TESTBIOTECH Background 17 - 7 - 2021

Testbiotech comment on EFSA GMO Panel Scientific Opinion on genetically engineered oilseed rape 73496 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2012-109) from Pioneer

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

Christoph Then & Andreas Bauer-Panskus

Introduction

Oilseed rape 73496 is genetically engineered to be herbicide-resistant to glyphosate. The oilseed rape expresses the glyphosate acetyltransferase protein GAT4621. Glyphosate inhibits the enzyme enolpyruvulshikimate-3-phosphate synthase (EPSPS), which is involved in the biosynthesis of aromatic amino acids. GAT proteins acetylate glyphosate giving rise to N-acetyl glyphosate, which has no herbicidal activity.

Implementing Regulation 503/2013 was not applied in this case.

Molecular characterisation

So-called open reading frames (ORF), which can give rise to various new gene products, were identified at the sites of insertion. The relevant DNA sequences were only assessed for potential new proteins and not for other biologically active DNA products, such as non-coding (nc) RNA.

Newly produced ncRNAs may cause RNAi effects on gene regulation within the intestinal microbiome, and also in mammalian cells after being taken up from the gut. In its reply to experts from Member States (EFSA, 2021b), EFSA declared this issue to be not relevant, but did not give a detailed assessment.

An inversion of a larger region of a chromosome was observed that was most probably due to the method of genetic engineering (biolistic transformation). This inversion affects the function of a gene involved in the functions of glycolytic enzymes needed for autotrophic growth in plants (it belongs to the gene family of triose phosphate transporter, tpt). It is unclear to which extent other gene copies can compensate for this function under environmental stress conditions, e.g. those caused by climate change. No data were made available to explore this issue although it is also relevant for the assessment of the phenotype and plant composition.

Moreover, the expression data for the newly introduced genes did not take into account the range of stressors, the higher rate of herbicide applications or any of the relevant bioclimatic regions that these plants will be exposed to in the countries where they are cultivated. Therefore, the data are inconclusive. This also affects assessment of the phenotype and plant composition, and raises the question to which extent amino acids are acetylated (see below).

Conclusion - molecular characterisation and gene expression

To gather reliable data on gene expression and functional genetic stability, the plants should have been subjected to a much broader range of defined environmental conditions and stressors. They should, in addition, have been tested in all relevant bioclimatic regions where the plants will be grown. EFSA should have further requested the applicant to submit data from field trials representing current agricultural practices, including high rates of spraying with the complementary herbicides.

In summary, the oilseed rape tested in field trials does not sufficiently represent the imported kernels and products. Consequently, the data presented by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental (stress) factors and herbicide applications. They are also insufficient to conclude on the impact that different genetic backgrounds have on gene expression and plant metabolism.

Based on the available data, no final conclusions can be drawn on the safety of the plants. Therefore, the data do not fulfill the requirements of Regulation 1829/2003.

Comparative assessment of plant composition and agronomic and phenotypic characteristics

Field trials were only performed in the US and Canada for just one year to generate the data on plant composition and for assessment of agronomic and phenotypic characteristics (EFSA, 2021a). Nevertheless, a large number of significant differences in comparison to the conventionally bred plants were identified.

Agronomic and phenotypic characteristics

Only a very low number of criteria (12) were assessed by the applicant, 5 of them showed significant differences if the plants were sprayed with the complementary herbicide (4 if the plants were not sprayed). While the number of statistically significant differences was found to be low, this would probably have been higher if the plants had been exposed to a sufficiently broad range of stressors.

In awareness of the unintended genetic changes (inversion of gene sequences, impacting important gene functions), it is likely that the plants, if grown in a wider range of environmental conditions, would exhibit more substantial and also more significant differences. Phenotypic changes due to the deficiency of tpt gene activity may, for example, impact growth, biomass and yield. At least two of the parameters (flowering duration and plant height) indicate possible growth retardation.

Therefore, without further trials, the data on agronomic and phenotypic characteristics are inconclusive.

