REVIEW

On: DOCTORAL DISSERTATION for awarding the educational and scientific degree "Doctor" in: Professional field 4.3. "Biological sciences", Scientific Specialty 01.06.16 "Plant Physiology"

to the author: **Dobrinka Anastasova Balabanova-Ivanovska**, part-time PhD student, Department of Plant Physiology and Biochemistry, Agricultural University in Plovdiv,

Topic:" **Physiological responses of sunflower Clearfield hybrids to the herbicide imazamox"**

Reviewer: Assos. Prof. Dr. Zarya Vasileva Rankova, Fruit Growing Institute – Plovdiv, appointed a member of the Scientific Jury by Order No. RD-16-1010, of the Rector of the Agricultural University.

Assistant Professor Dobrinka Anastasova Balabanova-Ivanovska was born on 18.05.1985 in Devin. She graduated from the Agricultural University – Plovdiv acquiring the educational and qualification degree Master in "Ecology of urban systems". In the period 2009-2010, she worked in the municipal administration of Devin. In 2011 she was enrolled in a PhD programme with a Scientific Supervisor Prof. Andon Vasilev by Order No. RD-26-47 of 15.10.2011. She was discharged with the right to defend the PhD Thesis by Order No. 26-50 of 20.05.2016, Scientific Supervisor: Prof. Dr Jaco Vangronsveld (University of Hasselt) and Co-Supervisor: Prof. Dr Ann Cuypers (University of Hasselt).

Topical issues of the problem

Weeds are one of the most limiting factors for global sunflower production causing considerable yield losses that are estimated at about 20-70%. Sunflower is usually planted at low densities and grows slowly during the first weeks after planting until canopy closure. For that reason it is a poor competitor and the weeds compete it successfully during these early growth stages and as many field crops, it is vulnerable to weed interference during the first 3 to 4 weeks after planting. Sunflower is relatively sensitive to herbicides and so far practically no selective chemical means against broadleaf weeds have been found for use during vegetation. Herbicides from the group of imidazolinone are distinguished by a high efficacy against weeds, but their selectivity is low to sunflower. Imazamox is a selective herbicide of the imidazolinone group used against annual and perennial grasses and broadleaf weeds applied in a wide spectrum of crops. Despite the relatively high tolerance of Clearfield® sunflower hybrids to imazamox, temporary yellowing and stunting of young leaves as well as a reduction of plant height may occasionally occur after imazamox application.

The PhD thesis treats major agricultural and plant protection problems in sunflower cultivation – weed control, based on Clearfield® production system, which determines its importance and topicality.

Purpose, objectives, assumptions, and investigation methods

The PhD thesis is written in 141 p. and includes the following Chapters: Introduction, Materials and Methods, Response of sunflower Clearfield hybrids to recommended and higher doses of imazamox herbicide; Monitoring on plant performance of imidazolinone resistant sunflower after single and combined application of the herbicide imazamox and branched-chain amino acids; Response of sunflower glutathione-mediated detoxification system to the herbicide imazamox; Photosynthetic performance of the imidazolinone resistant sunflower exposed to single and combined treatment by the herbicide imazamox and an amino acid extract; General discussion and perspectives and References.

In Chapter Introduction, the PhD student has done thorough analysis of the problem – the significance of sunflower as an oilseed crop, areas and yields and problems with weed infestation and their control in order to reduce the production cost. Chemical weed control in sunflower production – Pre-sowing herbicide treatment, Pre-emergent herbicides, Post-emergent herbicide treatments; The mechanisms of herbicide selectivity; The mechanisms for herbicide detoxification in plants – Glutathione (GSH) mediated detoxification system, Glutathione S-transferases mediated detoxification system, Antioxidative defense mediated detoxification system, Acetohydroxyacid synthase (AHAS) – a target of herbicide action, General properties of AHAS inhibiting enzymes , Mode of action of imidazolinones, Functional disorders in target plants induced by AHAS inhibitors have been discussed in details.

Imazamox metabolism in plants and Clearfield crop production system-Clearfield® production system based on *Imisun* trait and Clearfield Plus® production system based on *CLPlus* trait are treated in details.

The possibilities to improve plant performance of herbicide-treated crops-Herbicide safeners and Plant biostimulants are discussed in the thesis.

Literature review covers 199 scientific sources and shows a thorough understanding of the problem. On this basis the purpose and objectives of the research are clearly defined.

