

REVIEW

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of a dissertation for **Doctor's** educational and scientific degree in the area of higher education 4.0 Natural sciences, mathematics and computer science, professional direction 4.3 Biological sciences, scientific specialty – Biology (Plant physiology)

Author of dissertation paper: **Dobrinka Anastasova Balabanova-Ivanovska**, PhD candidate by correspondence at the department of Plant Physiology and Biochemistry at the Agricultural University of Plovdiv.

Topic of the thesis: „Physiological responses of sunflower Clearfield hybrids to the herbicide imazamox”

Reviewer: Prof. Dr. Malgozhata Jan Moetska-Berova, Agricultural University of Plovdiv, area of higher education 4.0 Natural sciences, mathematics and computer science, professional direction 4.3 Biological sciences, scientific specialty Physiology of plants, appointed as member of the scientific jury by virtue of ordinance № RD-16-1010 of 24 October 2016 of the Rector of AU-Plovdiv.

1. **Brief introduction of the candidate**

Dobrinka Anastasova Balabanova-Ivanovska was born on 18 May 1985 in Devin. In 2008 she finished her studies in the specialty of Ecology and Environmental Protection and in 2009 obtained a Master's degree in Ecology of town systems at the Agricultural University of Plovdiv.

As a student for Bachelor's educational and qualification degree she obtained the award „Green graduates of Carlsberg Bulgaria for her diploma thesis Studies of the phytotoxicity of the heavy metals Cd, Cu, Zn in *Arabidopsis thaliana* and sunflower (*Helianthus annuus*)”.

Within the period 2009-2010 she worked as a junior specialist at Municipal Administration in Devin. Since 2011 she has been a full-time postgraduate student at the department of Plant Physiology and Biochemistry at the Agricultural University of Plovdiv. Since 2014 she has been working as an assistant in the same department. At the same time, pursuant to legislative provisions, she modified her method of attendance to studies by correspondence.

She has attended two long-term specializations abroad: March 2010 — September 2010 — at German Research Center for Environmental Health, Munich, Germany (Scholarship Program by German Federal Environmental Foundation), May 2007 – August 2007 r. - at Hasselt University, city of Hasselt, Belgium (Erasmus European

programme for student mobility). She speaks English and German languages. Married, with one child.

2. Actuality of the problem

Weeds, along with the diseases and pests, cause serious damage to agriculture and reduce the yield of agricultural crops. Struggling with them is absolutely mandatory in contemporary agriculture. The integrated struggle against weeds includes agrotechnical events (crop-rotation, soil cultivation, etc.) and use of chemical substances (herbicides). Along with the application of herbicides in the struggle against weed, new and more efficient solutions of this problem have been constantly pursued. One of the innovative solutions for the oleaginous crop *sunflower* (subject of the present thesis) is the technology Clearfield®, which uses the herbicide imazamox and imidazoline-resistant hybrids. Currently around 25% of the sunflower areas are grown under the Clearfield technology.

Imazamox is one of the five imidazoline herbicides which block the synthesis of branched chain amino acids by inhibiting the activity of the enzyme acetohydroxyacid synthase (AHAS). The mechanism of activity of imazamox is well known, however the consequences of inhibition of AHAS enzyme and the disrupted synthesis of the amino acids leucine, valine and isoleucine on main physiological processes in plants are not completely clarified. There is scarce information on the metabolization of the herbicide imazamox in sunflower. There is limited information on the protective effects of various bio-stimulants on crops treated with imidazoline herbicides, including sunflower.

In this aspect I believe that the present dissertation is very topical and has a proven scientific and applied nature.

2. Objective, tasks, hypotheses and methods of study

The objective of the present thesis is related to the study of (1) the physiological response of sunflower Clearfield hybrids towards the herbicide imazamox and (2) opportunities for improvement of the physiological status of imazamox treated plants through exogenically added branched chain amino acids or foliar application of bio-stimulants from the group of protein hydrolisates.

