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Review of Bachelor thesis „Traditional plant breeding, advanced biotechnological engineering with emphasis on GM crops and GM environmental risk assessment” by Klára Kopicová

The bachelor thesis is written in a form of literature review, which is combined with a proposal of assessing the risk of horizontal gene transfer from GMO plants to the environment. The title is concise and corresponds to the subject described in the thesis. Author describes the traditional and advanced plant breeding techniques, positive and negative opinions toward GM plants, followed by a proposal of environmental risk assessment of unwanted horizontal gene transfer. The aim of the thesis is clearly stated. Thesis is written in a standard form and includes all sections recommended for scientific writing, however I would prefer the word „abstract“ instead of „preface“ in the beginning of the thesis. What is missing in the Introduction is a description of the mechanism and examples of the horizontal gene transfer. Considering that the main goal of the proposal is assessing the risk of *cry* gene transfer from GMO plants to arthropods, introducing this subject is vital. Thesis is summarized at the end by clear conclusions. The thesis is much improved by comparison to the earlier version. The proposal written by Author is much more coherent and clear. Author depicts goals and methods clearly and discusses potential problems later in the discussion section. The project, with some modifications, could presumably be used in practice. However I have some comments toward the planned experiments, which could be answered during the defence.

1. Is there any evidence that the horizontal gene transfer exists in eucaryotes? How do you imagine, that *cry* toxin gene is transferred from plants to the invertebrates, what would be the mechanism? In the section **Stated hypotheses** in the paper that you cite, mostly speculations are provided. Are there examples where non-GM plants were studied?
2. GM plants are grown for several decades already. Are there really no field studies evaluating the possibility of the horizontal gene transfer?
3. Why only non-GM plot would be treated with pesticides? Why should be at all? If treated, this plot cannot be used as proper control for experiment.
4. The wages for the full time experienced scientist are a little bit low – if I calculate correctly it is around 18 000 CZK per month.
5. As a bonus question - could Author shortly explain how transgenesis is achieved in plants? For example, how to insert *cry* toxin gene into wheat.

Formal assasment of the thesis:

The major disadvantage of the thesis is the description of methods used for genetically modified plants production in the section Advanced biotechnological engineering, which shows that the Author lacks completely understanding of the subject, for example what transgenesis is. There are several essential errors concerning description of buildup of genetic constructs, or Zinc Finger Nucleases and information provided there are just false. CRISPR/Cas9 is described in a chaotic way, where it is partially shown how it works in bacteria, not how it is used for genome editing in plants or animals.

The thesis is written in a mixed styles of languages (scientific and non-scientific) with the style prevailing for popular science. Sometimes the composition of the sentences or choice of words does not allow for understanding of the meaning of the sentence, or it can even provide false information like that „in

potatoes, there is natural accumulation of carcinogenic acrylamide“. In many cases sentences are lacking factual detailed information about results obtained by scientists. For example „There are also several publications that found serious negative effect of GM crops or connected with GM crops“, where is no details provided about what negative effects were found.

Thesis is written grammatically, however sometimes, as mentioned before, the word selection is poor, affecting the understanding of the meaning. Several typos and editorial errors were found, but the amount does not diminish the value of the work.

Figures and tables are properly described and cited in the text.

The Author is using all kind of available literature, books, publications and web pages including Czech and English sources, which are mostly properly chosen. Publications are from verified journals like Science as well as from web pages which are publishing popular science (for example Ujj, 2016, which is published on a web-page not in peer-reviewed journal and show sociological, not biological point of view). Author is sometimes citing the source just to state the obvious fact, which should be known already for a Biology student, for example that DNA is composed of A,T,G,C (Jobling et al., 2014) or Epigenetic is a next factor that can further significantly affect the phenotype (Pikaard & Scheid, 2014) or that „before the laws of heredity were defined, natural crossing was the only possible way of breeding (Roberts 1929). Some information lack citations.

I think that publications, web page links and Acts concerning GMO plants should be put in 3 different subgroups in the Literature section. Author is using information from links which do not work anymore. for example isaaa.org/gmapprovaldatabase/croplist is not available.

Examples impossible to understand:

- „If facing on stress (especially diseases), modern cultivars (monomorphic for most of their genes) are unable to adjust adequately (Bressegello & Coelho, 2013)“.

Comment: This sentence is understandable – however then follows another sentence:

- „With that said, the modern breeding methods basically suppress the sensitiveness in cultivars making them intact“.

Comment: To me does not make any sense.

- „Those of actively designed anti-insecticidal crops formatted from *B. thuringiensis* have been processing to face on commercial biological pesticides, and further re-evaluating from the environmental point of view for over 50 years (Koch et al., 2015)“.

- „They found serious negative effect of management connected with growing herbicide tolerant corn – application of glyphosate that was served in the diet to rats“.

Example of false information

- „Whereas the "traditional" GM hybrids are formed by the method of transgenesis, the newly available New Plant Breeding Techniques rather display a higher success in gene transfer in comparison with transgenesis“.

Comment: The process of introducing an exogenous gene is called transgenesis. So insertion of a new gene by homology directed repair after ZFN or TALEN or CRISPR or just recombination is transgenesis.

- “the target specific part recognizes its binding motif and based on the compatibility associates with the DNA template, the spacer causes the catalytic reaction which ends with site-specific DSBs.”
- “ZFN domains bind randomly (only every 200bps), but the consequently produced DSBs also happen randomly”

Comment: both above statements are just not true.

Lack of actual information

- „Opponents of GM technology often argue that introduction of GM crop raised up the numbers of suicides enormously in India“.

Comment: I don't see what is the connection between those two facts.

- „In case of on-whole-plant-feeding herbivores matters if the concentration of Cry toxin reaches the higher levels, e. g. larvae of pest *Spodoptera littoralis*... “

Comment: What does it mean „matters“? What is the effect?

Summarizing:

The thesis meets the requirements for bachelor's theses submitted to the Faculty of Science of the University of South Bohemia, and therefore I recommend it for defense.

The work is evaluated by the classification level:

DOBŘE

Datum: 30.06.2020

Podpis oponenta:

