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RESEARCH TO DETERMINE THE IMPACT OF BT TECHNOLOGY APPLIED TO MAIZE MON 810 ON SOIL QUALITY

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Abstract

Develop methodology for assessing the impact of Bt technology applied to maize MON 810 on soil quality parameters, and particularly, microbial diversity was done according to the following objectives: study the influence of soil type by its physical and chemical parameters defining, transgenic plant cultivation study on microbial diversity, the study of transgenic plant cultivars of the main soil chemical properties. The criteria for selection of soil materials on which to conduct the impact study of Bt biotechnology were favorability for species taken in the study and the presence of physical (content of clay) and chemical (reaction) properties as contrasting. Thus, were used three soil types: Eutric Fluvisols, Fluvi-Eutric Cambisols and Haplic Chernozems. Research has pursued: identifying correlations between the main physical-chemical and biological attributes of soil and plant genotypes (GMOs or non-GMOs) cultivation, environmental impact assessment of Bt protein on essential soil biological processes by investigating the biological activities associated with plant debris decomposition and determination of carbon, nitrogen, phosphorus and potassium in the soil, assessing the impact of Bt technology taxonomic and genetic diversity of soil micro-organisms, assessment of possible modifications of the main physical features, chemical and biological properties of soil under the influence of Bt technology.

INTRODUCTION

GMOs (plants, microbes and animals) with useful characters are considered to be a powerful technology for the future development of sustainable agricultural systems. Plants have been genetically modified to resist insect and fungal pathogens, withstand specific herbicide application (better weed management) or environmental conditions (e.g. water logging), to improve crop quality, for biomolecule production and for bioremediation/phytoremediation of polluted soils. MON 810 is a maize variety with a single transformation event, genetically modified to express the Bt insecticidal toxin gene that confers plant resistance to

attack by Ostrinia nubilalis. MON 810 has been available for commercial cultivation in European Union since 1998.

While reduced pesticide/herbicide use associated with Bt maize is, clearly beneficial, very little is known about potential non-target effects of Bt maize plants on the functional groups of biota and biological processes that are critical for plant health, and essential ecosystem functions including ecosystem health. Pre-release evaluation of GM plant varieties is generally concentrated on the genetic stability of gene insertions and agronomic aspects of GM varieties.

However, comparatively little experimental (especially quantitative) data are available on: environmental consequences of the introduced gene function and associated changes in management practices/farming systems on essential ecosystem functions and the fate of the products of engineered genes from genetically modified organisms (GMOs) e.g. persistence in the environment and gene transfer to other organisms. This needs to be an essential part of the risk assessment of any GMOs release.

Soil organism's communities, which are among the most diverse groups of earth's biota, regulate a number of processes in terrestrial ecosystems that are not only critical for productivity, but are, also, essential for maintenance of ecosystem health [3]. Micro organisms and microbial activity have a key role in stable aggregate formation. Water-stable aggregates are essential for good soil structure in all types of soils. Good soil structure is necessary to reduce soil erosion. Very few biological processes are mediated by individual species of biota; therefore, the successful functioning of most ecosystem processes requires a balance of biota interactions in the complex soil biota community. The availability of energy (carbon), the most important regulating factor of biological activity in soils, affects the composition of the soil biota community and food web structure [6, 5]. In addition, the number of trophic levels in a terrestrial food-web community and the stability of this complex community depend upon the amount and quality of carbon input and the level and type of disturbance (e.g. tillage, GM crops and use of agrochemicals).

Plant residues are one of the primary sources of carbon in soils and the majority of biota populations are concentrated near crop residues and in the plant root rhizosphere [13]. Therefore, any change to the quality of crop residue and rhizosphere inputs will potentially modify the dynamics of the soil biota composition and activity. Soil microoorganisms perform a number of key functions essential to plants, organic mater mineralization, nutrient cycling, disease regulation, agrochemical degradation, and the development and maintenance of physical and chemical properties of soil. Therefore, any change to the quality of rhizosphere exudates will potentially modify the dynamics of the soil biota composition (biodiversity) and activity and may cause changes to both deleterious and beneficial microflora and micro fauna [10, 12, 2].