This assumption is also reinforced by germination tests which showed substantial differences (lower rates in germination) between the seeds produced by the GE oilseed rape compared to conventionally bred plants. These differences were dependent on the temperature. The reason for the differences in germination was not investigated. Seed dormancy was not assessed.

Plant composition

Of 103 compounds which were subjected to statistical analysis to assess changes in plant composition, 53 were significantly different in plants not sprayed with the complementary herbicide compared to 56 in those sprayed with glyphosate. Several differences were considered to be outside the range of expected values, especially in regard to acetylated derivatives of several amino acids. Concentrations of N-acetylaspartate (NAA), N-acetylglutamate (NAG) and N-acetylthreonine (NAT) were much higher compared to data from conventionally bred plants. This was explained as a side effect of the additionally inserted gene sequences and enzymes produced thereof. These enzymes are known not only to intentionally acetylate glyphosate, but also to unintentionally acetylate amino acids.

As a result, the GE plants are not comparable to their conventional counterparts and must undergo a much more detailed risk assessment, which should include the systemic impact on plant metabolism. A further factor supporting the case for more detailed risk assessment is the fact that the gene insertion process caused the loss of function of an important gene. However, no further omics data were presented.

It is also concerning that no data were provided on the concentration of N-acetyl glyphosate. These data are meant to show there is no longer any herbicidal activity; they are nevertheless also relevant for toxicity assessment (EFSA, 2009).

Finally, the data that were presented did not take into account the cultivation of the GE oilseed rape in all relevant producing countries or cultivation in more extreme climate conditions, e.g. due to the effects of climate change. The range of differences and their significance are likely to be substantially increased if the plants are exposed to a wider range of regional and environmental conditions. In addition, EFSA should have requested the applicant to submit data from field trials, including several sprayings with higher dosages of the complementary herbicide.

Conclusion on comparative assessment of plant composition and phenotypic and agronomic characteristics

The data provided show that the GE oilseed rape plants are quite different to their conventional comparator. Therefore, much more data should have been requested, including on the systemic effects of genetic and metabolic differences.

Furthermore, the data provided by the applicant and accepted by EFSA are insufficient to conclude on the impact of environmental factors, herbicide applications and genetic background on gene expression, plant metabolism, plant composition or agronomic and phenotypic characteristics.

To gather reliable data on compositional analysis and agronomic characteristics, the plants should have been subjected to a much broader range of defined environmental conditions and stressors. Whatever the case, they should have been tested in all relevant bioclimatic regions to which these plans will be exposed in the countries where they are cultivated.

Furthermore, EFSA should have requested the applicant to submit data from field trials representing current agricultural practices, including higher rates of spraying with the complementary herbicides.

In summary, the GE oilseed rape tested in field trials do not sufficiently represent the imported kernels and products. Consequently, the data presented by the applicant and accepted by EFSA are insufficient to conclude on the impact environmental factors, herbicide applications and different genetic backgrounds will have on plant composition and agronomic characteristics.

Based on the available data, no final conclusions can be drawn on the safety of the plants. Therefore, the data do not fulfill the requirements of Regulation 1829/2003.

Toxicology

Acetylated derivatives of several amino acids are, for example, known to be involved in brain and kidney disorders. Furthermore, a previous 90-day rat study reported an impact on the salivary glands in both male and female rats orally exposed to 500 mg NAA/kg bw.

Nevertheless, EFSA believes that the data from risk assessment in combination with existing data on consumption habits do not raise health concerns.

EFSA (2021a) only suggests carrying out post-market monitoring (PMM) which should be focused on the "*collection of import data to Europe of oilseed rape 73496 and/or its products, entering the food and feed supply chains. If imports are identified, consumption data should be collected for humans and animals (e.g. through dietary surveys) on oilseed rape 73496 and/or its food and feed products to confirm the predicted consumption data and to verify that the conditions of use are those considered during the pre-market risk assessment.*"

EFSA is referring here to data provided by the applicant which are, however (see above), unlikely to sufficiently represent the true range of acetylated derivatives of amino acids in the harvested kernels and derived products. Therefore, the EFSA calculation on uncertainties and limits of exposure are based on insufficient data and hence not reliable. This will also undermine the reliability of the planned PMM.

Furthermore, EFSA (neither the GMO panel nor the pesticides panel) does not present any risk assessment data on N-acetyl glyphosate, which is meant to show there is no longer any herbicidal activity, but is also relevant for toxicity assessment of the products derived from the GE oilseed rape. While these data were made available for maize and soybean (EFSA, 2009), they seem to be absent for GE oilseed rape.

Therefore, safety of the GE oilseed rape kernels and related products at the stage of consumption is not sufficiently demonstrated.

Environmental risk assessment

Transgenic oilseed rape is known to be established independently from cultivation in countries such as Canada, the US, Japan, Australia and Switzerland (Bauer-Panskus et al., 2013) and more recently in Argentina (Pandolfo et al., 2018). Oilseed rape (*Brassica napus*) can spread via pollen and seeds, and seeds can remain viable in the soil for more than ten years (seed dormancy). Europe is the centre of origin and genetic diversity for the group of Brassica plants to which oilseed rape belongs. Some native plant populations, such as *Brassica rapa* (turnip), can hybridise with oilseed rape. *Brassic napus* itself occurs mainly as a cultivated plant, but still maintains significant characteristics of a wild plant. Disturbed soil promotes the establishment of *Brassica napus* beyond the fields, whereas dense vegetation will hinder establishment. However, *Brassica napus* growing in the wild is found primarily in habitats where wild relatives of the Brassica genus and related genera grow. In addition, many related species which can hybridise with oilseed rape occur in environments such as road verges, industrial or feral sites. Gene flow to wild relatives is possible and likely to happen, even if *Brassica napus* itself only has a reduced potential to spread in a densely vegetated environment (Bauer-Panskus et al., 2013).

The plants are mostly pollinated by insects, such as flies, honey bees and butterflies, which can also carry the pollen over many kilometers. Wind is also relevant for pollen drift: the farthest pollen-mediated outcrossing distance measured to date is 26 kilometres, recorded in a field trial with sterile male plants (Ramsay et al., 2003). Furthermore, the seeds remain viable in the soil for more than ten years (Lutman et al., 2003). Consequently, oilseed rape has a high potential for volunteer plants even many years after the first sowing.

Oilseed rape can appear in ruderal populations along field edges and roadsides. Pivard et al. (2008) found that ruderal populations are self-sustaining in a semi-permanent form. In Japan, GE oilseed rape from imports was found over a period of ten years near transportation roads (Nakajima et al., 2020). According to a recent study, these plants show considerable diversity, as they may have hybribized with nearby GE and non-GE rapeseeds, "*resulting in a broad diversity of GM feral populations*" (Chen et al., 2020).

According to Munier et al. (2012), herbicide tolerant oilseed rape is a weed. There are weedy forms of *B. rapa* and *B. olereracea*. The wild relative species *Sinapis arvensis*, *Raphanus raphanistrum* and *Hirschfeldia incana* are also considered to be weeds (OECD, 2012). Recent science shows that also gene flow rates between *B. napus* and relatives like black mustard (*B. nigra*) are higher than previously assumed and may have been underestimated in risk assessment (Marotti et al. 2020).

It cannot be ruled out that the plants will persist in the environment after spillage and start to propagate. This would allow next generation effects to emerge that were neither assessed by the applicant nor by EFSA (Bauer-Panskus et al., 2020). Therefore, the EU Commission should not allow the import of viable kernels.

Others:

Implementing Regulation 503/2013 came into force in December 2013. While this application was filed before then, the Regulation, after such a long period of time, should nevertheless have been applied to avoid risk assessment based on outdated data and insufficient standards. It should not be overlooked that Regulation 1829/2003 only allows market access (without any possibility of deviating) only "*after a scientific evaluation of the highest possible standard.*" (Recital 9 of Regulation 1829/2003)

Conclusions:

Importing viable kernels of oilseed rape 73496 cannot be allowed. Furthermore, the application has to be rejected since the safety of food and feed products derived from the kernels was not demonstrated.

