



Agricultural University Plovdiv  
Agronomy faculty  
Department of Plant physiology and Biochemistry

**Rositsa Cholakova-Bimbalova**

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

## **ABSTRACT**

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Supervisor:

Prof. Dr. Andon Vassilev, PhD

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The dissertation is written on 122 pages and contains 21 tables and 11 figures. Use 216 literature sources, of which 6 in Cyrillic and 210 in Latin.

Research was reviewed in the period 2014-2019 in the laboratory of the Department of Plant Physiology and Biochemistry at the Agricultural University of Plovdiv and in the Laboratory of "Artificial Climate" - Moscow Agricultural Academy "K.A. Timiryazev", Russia.

The dissertation was discussed and scagualled for defense at a meeting of the Department of Plant Physiology and Biochemistry, Agricultural University - Plovdiv.

The defense of the dissertation will be obtained at..... ..on ..... at.....  
.....

The specialized scientific jury, appointed by the Rector of the Agricultural University with document..... will be, as follows:

The materials of the defense will be available in the library of the Agricultural University of Plovdiv and published on the website of the university.

I would like to express my sincere gratitude to my supervisor Prof. Dr. Andon Vassilev, to the colleagues from the Department of Plant Physiology and Biochemistry and to the colleagues from the Department of Plant Physiology - Moscow Agricultural Academy "K.A. Timiryazev", Russia, for the assistance provided during the development of current dissertation.

## I. INTRODUCTION

Maize (*Zea mays* L.) is a major cereal in many parts of the world, the production is around 750 - 800 million tons and represents over one third of the world production of cereals. In Bulgaria, corn production has been progressively declining in recent years (Slavova, 2015).

One of the reasons is its sensitivity to low positive temperatures in the early stages of growth and development. Temperatures in the range of 5–15 °C during the early phenophases of maize development are suboptimal and capable of causing various functional disorders and impairments (Leipner, 2009; Stamp, 1984; Zaidi et al., 2010). The physiological response of maize to low temperatures depends on many factors - the sensitivity of the hybrid, the age of the plants, the duration of exposure and more. (Farooq et al., 2009).

The main adverse effects include cell membrane damage (Lukatkin, 2003; Grzybowski et al., 2019), reduced uptake of water and mineral elements (Aroca et al., 2005; Moradtalab et al., 2018), oxidative damage (Fryer et al., 1998), decreased photosynthetic activity (Long and Spence, 2013) and others. The integral result is the inhibition of growth, development and productivity of the culture (Stamp, 1984; Lobell and Asner, 2003; Wijewardana et al., 2016).

The harmful effects of low temperatures can be limited by two main approaches: selective, aimed at creating more tolerant maize hybrids (Rodríguez et al., 2007), and technological, based on the application of various innovative materials, with aim to improve plant tolerance (Wang et al., 2018; Moradtalab et al., 2018).

The sensitivity of maize to low positive temperatures has been a hot topic for the last 30 years. The main studies were performed in order to select more tolerant hybrids. (Huang et al., 2013; Wijewardana et al., 2015; Sobkowiak et al., 2016; Riva-Roveda et al., 2016) Big number of authors have found that the more tolerant maize hybrids have a better physiological status under stress conditions. (Verheul et al., 1995; Ribas-Carbo et al., 2000; Pietrini et al., 1999) and recover more quickly from stress than the sensitive hybrids (Aguilera et al., 1999; Aroca et al., 2001; Haldimann, 1999).

However, even it is less common as a method, for ameliorating the negative effects of low temperature, recently have been obtained some data, which shows that those materials have positive effects on plants exposed to various stress factors, including low temperatures. The studies have been conducted in three areas: pre-sowing seed treatment (Guan et al., 2015; Bradačova et al., 2016; Hacisalihoglu et al.,

2018), sowing fertilization (Moradtalab et al., 2018) and foliar fertilization and / or stimulation with foliar fertilizers and biostimulants (Polo et al., 2006; