TESTBIOTECH Background 25 – 5 - 2018

Testbiotech comment on EFSA GMO Panel, 2018, Scientific opinion on the assessment of genetically engineered maize MON 87403 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-BE-2015-125)

Testbiotech e. V.
Institute for Independent Impact Assessment in Biotechnology

Christoph Then & Andreas Bauer Panskus

Maize MON87403 is genetically engineered to increase biomass and yield through insertion of a truncated gene sequence derived from another plant species (*Arabidopsis thaliana).* This produces a protein (AtHB17Δ113) that can bind at a specific DNA sequence and thereby influence the expression of several genes in the plants. The aim is to increase the size and biomass of the ears (which become the corn cob for harvest).

The basic mechanism as explained by Monsanto (Rice et al., 2014) is that the newly expressed protein will act in competition with a similar natural protein, which is characterised as a HD-Zip II transcription factor. This natural protein is involved in growth control of the ears: it is meant to control gene regulation and growth of the plants so that the size of the ears is 'normal'. Both proteins, the natural and the newly produced protein, are binding at the same DNA site. By outcompeting the natural protein at this specific binding site, its controlling effect is diminished. Therefore, the ears of corn supposedly grow faster. The supposition is that maize MON87403 maize plants will produce more kernels and have a higher biomass. However, as the available data show, the effects are small and inconsistent.

Molecular data

The comments from the experts of the Member States (EFSA, 2018b) show that much more detailed information would be needed to understand the exact molecular mechanisms involved in the expression of AtHB17Δ113, which influences ear biomass at an early development stage and, therefore, potentially the yield at harvest. For example, experts from the German authority BVL (EFSA, 2018b) request, "*in this regard molecular mechanism, genes regulated and the role of environmental factors should be addressed.*"

In addition, experts from the German Federal Agency for Nature Conservation (BfN) spell out some unknowns in detail:

> *"Currently the mechanisms that regulate the transcriptional activity of HD-Zip I and HD-Zip II transcription factors in vivo are largely unknown (Harris et al. 2011, Turchi et al. 2015). It has been shown however that environmental and stress conditions such as water status, light conditions, nutrient status, temperature and the concentration of toxic compounds play a crucial role in its regulation. In addition evidence is emerging that these transcription factors are integrated in phytohormone-regulated developmental networks. (Harris et al. 2011). Thus for the characterization of the genetic modification plant material from different growth conditions should be examined and the mode of action of the trait should be characterized."*

Indeed, the most relevant publication prepared with experts from Monsanto and Dupont (Rice et al., 2014) does not answer crucial questions regarding the underlying mechanisms. This publication shows that the expression of several plant genes and related proteins are changed in the genetically engineered maize. Some of these natural proteins have similarity with heat shock proteins, others are regulatory proteins; some are involved in cell wall organisation or are just of hypothetical nature. The exact mechanisms causing the supposedly intended effects and their possible side effects remain a matter of uncertainty and non-knowledge. As the authors conclude: "*It is not yet clear what role these proteins may play in ear growth and development in maize.*" Further, the overall effects are described as minor: "*Overall, the observed effects of AtHB17Δ113 on the maize ear inflorescence and ear transcriptome were very small.*"

Furthermore, the outcome of the field trials shows that the observed effects were not only small, but also inconsistent. As an analysis of the data from the field trials shows, ear biomass and kernel weight developed differently and were dependent on the specific site of the field trial. In several of the field trials, no statistically significant effects could be observed in regard to the expected effects. EFSA (2018a) summarises:

> *"The GMO Panel acknowledges that the change due to the intended trait is known to be of limited amplitude, and that the AtHB17Δ113 protein is expressed in maize MON 87403, which suggests that the manifestation of the trait may depend on environmental conditions in the field trials."*

In conclusion, risk assessment on a molecular level shows several major uncertainties regarding the intended molecular mechanisms and unintended changes in biochemical pathways. Furthermore, gene expression depends on environmental conditions. No data were provided on whether gene expression also depends on the genetic background of the specific varieties.

Risk assessment cannot be concluded under these circumstances. To reduce uncertainties, the plants should be investigated under a wide range of defined environmental conditions taking into account potential extreme stress conditions, such as those caused by ongoing climate change. In addition, more varieties should have been included in the trials since it is known that the genetic background of the varieties can influence the level of expression of any inserted genes. Furthermore, much more data would be needed to assess the effects of the additional DNA on the genome of the plants, the transcriptome, proteome and metabolome.

Comparative assessment (for compositional analysis and agronomic traits and phenotype)
As EFSA (2018a) points out, the data from the field trials indicate that the magnitude of the effects is dependent on environmental factors.

However, the maize was only grown for one year and all the field trials were carried out in the US, leaving aside other important maize producing countries. As a result, the plants were grown under a too narrow range of environmental conditions that do not allow any conclusions to be drawn upon the quality and safety of the plants under different conditions, such as those related to climate change. Furthermore, according to EFSA, the data on biotic and abiotic stressors occurring during the field trials were not statistically analysed.