References:

Bauer-Panskus, A., Breckling, B., Hamberger, S., Then, C. (2013) Cultivation-independent establishment of genetically engineered plants in natural populations: current evidence and implications for EU regulation, Environmental Sciences Europe, 25: 34. https://doi.org/10.1186/2190-4715-25-34

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(1): 1-15. https://doi.org/10.1186/s12302-020-00301-0

Chen, R., Shimono, A., Aono, M., Nakajima, N., Ohsawa, R., Yoshioka, Y. (2020) Genetic diversity and population structure of feral rapeseed (Brassica napus L.) in Japan. Plos one, 15(1): e0227990. https://doi.org/10.1371/journal.pone.0227990

EFSA (2009) Modification of the residue definition of glyphosate in genetically modified maize grain and soybeans, and in products of animal origin on request from the European Commission. EFSA J, 7(9): 2009. https://doi.org/10.2903/j.efsa.2009.1310

EFSA (2021a) Scientific Opinion on the assessment of genetically modified oilseed rape 73496 for food and feed uses, under Regulation (EC) No 1829/2003 (application EFSA-GMO-NL-2012-109). EFSA J, 19(6): 6610. https://doi.org/10.2903/j.efsa.2021.6610

EFSA (2021b) Application , Comments and opinions submitted by Member States during the three-month consultation period. OpenEFSA portal, https://open.efsa.europa.eu/questions/EFSA-Q-2012-00617

Lutman, P.J.W., Freeman, S.E., Pekrun, C. (2003) The long-term persistence of seeds of oilseed rape (Brassica napus) in arable fields. The Journal of Agricultural Science, 141(2): 231-240. https://doi.org/10.1017/S0021859603003575

Marotti, I., Whittaker, A., Benedettelli, S., Dinelli, G., Bosi, S. (2020) Evaluation of the propensity of interspecific hybridization between oilseed rape (Brassica napus L.) to wild-growing black mustard (Brassica nigra L.) displaying mixoploidy. Plant Science, 296: 110493. https://doi.org/10.1016/j.plantsci.2020.110493

Munier, D.J., Brittan, K.L., Lanini,W.T. (2012) Seed bank persistence of genetically modified canola in California. Environmental Science and Pollution Research, 19(6): 2281-2284. https://doi.org/10.1007/s11356-011-0733-8

Nakajima, N., Nishizawa, T., Aono, M., Tamaoki, M., Saji, H. (2020) Occurrence of spilled genetically modified oilseed rape growing along a Japanese roadside over 10 years. Weed Biol Manag, 20(4): 139-146. https://doi.org/10.1111/wbm.12213

OECD (2012) Consensus Document on the Biology of Brassica crops (Brassica spp.). Organisation for Economic Co-operation and Development. https://www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2012)41&doclanguage=en

Pandolfo, C. E., Presotto, A., Carbonell, F. T., Ureta, S., Poverene, M., & Cantamutto, M. (2018) Transgene escape and persistence in an agroecosystem: the case of glyphosate-resistant Brassica rapa L. in central Argentina. Environmental Science and Pollution Research, 25(7): 6251-6264. https://doi.org/10.1007/s11356-017-0726-3

Pivard, S., Adamczyk, K., Lecomte, J., Lavigne, C., Bouvier, A., Deville, A., Gouyon, P.H., Huet, S. (2008) Where do the feral oilseed rape populations come from? A large-scale study of their possible origin in a farmland area. J Appl Ecol, 45: 476-485. https://doi.org/10.1111/j.1365-2664.2007.01358.x

Ramsay, G., Thompson, C., Squire, G. (2003) Quantifying landscape-scale gene flow in oilseed rape. Final Report of DEFRA Project RG0216: An experimental and mathematical study of the local and regional scale movement of an oilseed rape transgene. www.scri.ac.uk/scri/file/EPI/Agroecology/Landscape_scale_geneflow_in_oilseed_rape_rg0216.pdf