14013 - *Case study from Santa Catarina, Brazil: GM contamination detected in maize variety*

Estudo de caso em Santa catarina, Brasil: Contaminação GM detectada em variedades de milho

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Abstract
Genetically modified (GM) maize has been planted in Brazil since the 2007/2008 growing season. Cross pollination from GM to non-GM maize, i.e. GM contamination, may have severe negative impact on genetic diversity, in addition to lost access to non-GM markets. Such contamination has previously been detected in Latin America, and is of great concern for farmers who wish to grow non-GM maize. In this study, we aimed to detect genetic contamination from GM maize to non-GM maize, and analyze the social context of such GM contamination, in a model system in Santa Catarina, Brazil. The transgenic protein Cry1Ab was detected in one non-GM maize field using lateral flow strips, and a Polymerase Chain Reaction confirmed the presence of the p35s promoter. In this case, lack of information about the national regulations of minimum distances between GM and non-GM maize might have enabled the GM contamination event.
Keywords: Transgenic; Lateral Flow Strips; PCR; Coexistence; Brazil

Resumo
Milho geneticamente modificado (GM) foi plantado no Brasil desde a safra 2007/2008. Cruzamentos entre milho GM e não-GM, ou seja, a contaminação transgênica, pode ter um grave impacto negativo sobre a diversidade genética, além da perda ao acesso a mercados não-GM. Esta contaminação foi detectada anteriormente na América Latina, e é de grande preocupação para os agricultores que querem cultivar milho não-GM. Neste estudo, buscou-se detectar a contaminação genética do milho GM para o milho não-GM, e analisar o contexto social de tal contaminação GM, em um sistema modelo em Santa Catarina, Brasil. A proteína Cry1Ab transgênica foi detectada em um campo de milho não-GM, utilizando tiras de fluxo lateral e reação da polimerase em cadeia, confirmando a presença do promotor de P35S. Neste caso, a falta de informações sobre as normas nacionais de distâncias mínimas entre milho GM e não-GM pode ter facilitado o evento da contaminação transgênica.
Palavras-chave: Transgênicos; tiras laterais de fluxo; PCR; Coexistência Brasil;

Introduction

Genetic diversity of domesticated crop species is not only essential for present-day crop production, but also provides genetic resources for genetic improvement in the future, and is thus essential for meeting future demands for world food supply . Genetic contamination refers to the unwanted process of transgenic alleles from GM crops moving to other organisms, crop varieties and ecosystems, and becoming established there . If the transgenic alleles provide changes (either costs or benefits) in fitness of the recipient plants, such GM contamination might cause loss of genetic diversity and reduction of the evolutionary and agricultural potential of the recipient non-GM population (LU, 2013). Social mechanisms might cause loss of diversity in the contaminated population . In addition to the possible loss of genetic diversity, such GM contamination might have juridical and socioeconomic impacts for the farmer contaminated . The owner of the contaminated maize might face exclusion from advantageous target markets with limited acceptance for GM contamination . To avoid such GM contamination is therefore a question of great importance for farmers who wants to plant non-GM and access markets where GMOs are not accepted (HEWLETT AND AZEEZ, 2008).

Brazil is the world's second biggest producer of genetically modified (GM) crops, with 36.6 million hectares planted with GM crops in 2012, and the production is growing . Five years after the legalization of the first GM maize in 2007, the adoption rate (i.e. percentage of total maize producing area that is planted with GM maize) was 76% in the 2011/2012 growing season . In Latin America, evidence for GM contamination in maize has been found in Uruguay and Peru . Evidence for such GM contamination of maize has also been found in the center of origin for maize in Mexico, although this latter case has created controversy . In 2010, a study from Brazil detected GM contamination in maize fields up to 120 meters away from the closest GM source , however, also these findings have been disputed . To the author's knowledge, no other studies have previously unambiguously detected GM contamination of maize in a field context in Brazil .

In this study, we aimed to answer the question: Is there evidence for GM contamination in a system for maize seed multiplication localized in Santa Catarina, Brazil? A follow-up investigation was conducted to examine the social context of such contamination. The results presented in this paper are preliminary.

