

## **Evaluation Form PhD thesis**

Name PhD student: Dobrinka Anastasova Balabanova-Ivanovska

Name supervisors: Prof. dr. Andon Vassilev, Agricultural University Plovdiv, Prof. dr. Jaco Vangronsveld, Hasselt University.

Name co-supervisor: Prof. dr. Ann Cuypers, Hasselt University.

Title PhD Thesis: " Physiological responses of sunflower Clearfield hybrids to the herbicide imazamox "  $^{\prime\prime}$ 

Member of the jury: Prof. dr. Jaco Vangronsveld (by order of the rector of the AUP  $N^{\circ}$  RD-16-1010 / 24.10.2016)

## Please give your general remarks, minor and/or major comments

General remarks

The candidate presents the results of an original investigation on the "Physiological responses of sunflower Clearfield hybrids to the herbicide imazamox".

A special merit of this work is that the investigations were performed at different biological organization levels, from the molecular responses up to morphological ones. The fact that the work included molecular, biochemical, biophysical and physiological aspects made it very challenging. It is clear that the candidate had to solve many practical and technical problems that were occurring during her work. Therefore, she had to acquire the necessary theoretical background and very carefully plan the different experiments.

In a first chapter, the tolerance of a number of sunflower Clearfield hybrids to the herbicide imazamox was evaluated by assessing the modulation of the photosynthetic performance of plants treated with the recommended field dose and double dose of the herbicide. Different levels of tolerance to the herbicide imazamox were observed in between the tested cultivars. The cultivar Mildimi, being the most tolerant hybrid, was selected for further experiments.

In a next chapter it was investigated to what extent specific and non-specific defense mechanisms are involved in the responses of sunflower plants to the herbicide imazamox. A comparison of the responses of IMI-resistant and IMIsensitive sunflower hybrids was made 24 h after application of imazamox. Several differences were observed in the fast stress responses and defense mechanisms in both hybrids. In the IMI-sensitive sunflower hybrid non-specific mechanisms such as the antioxidative defense system ware activated, while in IMI-resistant plants predominantly the xenobiotic detoxification mechanisms were increased. The physiological effects of the herbicide imazamox were investigated by monitoring growth and changes in the physiology of imazamox-treated IMIresistant sunflower hybrids during stress and recovery phases. The results showed that the herbicide imazamox caused a transient inhibition of the photosynthetic performance (both light-dependent photosynthetic redox reactions and leaf gas exchange processes) of sunflower IMI-R plants. Analyses of growth performance of IMI-resistant sunflower hybrids treated with imazamox demonstrated that the growth retardation was most pronounced on 7 days after

treatment, and that on 14 days after treatment the plants were already recovering. Further, the specific activity of the AHAS enzyme, *AHAS1* gene expression and imazamox residues were determined.

Supplementation of the growth medium of imazamox-treated IMI-resistant sunflower plants with BCAA reduced the negative effect of imazamox on leaf area, fresh and dry weights. Analyses of gas exchange and chlorophyll fluorescence indicated that application of BCAA improved the photosynthetic performance of the imazamox-treated plants.

A chlorophyll fluorescence technique (OJIP test) was used to examine the effects of a commercially available amino acid extract on the photosynthetic performance of IMI-tolerant plants. The results showed that combined application of imazamox and the amino acid extract diminished the negative effects of the herbicide.

In general, this thesis is well-written.

Part of the work presented in this thesis has already been published in international peer reviewed journals, and there are still high quality data that can be valorised in good ranking international journals.

My conclusion is that this PhD thesis is of high scientific quality and proves that the candidate is able to deal with research from the molecular up to the plant production level. She also demonstrated that she is able to report her work in oral and poster presentations and in good scientific publications in international journals.

Main Comments (e.g., add or rewrite a section of chapter, change structure of the thesis, perform additional research and/or experiments, ...)

Being a supervisor of this thesis, I had the opportunity to discuss the results in an extensive way with the candidate. I also proofread the manuscript and provided my comments/suggestions before the final version was printed. Therefore, I don't have more comments now.

Minor Comments (e.g., typos, numbering figures & tables, referencing errors, rephrase sentences, remarks about the layout,...)

Please indicate in the table below your final score

Decision Decision	No.
	Implication
X Accepted without revision or with minor revision  Accepted with major revision  Not accepted	No review of the revised version is necessary. Permission for public defense is granted immediately. The promoter will check if the PhD student has made the requested revisions.  The jury must confer. Based on this discussion, two options are possible: a) review of the revised version is necessary before the candidate can be allowed to defend the work in public, b) the procedure is stopped due to major flaws and/or deficiencies in the work.

November 16th, 2016