GM plants, through the products of introduced genes, modified rhizosphere chemistry, or altered crop residue quality, have the potential to significantly change the microbial dynamics and essential ecosystem functions such as nutrient mineralization, disease incidence, and carbon turnover and plant growth [13]. For example, a decrease in specific microbial populations would lead to a decrease in decomposition processes, have secondary effects on plant pathogen survival, and build up, as well as soil organic matter level and composition [17]. However, little experimental data are available on the consequences of plant-microbe-soil interactions due to the sustained expression and/or presence of Bt toxin in the rhizosphere. Gupta et al. [11, 14] have found significant changes in the composition of bacteria in the rhizosphere of Bt cotton compared to that of its non-GM parent variety.

There is no ongoing research on the impact of Bt maize on soil biological processes in Romania. Limited research in Europe and North America suggests significant effects of GM crops on specific soil biota. Stotzky [16] in a recent review recommends a thorough evaluation of the persistence of GM products such as Bt toxins in soil and their effects on the inhabitants of soil and other habitats.

Due to the differences in soil and climatic conditions, and the biota composition, the evaluation of GM plant effects on soil biodiversity under Romanian conditions is necessary.

MATERIAL AND METHODS

Develop methodology for assessing the impact of Bt technology applied to maize MON 810 on microbial diversity in soil was done according to the following objectives: study of the soil type influence, due to its physical-chemical parameters, on persistence and degradation of Bt insecticidal protein; study of transgenic crops on microbial diversity and study of transgenic plants cultivation on the main soil chemical properties.

The effects of maize (*Zea mays* L.), genetically modified to express the Cry1Ab crystal toxin protein, on soil microbial communities were assessed in a glasshouse experiment. Soil for the experiment was taken from three field sites where maize is usually cultivated. Plants were grown in contrasting soils in terms of clay content, and soil samples taken at the five-leaf stage and maturity.

Three soil types: Fluvi-eutric Cambisols, Eutric Fluvisols and Haplic Chernozems were used. Soil samples were analyzed by ICPA methodology [8] developed to assess main physical (particle size) and chemicals soil properties: organic carbon and humus - Walkley-Black method (modified by Gogoasa), total nitrogen content, mobile phosphorus and potassium content - Egner-Riehm-Domingo method, pH (H_2O), ratio soil/water 1/2.5 – electromechanical method using glass electrode. Also, microbiological analyses: quantitative determinations of heterotrophic

bacteria (total bacteria number method) using traditional culturing methods and taxonomic determinations by usually identification methods, optical microscopy, determination keys and physiological tests [1, 9], were carried out.

Data were analyzed using standard analysis of variance (ANOVA) and presented as means with an associated least significant difference (LSD, at the 5% level), using as factors: soil type, and plant type.

RESULTS AND DISCUSSION

Choosing of the three soil types for experimentation was made considering the texture, respectively, different clay content, and reaction. Thus, the first soil type, a Eutric Fluvisols (FLeu*) has a clayey-loamy texture, argyle with Φ <0.002 mm content between 34.0-39.3% and moderate acid reaction; the second soil type, an Fluvi-Eutric Cambisols (CMeu-fv) with low argyle content about 15.1-20.0%, has a sandy-loamy texture and a slightly alkaline reaction, and, finally, the third soil type, a Haplic Chernozem (CHha) has a silty clay loam texture, argyle content between 39.3-41.6%, and weakly acid reaction.

Soil reaction

Have been recorded relatively minor variations of soil reaction in the experimental variants with GM corn, compared with non-GM corn, direction and magnitude of these changes being caused by soil type on which plants were grown.

The biggest difference of soil reaction by 0.45 pH units, was recorded in Fluvi-Eutric Cambisols (CMeu-fv) at 5 leaf stage, when soil cultivated with GM maize was acidified as compared with soil from the non GM maize variant (Figure 1). Variance analysis showed that there were no significant variations of soil reaction caused by the crop type (GM or non-GM) in any stage of analysis, but there were significant variations of pH values between the three soils types used for experimentation.

Humus content

Humus content varied considerably, with very significant differences between the three types of soil. Haplic Chernozem (CHha) is the richest in humus, compared with the other two soils used for experimentation: Eutric Fluvisols (FLeu) and Fluvi-Eutric Cambisols (CMeu-fv). Plant type (GM or non-GM hybrid) has not generated considerable variation of humus content in the two soil types. But in Haplic Chernozem, in both stages of soil analysis, humus content increase was significant and was noted in GM corn variant (Figure 2).

* Symbol according to WRB-SR-1998 in Florea & Munteanu, 2003 [7].