In chapter MATERIALS AND METHODS the plant material; chemicals used in the study – Imazamox /Pulsar 40/, branched chain amino acids and Amino acid extract /Terra-Sorb foliar/ are presented in detail. The applied research methods, the indicators analyzed and statistical analyzes are thoroughly discussed. Methodical staging is correct and meets the goal and objectives of the study.

CHAPTERS 3, 4, 5 and 6 are constructed as Abstract, Introduction Experimental design, Results and Discussion and Conclusions. Structured in this way as separate sections, they present exhaustively the various experimental

accumulation of BCAA, suppressing its activity up to 14 days after treatment (DAT). On the molecular level the expression of *AHAS1* gene was not significantly influenced by imazamox treatment, while single BCAA treatment inhibited its gene expression. The plants recovered their growth and photosynthetic rate after imazamox inhibition until 14 DAT, which may be due to the rapid imazamox degradation/metabolization.

CHAPTER 5 – Response of sunflower glutathione-mediated detoxification system to the herbicide imazamox

The aim of the study was (1) to monitor the herbicide-induced stress responses and recovery in IMI-R and IMI-sensitive (IMI-S) sunflower plants; and (2) to understand whether and to what extend root application of BCAA could improve performance of herbicide-treated plants. For this purpose the IMI-R and IMI-S sunflower plants are subject to single and combined application of imazamox and BCAA and growth rate, lipid peroxidation, activities of some oxidative stress responsive enzymes, GST, GSH content and its redox state, and expression of genes related to the GSH metabolism were investigated. Responses of growth, the antioxidative defense and glutathione-mediated detoxification systems were examined following imazamox and combined imazamox/BCAA application. A temporary slight increase of oxidative stress was observed after imazamox application, which was suppressed by antioxidative enzymes such as superoxide dismutase (SOD) and peroxidases (POD). Glutathione (GSH) does not seem to be involved in combatting ROS, since no increases in the activity of glutathione reductases (GR) as well as glutathione peroxidases (GPX) and no significant changes of the GSH redox state were observed. Increased GSH concentration, induction of *GSH1* and *GSH2* genes as well as increased glutathione-S-transferases (GSTs) activity on one hand, and no significant changes in the GSH redox state on the other hand, indicated that GSH produced in imazamox-treated sunflower plants, participated in phase II of the herbicide detoxification pathway, in the herbicide-GSH conjugate reaction catalyzed by GSTs.

Chapter 6 – Photosynthetic performance of the imidazolinone resistant sunflower exposed to single and combined treatment by the herbicide imazamox and an amino acid extract

The aim of the study was to describe the functional status of the photosynthetic apparatus of imazamox-treated IMI-R sunflower plants. Considering the lack of information concerning the effects of protein hydrolysates against herbicide stress, the second aim of the study was to explore whether the application of PHs-based biostimulants could improve photosynthetic performance and growth of these plants.

Therefore, more information about the photosynthetic performance of the herbicide-treated plants could be valuable for the further improvement of the Clearfield technology. Plant biostimulants have been shown to ameliorate

settings, included in the study. This is an original approach that allows carrying out the research properly and receiving and interpreting the results accurately. In these four sections, the obtained results are presented in **6 Tables and 18 Figures,** including colour photos, allowing better visualization. Statistical analysis was performed using one way ANOVA (for $P< 0.05$). Based on ANOVA results, a Duncan test for mean comparison was performed, at 95% confidence level, to test for significant differences among treatments.

Discussion of the results

CHAPTER 3 - Response of sunflower Clearfield hybrids to recommended and higher doses of the imazamox herbicide.

The aim of this study was to evaluate both growth and photosynthetic responses of Clearfield sunflower hybrids to the recommended dose and double dose of the herbicide imazamox. Five hybrids were studied: LG 56.58, Tektonic, Alego, Mildimi and Primis. The following doses of imazamox were applied: untreated control, the recommended dose of imazamox (4.8 g a.i. / da = 120 ml /da Pulsar 40) and doubled imazamox doses (9.6 g a.i. / da = 240 ml / da Pulsar 40). The herbicide was applied at the 4-6 leaves stage. The biometric and photosynthetic analyses were carried out after the treatment. Photosynthetic leaf gas exchange, quantum yield of PSII photochemistry and growth parameters (height, fresh weight and leaf area) were determined. The obtained results showed that treatment with the recommended dose of imazamox caused a temporary negative impact on photosynthetic performance and growth of sunflower plants, which recovered after 14 days. The double dose of imazamox seriously damaged the existing top leaves of sunflower plants, but the newly developed leaves did not show toxicity symptoms. The studied sunflower cultivars demonstrate a good tolerance to imazamox, when applied at the recommended dose. Mildimi hybrid was selected as tolerant and as such it was used in subsequent analyses.