Remarkably, EFSA raises the question of whether the data from these trials can be used for risk assessment at all, since the intended effects were only observed in some of the field trials:

> *"Based on the provided data, four out of seven sites from which samples were taken for the compositional analysis, phenotypic manifestation of the intended trait was realised. For*

these sites, the ear biomass (at the R1 or R6 stage) [explanation by Testbiotech: these are specific stages of growth] was higher. However, only for one site the increase in ear biomass was statistically significant at the R1 and R6 stages, which raised the question on whether compositional data obtained from the field trials would allow a thorough risk assessment."

Indeed, the relatively low number of differences in plant composition derived from comparison with the conventional plants might be due to failure of the intended additional gene function.

In conclusion and in awareness of the uncertainties in the assessment on the molecular level, the risk assessment cannot be completed. Any assumptions that products derived from MON87403 do not show unintended effects that would raise safety concerns are not based on sufficient evidence. In light of the facts, EFSA´s final conclusions on comparative assessment are nothing more than a kind of guessing game in a situation of profound non-knowledge:

"The GMO Panel concludes that the agronomic, phenotypic and compositional analysis did not identify issues requiring further assessment regarding food and feed safety and its environmental impact."

Toxicology

The maize used for the 90-day feeding study was not sufficiently assessed in regard to its biological characteristics and the magnitude of the intended effects. It is unclear whether the maize used in the diet is representative for the products that might be derived from MON87403 grown under practical conditions.

Consequently, the data provided from feeding studies cannot be considered to be sufficient to show the safety of the product.

Allergenicity

No tests were conducted to assess whether the concentration of known maize allergens was increased due to the insertion of the additional gene construct. Since the introduced trait interferes with the plants metabolism on several levels, more data are needed to show to which extent the maize is changed in regard to its allergenic properties.

Nutritional Assessment

The maize used for the feeding study with poultry was not sufficiently assessed in regard to its biological characteristics and the magnitude of the intended effects. It is unclear whether the maize used in the diet is representative for products that might be derived from MON87403 grown under practical conditions.

Consequently, the data provided from the feeding studies cannot be considered to be sufficient to show the nutritional quality of the product.

Environmental risk assessment

EFSA discusses the risk of gene flow from maize MON87403 to teosinte plants. These plants have been found growing in Spain for more than a decade and are wild relatives (ancestors) of cultivated maize. Depending on the subspecies of teosinte, gene flow is more or less likely to occur. However, the subspecies occurring in Spain has not been fully identified and seems to be a hybrid between maize and teosinte. Its actual potential for gene flow with maize in the fields is not known Trtikova et al. (2017).

MON87403 is not allowed for cultivation in the EU, however, spillage of imported kernels might lead to spontaneous transgenic plant populations. Pollen from these plants – under some circumstances - could enable gene flow to teosinte plants.

Without having any data on gene expression and possible effects on teosinte plants, EFSA (2018a), nevertheless, indicated that gene flow from maize MON87403 to teosinte would not cause problems because, as yet, teosinte has only been observed in the fields and not outside cultivated fields.

> *"Vertical gene transfer from maize is limited to Zea species. Wild relatives of maize outside cultivation are not known/reported in Europe (…). Therefore, potential vertical gene transfer is restricted to maize and weedy Zea species, such as teosintes and/or maize-teosinte hybrids, occurring in cultivated areas."*

A paper published by Devos et al. (2018) was one of the publications used for the EFSA assessment. It was written by Yann Devos, who works for EFSA, together with Alan Raybould, who works for biotech company Syngenta, which sells genetically engineered maize. Other experts from EFSA were also involved, including Antoine Messéan, Jeremy Sweet and Elisabeth Waigmann. It is remarkable that Devos, Sweet and Messéan were also involved in the risk assessment of MON87403. There are clearly several reasons why this kind of expert involvement needs to be regarded as a conflict of interest.

Whatever the case, the assumptions of EFSA and of Devos et al. (2018) are biased and lead to the wrong conclusions. Devos et al. (2018) acknowledge that currently there is no "*information of the expression of the transgenes in the hybrid plants*". They do not deem such data to be necessary. Instead, they simply state that a "*worst-case assumption is that any teosinte × GM maize hybrids will express/manifest the traits that the transgenes confer*".

Thus, these experts assume that once the transgenes have escaped to teosinte they will somehow preserve the intended biological trait originally inserted. They seem to think of the transgene as an inert BioBrick, which has a predictable function that is independent of the rest of the organism and its interaction with the environment. This is wrong. For example weedy rice, derived from Bt rice (Cao et al., 2009) and from glyphosate resistant rice (Fang et al., 2018) is known to show enhanced fitness that is not related to the intended trait.