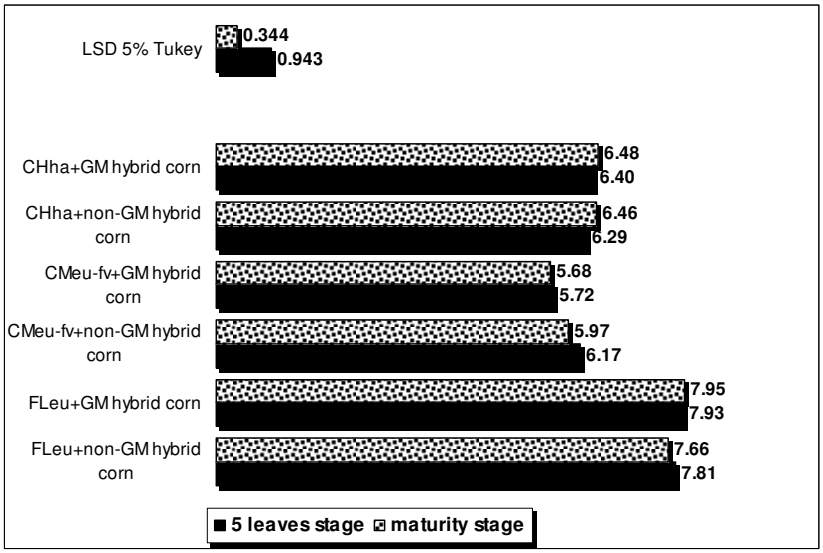

Fig. 1. pH variation in three contrasting soils, planted with GM (MON 810) and non-GM corn

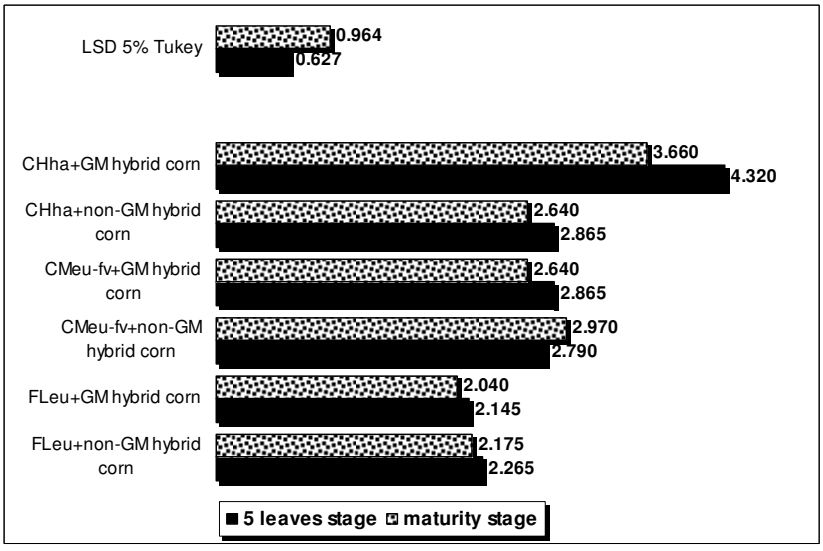

Fig. 2. Humus (%) variation in three contrasting soils, planted with GM (MON 810) and non-GM corn

Total nitrogen content

Total nitrogen content varied in the first stage of analysis, only in case of Fluvi-eutric Cambisols (CMeu-fv), where, in variant cultivated with GM hybrid was

recorded an increase in total nitrogen content from 0.132 to 0.151%, without statistical significance (Figure 3). In the other two soils, total nitrogen content values were approximately equal in both type of variants (planted with GM corn or non-GM corn).

At plant maturity stage, analyses have shown, however, a very significant difference in terms of total nitrogen content in the Haplic Chernozems variants. Here, total nitrogen content marked a very significant increase in GM maize variant (0.176%) compared with those recorded in non-GM maize variant (0.146%). Because of plant growth parameters were quite close; the difference in soil nitrogen reserve may be due to different nitrogen nutrition requirements of the two types of plants.