CHAPTER 4 - Monitoring on plant performance of the imidazolinone resistant sunflower after single and combined application of the herbicide imazamox and branched-chain amino acids.

The objectives of this study were: (1) to monitor the herbicide-induced stress responses and recovery in IMI-R sunflower hybrids; and (2) to understand whether and to what extend application of BCAA to the roots can improve growth and photosynthetic performance of herbicide-treated plants. For this purpose the subjected IMI-R sunflower hybrids were subject to single and combined application of imazamox and BCAA and AHAS activity and *AHAS1* gene expression, imazamox degradation, photosynthetic performance and plant growth were investigated. The obtained results showed that addition of BCAA to imazamox-treated sunflower plants ameliorated their growth and photosynthetic performance. AHAS activity is negatively influenced by the

damages caused by different stress factors on plants, but very limited information exists about their effects on herbicide-stressed plants. In order to characterize the photosynthetic performance of imazamox-treated sunflower IMI-R plants, experiments were carried out including both single and combined treatments by imazamox and the plant biostimulant. The results show that imazamox application at a rate of 132 μg per plant (equivalent of 40 g active ingredient ha^{-1}) induced negative effects on both light-light dependent photosynthetic redox reactions and leaf gas exchange processes, which was much less pronounced after the combined application of imazamox and amino acid extract.

Based on the results of various experimental settings, the PhD student has done in-depth analysis in the **CHAPTER 7. GENERAL DISCUSSION AND PERSPECTIVES.** It is worth underlining the skill of the PhD student to independently interpret and summarize the obtained results. One of the main conclusions is that the application of biostimulant of the protein hydrolysate group (Terra-Sorb Foliar®) has a protective effect on the sunflower plant in combined application with imazamox. Based on the obtained results demonstrating a positive effect of the amino acid extract (commercial product Terra-sorb) on imazamox-treated sunflower plants, it is recommend for foliar application at a rate of 3 L/ha given at 2-3 leaf for improving their growth and photosynthetic performance. This gives grounds to assume, that despite the prevailing fundamental character of studies, the results could be applied in practice to overcome the negative effects of treatment with herbicides.

I have some questions:

1. Could the level of the applied farming practices (fertilization, irrigation, climatic stress factors) affect the behavior of plants after treatment with imazamox (Pulsar 40)?
2. Are there other herbicides used in practice, whose mechanism of action is associated with inhibition of the synthesis of amino acids?

Scientific Achievements

The formulated Conclusions and Scientific achievements are well-founded and match with the results. The above scientific and applied achievements will enrich the knowledge about the possibilities for applying the herbicide imazamox in the sunflower crop and for reducing the negative effect.

This gives me the opportunity to assure the members of the Scientific Jury that the results obtained are largely a personal contribution of the candidate. I must say that Dobrinka Balabanova is a researcher, possessing the ability to work in a team, which is especially important for modern research. She has a profound knowledge of contemporary weed science, plant physiology, modern genetic research methods, statistical methods, and excellent skills for

independent scientific work. She is able to interpret scientific literature fluently and properly.

The main part of the thesis is published in 3 articles – in Front Plant Science, Agricultural Science and Acta Physoilogicae Plantarum, in a co-authorship, the PhD student being the first author. The PhD student has taken part in two scientific conferences.

The author's summary is prepared according to the requirements and it is well illustrated with 12 figures and 4 tables. In a concise form, it properly reflects the conducted research presented in the thesis, including the scientific and applied achievements.

Conclusion

Based on the presented review of the thesis, I think that the PhD student Dobrinka Anastasova Balabanova-Ivanovska has thorough theoretical knowledge and skills to apply different methods of study and to evaluate, analyze and summarize the results. I believe that the submitted dissertation meets the requirements of the Development of Academic Staff in the Republic of Bulgaria, Act and the Regulation of the Agricultural University for its implementation, which allows me to assess it positively.
I dare to propose to the respected Jury to vote positively for the award of the educational and scientific degree "Doctor" in the Professional Field 4.3. "Biological sciences" and Scientific Specialty 01.06.16 "Plant Physiology" to the PhD student Dobrinka Anastasova Balabanova-Ivanovska.

Reviewer

16.11.2016

Assos. Prof. Dr. Zarya Rankova