To this end, the following tasks have been set: (1) determination of the tolerance of widespread Clearfield sunflower hybrids towards imazamox by studying the photosynthetic activity of the plants after herbicide treatment; (2) study of the activity of enzyme systems (*acetohydroxyacid synthase*, *glutathione S-transferase*, *superoxide dismutase* etc.) and determination of the content of metabolites (glutathione), related to the decomposition of the herbicide and anti-oxidizing protection of the cells in sunflower plants treated with the herbicide in hybrids differing by their tolerance; (3) study of the effect of exogenically added branched chain aminoacids (valine, leucine and isoleucine) on the physiological status of imazamox treated sunflower plants; (4) study of the effect of foliar fertilizers containing L-amino acids on imazamox treated sunflower Clearfield hybrids.

The studies included in the present dissertation have been carried out under different methodological settings. They include hydroponic experiments with young sunflower plants and vegetation soil experiments. The methodological settings were appropriately selected, as a result of which valuable information of scientific and applied nature has been obtained.

A wide range of contemporary methods of study has been used, including molecular, bio-chemical, physiological and other analyses. This ensures the complex nature of the studies.

The level of statistical processing of the experimental material is very good. The processing of the obtained results has been carried out using ANOVA and SPSS software, as well as additional tests (Duncan's test).

4. Visualization and presentation of the obtained results

As a result of the studies extensive experimental material has been obtained, summarized and presented in several separate parts.

The tolerance of five sunflower Clearfield hybrids (LG 56.58, Tektonic, Alego, Mildimi, Primis) towards the herbicide imazamox, assessed through measurement of the photosynthetic activity of the plants treated with a recommendable and doubled dose, is presented in **Chapter 3**. In order to determine what specific and non-specific protective mechanisms are activated by the herbicide imazamox in sunflower plants, one imazamox-tolerant and one sensitive hybrids were observed 24 hours after their treatment with the herbicide. The results of the reaction of the two studied hybrids are presented in **Chapter 5**. The specific activity of AHAS enzyme was determined, as well as the expression of the gene *AHAS1* and the residual quantities of the herbicide imazamox, and the obtained results are presented in details in **Chapter 4**. The influence of the subsequently added branched chain amino acids on the physiological status of the imazamox-treated sunflower plants has been traced in **Chapter 4** and **Chapter 5**. The influence of the foliar treatment with bio-stimulant (amino acid extract) on imidazolinon-tolerant sunflower plants treated with imazamox is presented in **Chapter 6**.

The obtained results are presented in well-structured tables (7 pcs.) and figures (16 pcs). The thesis has been visualized with 7 original colour photos.

5. Discussion of the results and literature

On the basis of the studied photosynthetic parameters, different levels of genotype tolerance to the herbicide imazamox have been found, provided that the hybrid Mildimi has proven to be the most tolerant one.

Activation of specific and non-specific protective mechanisms was studied in one imazamox-tolerant and one sensitive sunflower hybrids. The obtained results show differences in the fast stress response of the two studied hybrids. In the sensitive hybrid mostly non-specific mechanisms were activated (the enzymes from the anti-oxidizing cell

protective system), while in the plants of the tolerant hybrid, the mechanisms of detoxification and metabolism of xenobiotics were activated.

With the help of biometric and photosynthetic parameters, the reaction of the imidazolin-resistant hybrid Mildimi towards the herbicide imazamox was studied in the phases of stress and recovery (7 and 14 days after treatment). It was found out that growth inhibition is best expressed 7 days after treatment and 14 days after that the plants recover their growth. It has been proven that the application of the herbicide causes temporary suppression of photosynthesis and this negative impact is available to the reactions of photosynthesis both during daylight and dark hours.

The influence of exogenically added branched chain amino acids (valine, leucine and isoleucine) on the physiological status of the sunflower plants treated with imazamox has been studied. It was proven that their addition considerably reduces the negative effect of the herbicide. The positive influence of the applied amino acids has been supported by the changes in the leaf gas exchange, the photosynthetic electronic transport and the content of photosynthetic pigments.

There has been studied the influence of foliar treatment with bio-stimulant (aminoacid extract) on imazamox-treated sunflower plants. With the help of OJIP test it was found out that the combined application of both components (imazamox and the bio-stimulant) reduces the negative effects of the herbicide and has a positive effect on the light-dependent processes of photosynthesis.

It is worth noting that the literature references (**Chapter 1**) and the skillful use of contemporary methods of processing of the obtained results have enabled the PhD candidate to interpret the information in a precise and critical manner and to formulate conclusions important in theoretical and practical aspects (**Chapter 7**).

6. Contributions of the thesis

As a result of the studies 3 scientific contributions have been made and 1 recommendation for the practice.

Scientific contributions

1. An overall picture of imazamox-induced injuries to sunflower, their consequences and recovery of the plants have been detailed by time course monitoring on the physiological responses of the plants subjected to both separate and combined application of imazamox and branched chain amino acids.
2. A novel fact, established in the conducted study, is the obtained evidence that the metabolite glutathione participates in the herbicide imazamox detoxification in sunflower plants by conjugation reactions catalyzed by glutathione S-transferase enzymes family. This fact complements the known detoxification pathway of imazamox in plants by cytochrome P₄₅₀ monooxygenase and glycosyl transferases.
3. Using a sensitive chlorophyll a fluorescence method (OJIP) several novel aspects

of imazamox-photosynthesis interactions have been described, in particular that imazamox slightly diminished the concentration of active PSII reaction centers and significantly decreased the relative part of active reaction centers compared to the total chlorophyll content.

Practical recommendations

1. Based on the obtained results demonstrating a positive effect of an amino acid extract (commercial product Terra-Sorb) on imazamox-treated sunflower plants, we recommend it for foliar application in a rate of 3 l ha⁻¹ given at 2-3 leaf pair for improving their growth and photosynthetic performance.

7. Critical notes and questions

I do not have any critical notes. I would like to express my satisfaction with the scientific study which fully covers the requirements for PhD dissertation under the European standard.

I have the following question to the PhD candidate:

1. What is the reason for the tolerance of the contemporary sunflower Clearfield hybrids to the herbicide Imazamox?
2. Which components, apart from glutathione, are incorporated in the non-enzymatic anti-oxidation protective system of the plants?

8. Published articles and quotes

Two scientific publications were made in connection with the dissertation:

- ✓ Balabanova, D., A. Vassilev. 2015. Response of sunflower Clearfield hybrids to both recommendable and higher doses of imazamox herbicide. Agricultural Sciences (Bulgarian journal), 8, 18:41-46;
- ✓ Balabanova, D., M. Paunov, V. Goltsev, A. Cuypers, J. Vangronsveld, A. Vassilev. 2016. Photosynthetic performance of the imidazolinone resistant sunflower exposed to single and combined treatment by the herbicide imazamox and an amino acid extract. Front Plant Sci. DOI: 10.3389/fpls.2016.01559.

One publication has been submitted for printing:

- ✓ Balabanova, D., T. Remans, A. Vassilev, A. Cuypers, J. Vangronsveld. Response of sunflower glutathione-mediated detoxification system to the herbicide imazamox. Submitted to Acta Physiologiae Plantarum.

The PhD candidate has attended two scientific conferences:

- ✓ Balabanova, D., T. Remans, A. Vassilev, A. Cuypers, J. Vangronsveld. Response

of sunflower glutathione-mediated detoxification system to the herbicide imazamox. 19th Plant Biology Europe FESPB/EPSO Congress. Dublin, Ireland. June 22nd to 26th 2014. Poster presentation;

✓ Balabanova, D., A. Vassilev. 2015. Response of sunflower Clearfield hybrids to both recommendable and higher doses of imazamox herbicide. Jubilee scientific conference with international participation 70th Anniversary Agricultural University – Plovdiv. October 29th to 31st 2014.

Quotations are not observed.

The author's summary reflects the main results, achievements and contributions in the thesis.

CONCLUSION

On the basis of the different methods of study learned and applied by the PhD candidate, the summaries and conclusions, I believe that the presented dissertation meets the requirements of the Law on Development of the Academic Staff in the Republic of Bulgaria and the Regulations of the Agrarian University for its application. This gives me good reason to assess it as POSITIVE.

I would like to propose to the honorary Scientific jury to vote positively and to confer **Doctor's** educational and qualification degree in Biology (Plant Physiology) to Dobrinka Anastasova Balabanova-Ivanovska.

Date: 15.11.2016
Plovdiv

REVIEWER:
(Prof. Dr. Malgozhata Berova)