic maize and landraces can occur more efficiently when compared to the ancestral teosinte (Baltazar *et al.*, 2005; Rojas-Barrera *et al.*, 2019). Unintentional gene flow through pollination has been observed

Table 1: SImmunochromatographic strip test results for the presence of Cry1F and Cry2Ab2 proteins

Varieties	**Cry2Ab2**	**Cry1F**
	Samples with six replicates (%)	
Avermelhado Amendoim	0	0
Vicente Avermelhado	0	0
Amarelo Bateias	0	0
Pipoca Preta	0	0
Crioulo Branco	0	0
Rajado	0	0
Roxo	0	0
Teosinte	0	0
UFVM200	0	0
PowerCore®	100	100

in Brazil between transgenic and non-transgenic varieties (Nascimento *et al.*, 2012). Another study reported the gene flow from GM maize to maize landraces and non-genetically modified hybrids (Chaparro-Giraldo *et al.*, 2015). Introgression between GM crops and landraces may affect genetic diversification at a regional scale, with risks related to reducing the genetic diversity of landraces (Rojas-Barrera *et al.*, 2019). Thus, continuous monitoring to detect possible gene flows between GM, and non-GM maize constitutes an important piece of ecological risk management for the genetic conservation of maize landraces.

Unintentional hybridization followed by introgression from GM maize to their wild relative (teosinte) or landraces may have ecological and agronomic risks whose implications are poorly understood (Todesco *et al.*, 2016; Hernández-Terán *et al.*, 2017). In our study, proteins Cry1F and Cry2Ab2 were not observed in teosinte leaves. According to Baltazar *et al.* (2005), gene flow occurs asymmetrically between maize and teosinte due to morphological and phenological differences. The direction of gene flow is more likely from teosinte to maize, and this asymmetry contributes to the coexistence of teosinte as a separate entity from maize. The introgression between maize and teosinte is an event that occurs in low frequency, but it is a significant environmental risk factor, mainly for the primary center of origin of maize species (Chavez *et al.*, 2012; Todesco *et al.*, 2016).

On the other hand, GM maize introgression into teosinte has the potential to cause the emergence of teosinte varieties as a noxious weed that, in addition, can express herbicide resistance genes (Le Corre *et al.*, 2020). We attribute the lack of expression of Cry1F and Cry2Ab2 proteins in landraces evaluated to the low density of cultivated areas with transgenic varieties. Our results may indicate that the Alto Jequitinhonha region presents favorable conditions for in situ conservation of maize landraces. However, risk management depends on continuously monitoring possible introgressions between GM maize and teosinte in geographical locations of coexistence between these species.

CONCLUSIONS

We evidenced the absence of Cry1F and Cry2Ab2 in landraces and an improved maize variety. This fact is positive, as it indicates no gene flow in natural conditions where these genotypes are cultivated for Cry1F and Cry2Ab2. We emphasize the importance of more studies, mainly using DNA-based approaches for transgenic detection. In addition, adequate sampling methods to offer representative results for the region must be developed and applied in new studies to monitor transgenic DNA in maize landraces. The results may be a starting point for monitoring maize landraces to guarantee the absence of gene flow that may cause loss of genetic characteristics essential for maize landraces. These results could be used to certify the non-presence of Cry1F and Cry2Ab2 for landrace maize in the studied regions.

ACKNOWLEDGMENTS

To José Carlos Barbosa for his contribution to Figure 1, Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Code Finance 001 for financial support.

FUNDING

This research received no specific grant from any funding agency.

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Agapito-Tenfen SZ & Wickson F (2018) Challenges for transgene detection in landraces and wild relatives: learning from 15 years of debate over GM maize in Mexico. Biodiversity and Conservation, 27:539-566.

Arteaga MC, Moreno-Letelier A, Mastretta-Yanes A, Vázquez-Lobo A, Breña-Ochoa A, Moreno-Estrada A, Eguiarte LE & Piñero D (2016) Genomic variation in recently collected maize landraces from Mexico. Genomics Data, 7:38-45.

Baltazar BM, Sánchez-Gonzalez JJ, Cruz-Larios L & Schoper JB (2005) Pollination between maize and teosinte: an important determinant of gene flow in Mexico. Theoretical and Applied Genetics, 110:519-526.

Baltazar MB, Espinoza LC, Banda AE, Martínez JMF, Tiznado JAG, García JG, Gutiérrez MA, Rodríguez JLG, Díaz OH, Horak MJ, Martínez JIM, Schapaugh AW, Stojšin D, Montes HRU & García FZ (2015) Pollen-Mediated Gene Flow in Maize: Implications for Isolation Requirements and Coexistence in Mexico, the Center of Origin of Maize. PLoS ONE, 10:e0131549.

Bauer-Panskus A, Miyazaki J, Kawall K & Then C (2020) Risk assessment of genetically engineered plants that can persist and propagate in the environment. Environmental Sciences Europe, 32:32.