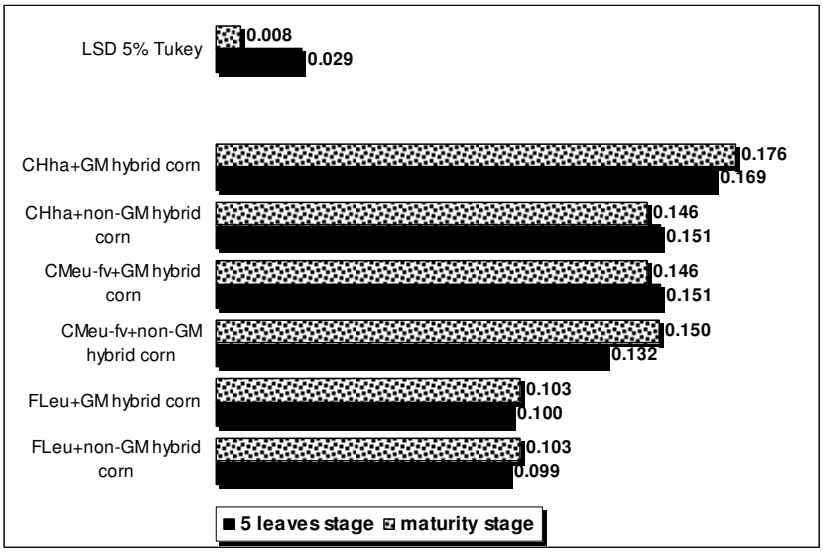

Fig. 3. Total nitrogen (%) variation in three contrasting soils, planted with GM (MON 810) and non-GM corn

Mobile phosphorus and potassium content

Phosphorus and potassium mobile contents showed variations less important to be taken into account between GM corn and the non-GM corn variants, in any stages of analysis. Significant differences were only between different degrees of initial supply of soil used for experimentation with these elements (Figure 4 and 5). Thus, Eutric Fluvisols is very well supplied with mobile phosphorus, while Fluvi-eutric Cambisols and Haplic Chernozems are significantly low supplied in mobile phosphorus, both compared with the first soil type and each other.

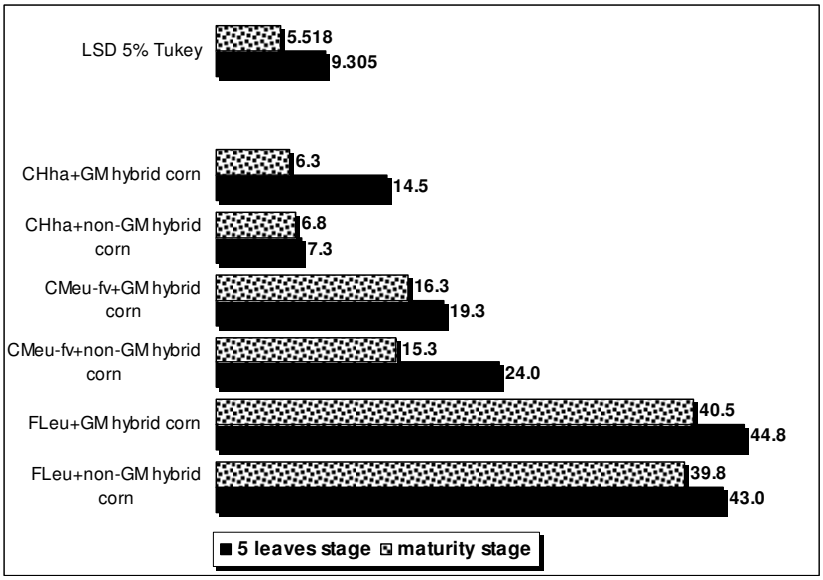

Fig. 4. Mobile phosphorus content (mg·kg^{-1}) variation in three contrasting soils, planted with GM (MON 810) and non-GM corn