Currently, there are neither EFSA guidelines nor methods for making detailed assessments of the risks associated with genetically engineered plants emerging from unintended crossings and next generation effects. Risk assessment as performed by EFSA only considers genetically engineered plants that are grown for just one season and are re-sown every year.

Devos et al. (2018) try to escape this factual complexity by stating that risk assessment "*focuses the assessment on the phenomena that are important for decision-makers and away from the multitude of other changes that may interest scientists, but which are irrelevant for ERA*". This approach is clearly failing by design: In many cases, there is no clear cut difference between environmental risk assessment (ERA) and basic research on the biological characteristics of genetically engineered plants.

Genetically engineered plants are mostly grown for just one season and re-sown every year. This enables the company to check the seeds in regard to their most relevant economic characteristics before they go into the fields. However, potential teosinte × GM maize hybrids and their offspring will not undergo any additional quality or safety checks before they appear in the fields. Instead, they are simply new, untested, never risk assessed transgenic plants. Therefore, they cannot be

allowed to emerge and persist in the environment. This problem does not depend on the question of whether teosinte will spread beyond sites of agricultural production.

Therefore, the ERA of EFSA has to be rejected due to significant methodological flaws and due to the bias caused by conflicts of interest.

Others
Metabolic pathways which interfere with plant growth are multifunctional and complex. They are connected to plant characteristics such as stress reactions, fitness and composition of the plants constituents. Under these circumstances, risk assessment has to be driven by the hypothesis that the biological characteristics of the plants as a whole will be changed by the genomic intervention.

The risk manager and the risk assessor need to be aware of these challenges. The pending application should be stopped and a comprehensive methodology of risk analysis for this category of plants should be developed.

Conclusion and recommendations:
The EFSA opinion does not identify the true range of uncertainties and the current limits of knowledge. The risk manager should therefore reject this opinion and not allow import of maize MON87043.

References:
Cao, Q.-J., Xia, H., Yang, X., Lu, B.-R. (2009) Performance of Hybrids between Weedy Rice and Insect-resistant Transgenic Rice under Field Experiments: Implication for Environmental Biosafety Assessment. J. Integr. Plant Biol. 51, 1138–1148. https://doi.org/10.1111/j.1744-7909.2009.00877.x

Devos, Y., Ortiz-García, S., Hokanson, K.E., Raybould, A. (2018) Teosinte and maize × teosinte hybrid plants in Europe−Environmental risk assessment and management implications for genetically modified maize. Agric. Ecosyst. Environ. 259, 19–27. https://doi.org/10.1016/j.agee.2018.02.032

EFSA GMO Panel (2018a) Scientific Opinion on the assessment of genetically modified maize MON 87403 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-BE-2015-125). EFSA Journal 2018;16(3):5225, 28 pp https://doi.org/10.2903/j.efsa.2018.5225

EFSA GMO Panel (2018b) Comments from the experts of the EU Member States on the Scientific Opinion on the assessment of genetically modified maize MON 87403 for food and feed uses, import and processing, under Regulation (EC) No 1829/2003 (application EFSA-GMO-BE-2015-125). EFSA Journal 2018;16(3):5225, 28 pp, accessed via the register of EFSA, http://registerofquestions.efsa.europa.eu/roqFrontend/login?1

Fang, J., Nan, P., Gu, Z., Ge, X., Feng, Y.-Q., Lu, B.-R. (2018) Overexpressing Exogenous 5-Enolpyruvylshikimate-3-Phosphate Synthase (EPSPS) Genes Increases Fecundity and Auxin Content of Transgenic Arabidopsis Plants. Front Plant Sci. 9, 233. https://doi.org/10.3389/fpls.2018.00233

Harris, J.C., Hrmova, M., Lopato, S., Langridge, P. (2011) Modulation of plant growth by HD-Zip class I and II transcription factors in response to environmental stimuli. New Phytologist 190, 823–837. https://doi.org/10.1111/j.1469-8137.2011.03733.x

Rice, E.A., Khandelwal, A., Creelman, R.A., Griffith, C., Ahrens, J.E., et al. (2014) Expression of a Truncated ATHB17 Protein in Maize Increases Ear Weight at Silking. PLoS ONE 9(4): e94238. https://doi.org/10.1371/journal.pone.0094238

Trtikova, M., Lohn, A., Binimelis, R., Chapela, I., Oehen, B., Zemp, N., Widmer, A., Hilbeck, A., (2017) Teosinte in Europe – Searching for the Origin of a Novel Weed. Sci. Rep. 7, 1560. https://doi.org/10.1038/s41598-017-01478-w

Turchi, L., Baima, S., Morelli, G., Ruberti, I. (2015) Interplay of HO-Zip II and III transcription factors in auxin regulated plant development. Journal of Experimental Botany, 66(16): 5043-5053. https://doi.org/10.1093/jxb/erv174