Bellon MR & Berthaud J (2006) Traditional Mexican Agricultural Systems and the Potential Impacts of Transgenic Varieties on Maize Diversity. Agriculture and Human Values, 23:03-14.

Bøhn T, Aheto D, Mwangala F, Fischer K, Bones IL, Simoloka C, Mbeule I, Schmidt G & Breckling B (2016) Pollen-mediated gene flow and seed exchange in small-scale Zambian maize farming, implications for biosafety assessment. Scientific Reports, 6:34483.

Brasil (2002) Registro Nacional de Cultivares – RNC: UFVM 200. Available at: <http://sistemas.agricultura.gov.br/snpc/cultivarweb/detalhe_cultivar.php?codsr=12367>. Accessed on: September 20th, 2021.

Chaparro-Giraldo A, Blanco M, Jennifer T & López-Pazos SA (2015) Evidence of gene flow between transgenic and non-transgenic maize in Colombia. Agronomía Colombiana, 33:297-304.

Chavez NB, Flores JJ, Martin J, Ellstrand NC, Guadagnuolo R, Heredia S & Welles SR (2012) Maize x Teosinte Hybrid Cobs Do Not Prevent Crop Gene Introgression. Economic Botany, 66:132-137.

Cruz JC (2010) Cultivo do Milho. 6ª ed. Sete Lagoas, Embrapa Milho e Sorgo. 10p.

Guzzon F, Arandia RLW, Caviedes CGM, Céspedes PM, Chavez CA, Muriel FJ, Medina HAE, Jara CTW, Molnar TL, Narro LLA, Narro LTP, Mejía KSL, Ospina RJG, Vázquez G, Preciado-Ortiz RE, Zambrano JL, Palacios RN & Pixley KV (2021) Conservation and Use of Latin American Maize Diversity: Pillar of Nutrition Security and Cultural Heritage of Humanity. Agronomy, 11:172.

Hernández-Terán A, Wegier A, Benítez M, Lira R & Escalante AE (2017) Domesticated, Genetically Engineered, and Wild Plant Relatives Exhibit Unintended Phenotypic Differences: A Comparative Meta-Analysis Profiling Rice, Canola, Maize, Sunflower, and Pumpkin. Frontiers in Plant Science, 5:2030.

IBGE – Instituto Brasileiro de Geografia e Estatística (2017) Instituto Brasileiro de Geografia e Estatística. Censo Agropecuário. Available at: <https://www.ibge.gov.br/estatisticas/downloads-estatisticas.html>. Accessed on: October 22nd, 2021.

Le Corre V, Siol M, Vigouroux Y, Tenaillon MI & Délye C (2020) Adaptive introgression from maize has facilitated the establishment of teosinte as a noxious weed in Europe. Proceedings of the National Academy of Sciences of America, 117:25618-25627.

Marques LH, Santos AC, Castro BA, Moscardini VF, Rosseto J, Silva O & Babcock JM (2019) Assessing the Efficacy of Bacillus thuringiensis (Bt) Pyramided Proteins Cry1F, Cry1A.105, Cry2Ab2, and Vip3Aa20 Expressed in Bt Maize Against Lepidopteran Pests in Brazil. Journal of economic entomology, 112:803-811.

Nascimento VE, Pinho EVRV, Pinho RGV, Souza JC & Júnior ADN (2012) Fluxo gênico em milho geneticamente modificado com resistência a insetos. Pesquisa Agropecuária Brasileira, 47:784-790.

Parentoni SN, Miranda RA & Garcia JC (2013) Implications on the introduction of transgenics in Brazilian maize breeding programs. Crop Breeding and Applied Biotechnology, 13:09-22.

Rojas-Barrera IC, Wegier A, González JJS, Owens GL, Rieseberg LH & Piñero D (2019) Contemporary evolution of maize landraces and their wild relatives influenced by gene flow with modern maize varieties. Proceedings of the National Academy of Sciences of America, 116:21302-21311.

Todesco M, Pascual MA, Owens GL, Ostevik KL, Moyers BT, Hübner S, Heredia SM, Hahn MA, Caseys C, Bock DG & Rieseberg LH (2016) Hybridization and extinction. Evolutionary applications, 9:892-908.

Van Heerwaarden J, Ortega DVD, Alvarez-Buylla ER & Bellon MR (2012) New genes in traditional seed systems: diffusion, detectability and persistence of transgenes in a maize metapopulation. PLoS One, 7:e46123.

Zhang L, Huo S, Cao Y, Xie X, Tan Y, Zhang Y, Zhao H, He P, Guo J, Xia Q, Zhou X, Long H & Guo A (2020) A new isolation device for shortening gene flow distance in small-scale transgenic maize breeding. Scientific Reports, 10:15733.