Methods and locality

Locality: The Brazilian Movement of Small Farmers (Movimento dos Pequenos Agricultores, hereafter MPA), is based on agroecological principles, promoting a sustainable agriculture without the use of GMOs (MPA, 2011). MPA, organizing farmers in 17 states of Brazil, initiated in 2007 a project for conservation and multiplication of landraces and open pollinated varieties of maize in the extreme east of Santa Catarina. Before the legalization of GM maize in 2007, 40 maize varieties were implemented in the MPA conservation system. The MPA system produced in 2011/2012 1483 tons of maize seeds, which are distributed to approximately a hundred thousand families all over Brazil (A. Muniari, pers. Comm., MPA 2011).In Santa Catarina, the adoption rate of GM maize was 90% in the 2012/2013 growing season . The coexistence of GM maize and non-GM maize opens the possibility for GM contamination of the maize varieties in the MPA conservation system, and is a main concern of MPA in Santa Catarina, as such GM contamination might be widely distributed, possibly with severe biological and socioeconomic consequences.

Sampling: Between June and December 2012, maize seeds from 39 fields delivering seeds to the MPA system were sampled. The fields were located in 18 municipalities in the extreme east of Santa Catarina. Depending on the maturity of the maize, the samples were taken from from fresh cobs in the field, from dried cobs in the field, or from a mixture of dry seeds. In the latter case, the samples were taken either from a storage room on the farm or from the MPA central in São Miguel do Oeste in Santa Catarina. Some samples taken from the MPA central contained seeds from several farmers, but all samples contained one variety exclusively. In total, 24 landraces and open pollinated varieties of maize were collected.

Analyses: From each dry seed sample, three subsamples of 100 seeds each were separately crunched to a fine powder. Between every sample, the mill was meticulously cleaned with 70% alcohol, and 100 non-GM maize seeds were crushed. From the fresh cobs, subsamples of 100 seeds each were crunched in separate mortars with liquid nitrogen before protein and DNA analyses were performed. The detection of the transgenic protein Cry1Ab was performed using AS003 BG Lateral Flow Membrane Strips (limit of detection: one GM seed out of 125 seeds), and the detection of the transgenic protein EPSPS was performed using AS 010 BG Lateral Flow Membrane Strips (limit of detection: one GM seed out of 200 seeds), both from Envirologix (Portland, Maine, USA), following the manufacturers protocol. The alleged limit of detection was confirmed with GM and non GM seeds, before two subsamples (100 seeds each) from each sample were analyzed.

In order to confirm the presence of transgenic DNA sequences in the samples that tested positive for transgenic proteins, a PCR was conducted using a primer pair (Table 1) specific for a part of the p35s region which is present in all but two of the 18 GM maize varieties commercially available in Brazil per June 2012 . DNA from the crunched subsamples was extracted using a CTAB protocol modified the following way: The extraction with Chloroform-Isoamyl alcohol (CIA) were performed twice, adding 1/10 volume of 10% CTAB solution to the samples between the CIA extraction. After precipitating the DNA with isopropanol, the samples were put for further precipitation in a -20 degrees fridge for 30 minutes. Washing of the end extract with ammonium acetate was not performed. A Zein specific primer pair was used in a multiplex reaction as an endogenous control. The detection level of the PCR reaction was verified by testing the reaction with an extract of 1% GM seeds (dry weight). A DNA sample from a hybrid between a landrace and a transgenic was used as a positive control in each PCR run. The resulting PCR product was run in a 2% agarose gel on 100 Volt and 400mA, and the bands were checked in an UV illuminator, type DigiDoc-It System (UVP Bioimagiging Systems, California, USA).

TABLE 1: Primer pairs utilized for transgene detection in maize

Gene	Amplicon	Sequence 5'-3'	Reference
P35s	123 bp	CCACGTCTTCAAAGCAAGTGG TCCTCTCCAAATGAAATGAACTTCC	Nikolić, Milošević et al. 2008
Zein	329 bp	TGCTTGCATTGTTCGCTCTCCTAG GTCGCAGTGACATTGTGGCAT	Gamarra, Delgado et al. 2011

Results and discussion

Detection of contamination: All samples were successfully tested for presence of Cry1Ab and EPSPS protein using Lateral Membrane Flow Strips. The protein Cry1Ab was detected in both subsamples of sample 29_10 as well as in both subsamples of the neighboring field 29_23. This result was confirmed in the PCR, which produced a band at around 123 bp, probably corresponding to the 123 bp amplicon in the p35s region. Neither the negative controls nor the non-GM seeds that were crunched between each sample showed signs of contamination. However, resampling, PCR and sequencing of the PCR product would be ideal in order to confirm the positive result and eliminate the possibility for contamination in the transport or in the lab. We conclude that the positive result of protein and DNA analyses is likely to reflect an actual presence of Cry1Ab protein and the p35 s region in all three subsamples of sample 29_10 and 29_23, thus indicating GM contamination.

Consequences of contamination: In this case, the owner of the maize sample testing positive for GM contamination will not be able to sell his seeds to MPA, or to any customer demanding non-GM seeds. He will thus be excluded from an economically advantageous market, with economic losses as a result.

Context of contamination: To better understand how this probable contamination event did happen, and to propose directions for further research, an investigation of the possible background for the contamination was initiated. Conversations were performed with the farmer involved (JB), as well as nine farmers, three agronomists and one employee in a company selling GM seeds, all from eastern Santa Catarina. The field that was found to be contaminated (sample 29_10) was planted with an open pollinated variety of maize that JB achieved from MPA in 2010. The neighboring maize field, five meters away (sample 29_23), was owned by the same farmer, and planted with a commercial hybrid variety bought in 2012. Due to our analyses, sample 29_23 from this commercial variety was also containing the Cry1Ab protein and the p35s promotor. Several scenarios could explain this scenario, such as seed or pollen mediated GM contamination from the field 29_23 to field 29_10, seed or pollen mediated GM contamination of both field 29_10 and 29_23 from a third field, or a GM contamination in the seeds that JB received from MPA in 2010, contaminating also field 29_23. However, JB explained that he knew that the seeds he had bought and planted (sample 29_23) were resistant to insects, but that he was not informed by the seller that they were genetically modified. When asked directly, he was not aware of any regulations on coexistence between GM and non-GM maize. No commercial hybrid on the Brazilian marked is resistant to insects without being genetically modified. It is therefore probable that the seeds JB planted in field 29_23 were in fact genetically modified. Due to the proximity of the maize fields 29_23 and 29_10, and the putative GM identity of field 29_23, this field is one plausible donor of GM seeds or pollen contaminating the non-GM variety in field 29_10.

Essential to the success of the coexistence between GM and non-GM maize is a realistic assessment of the risk of GM contamination, followed by corresponding regulations to avoid such contamination. Such measures should consider the social context to which the GM technology and corresponding regulations are implemented, and to what degree the regulations will be complied . Due to the Normative Resolution nº 4 by National Technical Committee on Biosecurity (CTNBio), Brazilian farmers have to plant GM maize minimum 100 meters away from the closest non-GM maize field, or plant at least 10 rows of conventional maize at a minimum distance of 20 meters from the closest non-GM maize field in order to avoid GM contamination. To be able to follow the national regulations on coexistence set by CTNBio, the farmers need to be unambiguously informed when they are buying GM seeds, and have access to information about the coexistence regulations. Only one out of the ten farmers that were interviewed in connection to this study was aware of these regulations. Analphabetism is overrepresented in marginalized groups, in small municipalities and in rural areas . For instance, in the rural municipality of São

Lourenco do Oeste, Santa Catarina, 9,7 % of the population was not able to read a simple note, due to data from 2012 . Analfabetism was mentioned by an agronomist as an explanation to why farmers were unaware of the regulations on GM maize: “A student [of the informant’s wife] interviewed 50 farmers, and it was scandalous how little they knew [about GM seeds]. Many can not read on the product package” (Agronomist, University of Chapecó, 2013).

The three agronomists working in the area confirmed that many farmers planting GM did not have access to information about the coexistence regulations. Some of the farmers also expressed the misconception that the commercial maize hybrids sold in this area were resistant to insects, but not genetically modified. One agronomist working for MPA said: "The word *genetically modified* makes a big impact and has a negative connotation. Many sellers of genetically modified seeds say to farmers that the seeds are hybrids resistant to caterpillars. They don’t mention that the seeds are transgenic, or that the crop farmer must respect the neighbor’s harvest, nothing. Farmers are not informed about the laws of coexistence, there is a lot of misinformation. "(Agronomist, MPA, 2013).

Conclusions:

This study has identified a probable case of GM contamination of a non-GM variety of maize in the extreme east of Santa Catarina, Brazil. In this case, the neighboring GM maize field is a plausible source of contamination. Lack of knowledge about GM maize, and/or lack of awareness of the existence of the CTNBio coexistence regulations as described in Resolução Normativa CTNBio nº 4, appeared common among farmers in the extreme east of Santa Catarina, Brazil. Further studies should be conducted in order to determine to what degree planters of GM maize are following the regulations of coexistence set by CTNBio, if not: why, and how the awareness of coexistence regulations is affecting the risk of GM contamination of maize.

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