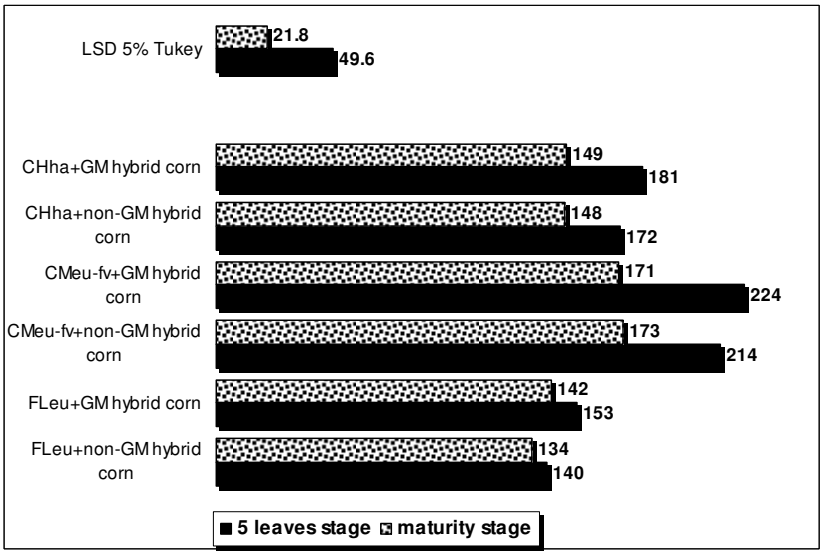

Fig. 5. Mobile potassium content (mg·kg^{-1}) variation in three contrasting soils, planted with GM (MON 810) and non-GM corn

In terms of mobile potassium content, between the three soils, the best supplied is Fluvi-eutric Cambisols, with a high content, followed by the other two soils with

medium contents. An interesting aspect was observed in tests made at plant maturity, when in the Fluvi-eutric Cambisols was noted a higher reduction of potassium content in soil compared both with the initial state of this nutrient supply, and the other two soils. It seems that in this soil type, corn plants had a higher consumption of potassium than in the other two soils used for experimentation.

Soil heterotrophic bacteria

Quantitative determinations of heterotrophic bacteria in the soil did not reveal significant differences between variants cultivated with GM corn as compared with those cultivated with non-GM hybrid nor in any of the soils, and even between stages of determination. Significant quantitative differences were observed only between the three soil types, significantly higher values being determined in Haplic Chernozems, and some smaller but very close in other two soils, Eutric Fluvisols and Fluvi-eutric Cambisols (Figure 6).

Differences between the total bacteria number values recorded in variant cultivated with GM maize compared with those cultivated with non-GM maize on Haplic Chernozems, can be interpreted only as a trend, not statistically assured.

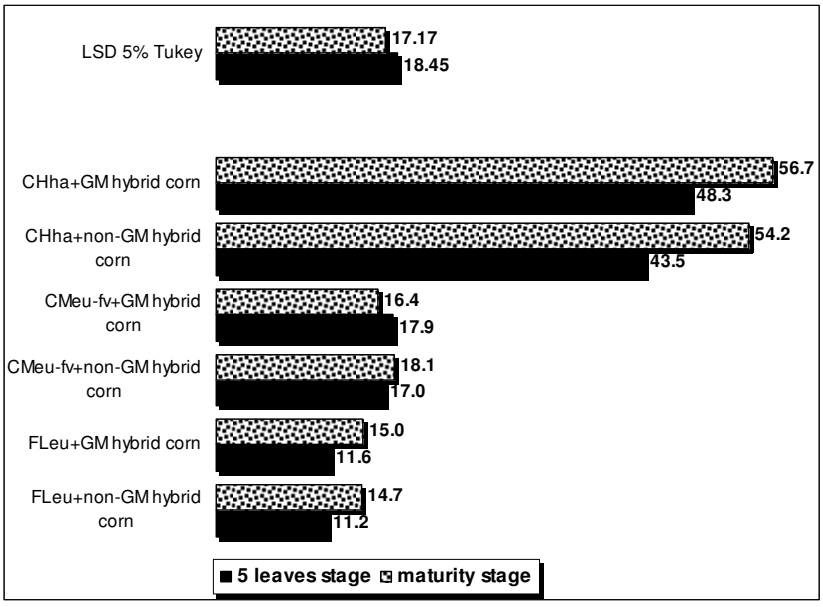

Fig. 6. Total bacteria number (colony forming units x 10^6/g dry soil) variation in three contrasting soils, planted with GM (MON 810) and non-GM corn

Soil microbial communities are very plastic in their species composition and structure and change constantly in different root zones, agricultural practices, and with respect to various other environmental variables [4, 15].

In terms of genus and species diversity of soil bacteria, no major differences were recorded between species composition of bacterial communities in soil cultivated with non-GM hybrid compared to soil cultivated with transgenic hybrid, the number of bacterial strains being quite close in samples analyzed (Table 1).

