# REVIEW

**of a dissertation paper for a PhD degree in: area of higher education – 6. Agrarian Sciences, professional direction – 6.1. Plant-growing, scientific area: Plant physiology**

**Author of the dissertation paper**: Rositsa Zhivkova Cholakova-Bimbalova – a PhD student by correspondence at the department of Plant Physiology and Biochemistry (currently Plant Physiology, Biochemistry and Genetics) at the Agrarian University of Plovdiv.

**Topic of the dissertation paper**: Study of the reaction of maize (*Zea mays L.*) to low temperature and the efficiency of subsequent leaf treatment

**Reviewer**: Prof. Malgozhata Jan Moetska-Berova, Dr, area of higher education - 6. Agrarian sciences, professional direction - 6.1. Plant growing, scientific subject - Plant physiology, elected for a member of the scientific jury by virtue of Ordinance No: RD 16-506 of 18 June 2020 of the Rector of the Agrarian University.

## 1. Brief introduction of the candidate

Rositsa Zhivkova Cholakova-Bimbalova was born on 7 June 1989 in Plovdiv. In 2012 she completed her studies in Plant Protection and in 2014 she obtained her Master's degree in Plant Protection at the Agrarian University of Plovdiv. Since 2014 she has been a PhD student at the department of Plant Physiology and Biochemistry at AU – Plovdiv and since 2016 she has been an assistant in the above department.

She has carried out a four-month specialization at Russian State Agrarian University "K. A. Timiryazev". She has knowledge of written and spoken English and Russian languages. She has good computer literacy.

## 2. Actuality of the problem

The dissertation paper is dedicated to a problem with outlined actuality, both theoretically and practically. It is well known that the production of maize constitutes one third of the world production of grain crops. In the last decades it has increased by almost 50%. Maize is also an economically important crop for the Bulgarian agriculture. It is grown mostly on non-irrigation areas, therefore drought and high temperatures are the main stress factors, limiting the yields of this crop.

The harmful impact of those factors may be avoided by early sowing, however it is restricted by the high sensitivity of maize to low temperatures in the early stages of its growth

and development. There is evidence that temperatures within the range 5-15 °C during that period are able to cause different functional disturbances and damages. They have a negative influence on the physiological status of the plants, especially on the mineral feeding, photosynthesis and a number of other processes, which leads to suppression of the growth, development and productivity of the crop.

Leaf nutrition is a traditional practice in plant feeding but is also a means of improvement of their physiological status in cases of stressful impacts.

Biostimulants are an innovative group of products and their use in agriculture has been significantly increasing in the recent years. They are not classical fertilizers but products which support the nutrition of the plants and at the same time increase their tolerance to stress factors.

According to the output resources and method of production, the products with biostimulating properties are divided into several groups, namely: (1) protein hydrolysates; (2) humic and fulvic acids; (3) seaweed extracts; (4) microbial products, etc.

The scientific information about the physiological effects of biostimulants is still insufficient. Currently there is no information about their influence on maize plants subjected to low-temperature impact. This has motivated the conduct of the study presented in the dissertation paper.

Considering the fact that under the conditions in our country, the young maize plants are often affected by temperatures below the optimal values, the actuality of the development (in a scientific and applied aspect) is unquestionable.

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

The purpose of the dissertation paper is to study the influence of the chronic low-temperature impact on the physiological status of young maize plants and the possibility for overcoming of the functional disturbances caused therein, by means of application of leaf fertilizers and biostimulants.

For the purpose of carrying out the set objective, the following tasks were formulated:

**1.** Tracing the influence of the low positive temperatures on the physiological and biochemical indexes of young maize plants (visual appearances, changes in the growth, mineral and water status, photosynthetic activity);

**2.** Determination of the effect of leaf products on the physiological and biochemical indexes of young maize plants (under optimal temperatures and under low-temperature impact).

The purpose and objectives ensue from the in-depth literature review presented in the dissertation paper and are logically formulated.

The methodological approach is correct and enables the conduct of the tasks set. As a result of this, significant scientific and applied information has been obtained.

The experiments have been carried out using a suitable subject. These are plants of two maize hybrids: Knezha 307, created in the Institute of Maize in Knezha, and early maize hybrid P9528 of Pioneer company (currently Korteva Agriscience).

Three biostimulating products have been used: Terra-Sorb Foliar®, Naturamin WSP and Amino Expert® Impulse, as well as one leaf fertilizer Polyplant 20/20/20.

The experiments have been carried out in laboratories of the department of Plant Physiology and Biochemistry of the Agrarian University of Plovdiv and the Laboratory of Artificial Climate of Moscow Agricultural Academy "K.A.Timiryazev", Russia.

The plants were grown as a water and hydroponic-substrate crop.

The preliminary experiments included a selection of suitable temperature (10 °C) and duration of the low-temperature impact (7 days) causing chronic stress on the young maize plants.

In the main experiments the plants were grown under two different temperature regimes: 25±1°C/20±1°C (day/night) and permanent 10±1°C temperature. Some of them were treated with leaf products for the purpose of recovery of the plant from the low-temperature impact.

At the end of the experimental period, the plants were analyzed with the help of a number of indexes. To this end a wide range of classical and contemporary research methods were used (physiological, electrophysiological, conductometric, biochemical and other analyses). They are interrelated, which undoubtedly secures the complex nature of the studies.

**4. Visualization and presentation of the obtained results**

As a result of the studies, rich experimental material has been obtained, which is summarized and presented in well-formulated tables (21) and figures (11). It is well visualized with photos made by the PhD student.

The level of statistical processing of the experimental material is very good. The data have been processed using a single-factor ANOVA analysis, followed by a Dunkan test.

