REVIEW

On dissertation work for obtaining the educational and scientific degree "doctor" in field of higher education 6. Agrarian sciences and veterinary medicine, professional field: 6.1. Crop science, scientific specialty: Plant Physiology

Author of the dissertaton. **Rositsa Cholakova-Bimbalova** - PhD student at the Departmant of Plant Physiology and Biochemistry at the Agricultural University, Plovdiv.

Thesis topic. "Research of the reaction of maize (Zea mays L) to low temperatures and the effectiveness of the following leaf fertilization"

Reviewer: Assoc. Prof. Dr Nevena Stoeva, Agricultural University, Plovdiv. Department of Plant Physiology and Biochemistry, professional field Crop science, scientific specialty Plant Physiology,appointed by the Rector of the Agricultural University, as a member of the Scientific Jury with Order No 16-506/18.06.2020.

1. Short introduction of the applicant.

Rositsa Cholakova-Bimbalova was born on 07.06. 1989 in Plovdiv. She completed her secondary education in 2008. In 2012 she graduated in Plant protection at the Agricultural University, Plovdiv, and in 2014 she completed her Masters's degree in Plant protection at the Agricultural University, Plovdiv

From 2014 to 2020 she is PhD student at the Departmant of Plant Physiology and Biochemistry at the Agricultural University, Plovdiv.

During hir doctoral studies Rositsa Cholakova-Bimbalova fulfilled the tasks included in the individual curriculum and mastered the training modules. She successfully passed the doctoral exam. The doctoral student has very good proficiency in English (B2C1) and Russian (B2).

Rositsa Cholakova-Bimbalova possesses as well the necessary computer literacy, uses a wide range of software products /Word, Excel, Power Point and ststistical processing programs; SPSS 19,0, Internet/.

2.Relevance of the prpblem.

One of the main environmental problems having a negative impact on the productivity and quality of maize yield, especially in the regions of drought, are the low temperatures. Maize is a thermophilic crop and very often in the early stages of development it is subjected to the influence of low positive temperatures. Maize is a main grain crop for our country. Its high sensitivity to low positive temperatures and its quality of a model subject create good opportunities for physiological studies of the reaction of the plants to low temperature stress and the possibility for improvement of their physiological status by means of leaf nourishing.

On the other side, the information about the influence of different bio-stimulants and more particularly of protein hydrolysates as a means of support of maize plants subjected to low temperature impact, are very limited or almost missing.

All of the above has motivated the PhD student to carry out a study of the physiological reaction of contemporary maize hybrids to low temperature impact and to assess the remedial potential of various bio-stimulants and leaf fertilizers, to what extent and in what phases of development they could be applied for plant recovery.

3.Purpose, tasks, hypotheses and methods of research.

The purpose of the study has been clearly and precisely formulated, namely: "To study the impact of chronically low temperature impact on the physiological status of young maize plants and the possibility for overcoming of the functional disturbances therein, by means of application of leaf fertilizers and bio-stimulants."

The above objective has been carried out with the performance of 6 main tasks, 4 of which are connected with the influence of low positive temperatures on the physiological and biochemical parameters of young maize plants, and 2 in connection with the influence of leaf products on the physiological and biochemical parameters of young maize plants.

My assessment of the methodological approach and the used methods of study is that they are correct, contemporary and fully complying with the set objective and tasks, namely:

- An appropriate model subject has been selected. Maize is a thermophilic crop, with high sensitivity to low positive temperatures which are a frequent phenomenon, especially in the early phases of crop development. On the other side, it is a suitable subject of physiological studies. The development covers two hybrids in order to give a more objective picture of the reaction of the variety.
- On the basis of the preliminary experiments and literature sources, the most appropriate temperature for achieving chronic low temperature stress in young maize plants is 10°C. The main experiments have been carried out under such temperature and exposure time 7 days.
- A large part of the experiments are dedicated to the main purpose of the development, studying the effect of some bio-stimulants (protein hydrolysates) as modifiers of low temperature impact on the maize hybrid Knezha 307.

A significant number of logically selected, contemporary methods and analyses have been used in the experiments (***electrophysiological and conductometric, biometric, biochemical, physiological***), which are described in details and precisely implemented using contemporary methodologies.
Most contemporary devices available in the department have been used.

The hybrids used, the methodical settings, the growing conditions have been described in details.

Most of the experiments have been carried out in the laboratories of the Plant Physiology and Biochemistry Department at the Agrarian University of Plovdiv and

part of them in the Artificial Climate Laboratory of the Moscow Agricultural Academy "K.A. Timiryazev", Russia, as a hydroponic and hydroponic-substrate crop.

Detailed specification of the bio-stimulants and fertilizers has been provided. All results were statistically processed using a one-factr ANOVA analysis, followed by a Dunkan test. The trustworthiness of the differences was determined at a significance level of 95% ($P < 0.05$).

4.Visualization and presentation of the results obtained.

The dissertation paper has been written in a sufficient volume of 131 standard pages. In a composition aspect it is correctly and comprehensively structured, thus facilitating the reading and the necessary references. The presentation follows a good scientific style. The obtained results are presented in 21 tables and 10 figures which are aesthetically developed. Their titles are clear and the symbols are correct. The photos of the experimental settings and the plants (19) are of good quality and contribute to the more convincing perception of the obtained results. All of the above shows the ability of the PhD student to systematize and illustrate the scientific information.

5.Discussion of results and literature used.

On the basis of an exceptionally detailed and **rich literature review**, containing 216 sources (only 6 in Cyrillic, 5 of them in Bulgarian language), the PhD student shows excellent knowledge of the contemporary literature on the problem, as well as of the methods of in-depth studies in the field of plant physiology.

It is worth noting that the PhD student shows abilities for critical analysis of the scientific information.

The above described effects of the low temperature stress on plants are accompanied by the necessary methodological explanations, revealing good theoretical preparation of the PhD student.

In the main section "Results and Discussion", extended experimental material has been presented, obtained from the experiments and laboratory analyses that have been carried out.

There is a comprehensive study of the bio-stimulators used in the development, including assessment of their positive effects on the plants under stress. The obtained results are interesting from a scientific point of view and are convincing because of the reliability of their statistical processing and logical connections between the changes in different physiological parameters. In many cases the discussion of the results in the separate sections has not only an ascertainment nature but also relates to the information about other parameters in previous sections. (Between the content of mineral elements and the lipid peroxidation in the roots; between the chlorophyl content and LP; between the chlorophyl fluorescence and the content of pigments, etc.).

The results related to the chlorophyl fluorescence and JP – test and their scientific interpretation and comprehensive interpretation in the conclusion made by the PhD student is especially interesting for the science. The measurements with MINI-PAM

give additional information about the condition of the photosynthetic apparatus in the stressed maize plants of both maize hybrids and describe in details the physiological reaction of the crop to low-temperature stress.

In 4 types of experiments carried out with different substances and combinations there are few differences in the degree of positive impact depending on the type of product (leaf fertilizer or bio-stimulant). Regardless of the fact that they do not influence the growth of the young maize plants, those substances improve their protection against low-temperature impact.

The conclusions are precise and correctly reflect the main results. They are compactly and clearly formulated and are sufficient for a dissertation paper; there are 10 of them, namely:

1. The applied low temperature stress (7 days, 10oC) reduced the relative growth rate (RGR) of maize plants from the hybrids Knezha 307 and P9528 by 36-38% in comparison with the plants of optimal temperature regime (25/20 oC; day / night).). The inhibitory effect is accompanied by chlorosis. In case of prolonged low-temperature stress (14 days) a violet color appeared on a part of the leaves, which is observed (3 days) after the stress has passed.
2. The negative influence of the low positive temperature is an integral result of caused disturbances in the oxidation-reduction status of the corn plants, their mineral nutrition and the photosynthetic process. In stressed plants, the activity of the enzyme guaiacol peroxidase (GPOD), lipid peroxidation, electrolyte leakage from the leaves increases, and the content of macro- and microelements decreases, but without reaching critical mineral levels.
3. The applied low temperature reduces the functional activity of maize plants, assessed by their bioelectrical reaction (BER), on the first day of exposure, and the effect is enhanced up to 3 days. After this period, acclimatization changes occur, which are reflected in the increase of total antioxidant activity (OAA) due to higher content of total phenols and anthocyanins in plants, as well as the effective dissipation of excess excitatory energy by non-photochemical quenching (increase in qN) and "Ungrouping" of light-collecting complexes (CCC2) by reaction centers (RCs) of photosystem 2 (increases to F0).
4. Low temperature exposure reduces by more than 60% the rate of net photosynthesis (A) in maize plants. The negative effect is due to the reduced content of photosynthetic pigments, suppressed speed of photosynthetic electronic transport (ETR) and mainly to the disturbances in the biochemical reactions of the Calvin cycle. Indirect evidence for this statement is the lower use of the final products of the light phase NADPH and ATP, which is expressed by the pronounced negative G peak in the induction fluorescence kinetics of stressed plants and the effective dissipation of excess excitatory energy (increase in qN). In addition, the rate of net photosynthesis (A) is limited by the severely inhibited growth of maize plants due to a change in their donor-acceptor relationships.
5. The young corn plants from the new Bulgarian hybrid Kneja 307 have a more disturbed physiological status in conditions of low temperature impact compared to the plants from the hybrid P9528, which is why we characterize them as more sensitive to this type of stress.

6. The 6. The foliar application of the biostimulants Terra-Sorb Foliar, Naturamin - WSP and Amino Expert Impuls and of the foliar fertilizer Polyplant during the low temperature exposure does not improve the growth of maize plants, but has a positive effect on their physiological status.
7. The positive effects of the applied foliar products on the corn plants subjected to low temperature stress are manifested by improving the foliar gas exchange (A, E, gs), increasing the content of photosynthetic pigments and activating the photosynthetic electronic transport (ETR). The biostimulant Terra-Sorb Foliar has a positive effect on the redox status of plants.
8. The positive effect of the applied biostimulants and fertilizers depends on the type of product, the nature of the components in the composition of the biostimulant, as well as on the individual composition of the specific protein hydrolyzate, but the differences between them are relatively small.
9. The improved physiological status of corn-stressed corn plants is a prerequisite for faster recovery in the post-stress period.
10. Terra-Sorb Foliar does not have a significant stimulating effect on the growth and physiological status of maize plants grown at optimum temperature.

6.SCIENTIFIC AND SCIENTIFIC-APPLIED CONTRIBUTIONS.

1. By analyzing the induction kinetics of chlorophyll fluorescence according to Strasser et al. (2004), for the first time in studies of maize plants, it has been shown that low positive temperatures reduce the connectivity between light-collecting complexes (CCC2) and reaction centers (RCs) of photosystem 2 (FS2) and increase the relative pool of available electronic acceptors of FS 1 (NADP molecules) and its relative content.
2. By using the electrophysiological method of Panichkin et al. (2009), for the first time the bioelectrical reaction of maize plants to low temperature influence was observed, as a result of which it was found that their functional activity decreases on the first day, the decrease increases to the third day and stabilizes at this level in the next period. due to the occurrence of acclimatization processes.
3. Through a complex of physiological and biochemical parameters it is shown that the new Bulgarian hybrid corn Knezha 307 has a high sensitivity to low positive temperatures in the initial period of growth and development of the crop.
4. It was found that foliar application of biostimulants Terra-Sorb Foliar, Naturamin - WSP and Amino Expert Impuls and foliar fertilizer Polyplant during low temperature exposure does not improve the growth of maize plants, but has a positive effect on their physiological status, which is prerequisite for faster recovery of growth in the post-stress period.

7.Critical notes and questions.

Regarding the dissertation paper of the PhD, I have the following remarks:

1. As some kind of omission it could be pointed out that the Bulgarian and other literature sources in Cyrillic are relatively insufficient – only 6. Moreover, 54 authors (25%) are from the past century.
2. In section V.1 the main experiments are 3 – it is not clear which task it corresponds to.
3. The results could have been more clearly integrated if the dissertation paper ended with a general discussion, unifying the separate parts of the study and with a recommendation for the implementation in practice. Which of the preparations, at what concentration and in which growth phases would increase the tolerance of the plants from maize hybrid Knezha 307 to low positive temperatures.
4.As all five publications are from participation in conferences, I recommend the PhD student, for the purpose of his future development, to publish in a magazine with an impact factor.

I have the following questions towards the PhD student:

1. Regarding photo 19, it is said that the leaves of the plants treated with bio-stimulants are larger and with better turgor. Isn't it evidence of a more intensive growth?
2. Does the PhD student intend to study in his future work the effect of some of the used preparations to a later phase of development of the plants?

8.Published articles and citations

In connection with the dissertation, the doctoral student presents 5 scientific publications, four of which are co-authored with the supervisor. Four of them are written in English.

During the PhD study, Rositsa Cholakova-Bimbalova participated as well in 5 International conferences, two of them held in Bulgaria, one in Moscow, one in Prague and one in Cacak.

The total number of points is 43 and are sufficient scientometric requirements for obtaining the educational and scientific Doctor's degree in accordance with the Law for the Development of the Academic Staff of the Republic of Bulgaria and the Regulations of the Agricultural University.

The presented self-report objectively reflects the structure and content of the dissertation.

No article citation has been submitted.

CONCLUSION:

Based on the conducted analysis of the scientific and applied activity on the doctoral student Rositsa Cholakova-Bimbalova, I consider that she meets the requirements of the Law for the Development of the Academic Staff of the Republic of Bulgaria and the Regulations of the Agricultural University for the acquisition of the educational and scientific degree "Doctor".

Rositsa Cholakova-Bimbalova has acquired basic knowledge and skills necessary for conducting of scientific researching and summarizing the scientific literature on a specific scientific problem, arranging and conducting of field experiments, taking into account the results of the performed experiments and chemical analyses, as well as

the most important summarizing the obtained results and the formation of scientifically reasoned conclusions.

During the work process the doctoral student has mastered and applied modern methods for statistical analysis of the results.

All this gives me a reason to evaluate her overall activity POSITIVELY and to offer the Scientific Jury also to vote positively and to award Rositsa Cholakova-Bimbalova the educational and scientific degree **Doctor** in the scientific speciality Plant Physiology.

Date: 20. 07.2020 **REVIEWER.....**

Plovdiv **/Assoc.prof. d-r N.Stoewa/**