Table 1

Diversity of bacterial communities in three contrasting soils, planted with GM (MON 810) and non-GM corn

Soil type/ Hybrid type	**Bacteria genus and species (in order of frequency)**		
	Before planting	**5 leaves stage**	**Maturity stage**
FLeu+ non-GM hybrid corn	*Pseudomonas sp**** *Bacillus megaterium*** *B. circulans, B. cereus* *Arthrobacter globiformis**** *Actinomyces sp**	*Pseudomonas* sp.*** *Bacillus megaterium**** *B. circulans B. cereus* *A. globiformis**** *Actinomyces* sp.*	*Pseudomonas* sp.*** *Bacillus megaterium, **** *B. circulans, B. cereus* *A. globiformis**** *Actinomyces* sp.
FLeu+ GM hybrid corn	*Pseudomonas sp**** *Mycobacterium roseum** *B. megaterium**** *A. citreus* A. globiformis**	*Pseudomonas* sp.*** *Mycobacterium roseum** *B. megaterium***, B. cereus* *A. globiformis**, A. citreus*	*Pseudomonas* sp.*** *Mycobacterium roseum* *B. megaterium**** *A. globiformis**, A. citreus**
CMeu-fv+ non-GM hybrid corn	*Pseudomonas* sp.*** *Mycobacterium roseum** *B. sphaericus, B. circulans* *B. megaterium,**** *A. globiformis*** A. citreus,** *Actinomyces* sp.	*Pseudomonas* sp.*** *Mycobacterium roseum* *B. sphaericus, B. circulans* *B. megaterium**** *A. globiformis*** A. citreus* *Actinomyces* sp.*	*Pseudomonas* sp.*** *Mycobacterium roseum* *B. sphaericus,* *B. megaterium**** *B. citreus B. circulans,* *A. globiformis**** *Actinomyces* sp.
CMeu-fv+ GM hybrid corn	*Pseudomonas* sp.***, *Mycobacterium roseum,* *B. megaterium*** B. cereus,* *A. globiformis** *Actinomyces* sp.**	*Pseudomonas* sp.***, *Mycobacterium roseum,* *Bacillus megaterium***,* *Arthrobacter globiformis** *Actinomyces* sp.****	*Pseudomonas* sp.*** *Mycobacterium roseum,* *Bacillus megaterium**** *Bacillus circulans* *Arthrobacter globiformis**
CHha+ non-GM hybrid corn	*Pseudomonas* sp.*** *Bacillus megaterium, **** *Bacillus circulans**** *Arthrobacter globiformis** *Actinomyces* sp.**	*Pseudomonas* sp.*** *Bacillus megaterium*** *Bacillus circulans*** *Arthrobacter globiformis*** *Actinomyces* sp.**	*Pseudomonas* sp.*** *Bacillus megaterium**** *Bacillus circulans*** *Arthrobacter globiformis*** *Actinomyces* sp.**
CHha+ GM hybrid corn	*Pseudomonas* sp.*** *B. megaterium**** *B. circulans, A. citreus* *Arthrobacter globiformis****	*Pseudomonas* sp.*** *Bacillus megaterium**** *Arthrobacter globiformis**** *Arthrobacter citreus,* *Actinomyces* sp.**	*Pseudomonas* sp.*** *B. megaterium**** *A. globiformis**** *A. citreus** *Actinomyces* sp.

CONCLUSIONS

1. Research on the impact of Bt technology applied to transgenic maize MON 810 on the main soil parameter quality were conducted in green house, using three contrasting soils in terms of clay content and reaction: Eutric Fluvisols, Fluvi-eutric Cambisols and Haplic Chernozems.
2. Soil chemical parameters: pH, humus and total nitrogen contents, the contents of mobile phosphorus and mobile potassium revealed significant differences between soil types only, not between the two types of maize hybrids, GM and non-GM, used in experiment.
3. Also, quantitative determinations of heterotrophic bacteria in the soil did not reveal significant differences between variants cultivated with GM corn as compared with those cultivated with non-GM hybrid nor in any of the soils, and even between stages of determination.
4. No major differences were recorded between species composition of bacterial communities in soil cultivated with non-GM hybrid compared to soil cultivated with transgenic hybrid, the number of bacterial strains being quite close in samples analyzed.
5. Research will be continued and data from chemical and biological analysis of soil will be correlated with determinations regarding the amount of insecticidal toxin Cry1Ab released into soil (through root exudates or plant debris along with the remaining plants in the soil after harvesting) and its persistence in the three soils chosen to investigate the impact of Bt technology on soil as a major component of the environment.

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