**5. Discussion of the results and literature**

The discussion of the obtained results is detailed, with logical connections between the studied indexes. It is supported by an analysis of the scientific information on the developed problem.

On the basis of the studies that have been carried out, it was proven that the 7-days' low-temperature impact decreases the relative growth rate (RGR) of the plants from the hybrids Knezha 307 and P9528 by 37% on average, compared to that under an optimal temperature regime.

It has been found out that the negative influence of the low positive temperature is an overall result of disturbances caused in the oxidation-reduction status of the plants, their mineral nutrition and photosynthetic process.

The applied low temperature decreases the functional activity of the maize plants. This effect has been proven even on the first day of its impact, by tracing the bioelectrical reaction of the plants. After the 3-days' period, aclimatic changes occurred resulting in increase in the total antioxidant activity, as well as the efficient distraction of excessive energy through non-photochemical quenching and "de-grouping" of light-collecting pigment complexes from the reaction centers of PS II.

It has been found out that the low-temperature impact reduces by 60% the speed of the net photosynthesis (A) in maize plants. The negative effect comes as a result of the decreased content of photosynthetic pigments, suppressed rate of the photosynthetic electronic transport (ETR) and of the disturbances in the biochemical reactions from the Calvin cycle.

The young maize plants from Knezha 307 hybrid are known by their more severely disturbed physiological status under the conditions of low-temperature impact, compared with the plants from the hybrid P9528, therefore the PhD student characterizes them as more susceptible to this type of stress.

It has been proven that the leaf application of the biostimulants Terra-Sorb Foliar, Naturamin – WSP and Amino Expert Impuls and of the leaf fertilizer Polyplant during the low-temperature impact have a positive effect on the physiological status of the treated plants.

The positive effects of the applied leaf products on the maize plants subjected to stress result in improvement of the leaf gas exchange, increase in the contents of the photosynthetic pigments and activation of the photosynthetic electronic transport.

It has been proven that the positive effect of the biostimulants and fertilizers depends on the type of product and the nature of their ingredients.

On the basis of the obtained results, the PhD student expresses an opinion that the improved physiological status of the maize plants stressed by low temperatures is a precondition for the faster recovery of the growth during the post-stress period.

I accept the presented results as trustworthy. All of them are statistically processed and there is a logical connection between the separate studied indexes.

The conclusions correctly reflect the obtained results.

## 6. Contributions of the dissertation paper

As a result of the studies, results have been obtained, some of which are important contributions both to science and practice.

### Scientific contributions

1. For the first time in our country studies with maize plants have been carried out, showing that the low positive temperatures decrease the relation between the light collecting complexes and the reaction centers of PS II and increase the relative pool of the accessible electronic acceptors of PS I (NADP molecules) and its relative contents. For this purpose, analysis of the induction kinetics of the chlorophyll fluorescence has been performed using the JIP method of Strasser et al. (2004).

2 . This is a first-time study of the bioelectric reaction of maize plants to low-temperature impact. As a result of the study it was found out that their functional activity is reduced even on the first day, the decrease lasts until the third day and stabilizes at that level in the next period due to the occurrence of aclimatic processes. To this end, the electrophysiological method of Panichkin, et al (2009) was used.

### Scientific and applied contributions

1. It has been shown that the new Bulgarian hybrid Knezha 307 has high sensitivity to low positive temperatures in the initial period of the growth and development of maize.

2. It has been found out that the leaf application of the biostimulants Terra-Sorb Foliar, Naturamin – WSP and Amino Expert Impuls and of the leaf fertilizer Polyplat during the low-temperature impact does not improve the growth of the maize plants but has a positive effect on their physiological status, which is a precondition for faster recovery of the growth during the post-stress period.

## 7. Critical notes and questions

The obtained results can serve as a theoretic basis for purposeful use of the biostimulants and fertilizers testes by the PhD student in the agronomic practice. Therefore I believe that a recommendation regarding the suitability of the studied products for application for the purpose of increase in the sustainability of the maize plants from hybrid Knezha 307 to low positive temperatures in the initial period of growth and development, is missing.

I have the following questions towards the PhD student:

1. The seven days' and fourteen days' low-temperature impact on maize plants has a similar effect with regard to the parameters of growth. What is the reason for the significantly

lower (by 42% on average) inhibitory effect of the low temperatures on the leaf gas exchange after a 14-days' impact in comparison with the 7-days' impact?

2. Please clarify the mechanism of physiological effect of the leaf products tested on the plants, depending on their ingredients.

**8. Published articles and citations**

The PhD student covers the science metric criteria for acquisition of PhD degree. In connection with the dissertation paper, 5 articles have been published: 3 in Bulgarian and 2 in foreign magazines. No information has been provided with regard to citations.

The PhD student has taken part in 5 international scientific forums (in Bulgaria, Serbia, Czech Republic and Russia), presenting materials on the dissertation paper.

**The author's abstract** objectively reflects the structure and contents of the dissertation paper (main results, achievements, contributions). It has been prepared in Bulgarian and in English language.

**Conclusion:**

The various methods of study, the correctly conducted experiments, the summaries and conclusions learned and applied by the PhD student allow me to state that the presented dissertation paper meets the requirements of the Act on the development of the academic staff in the Republic of Bulgaria and the Regulations of the Agrarian University regarding its application. This gives me a reason to assess it **POSITIVELY**.

I allow myself to propose to the honorable Scientific jury to also vote positively and award Rositsa Zhivkova Cholakova—Bimbalova with the **PhD** degree in the scientific subject Plant Physiology.

Member of the scientific jury:

23.07.2020 Prof. M. Berova, Dr

Plovdiv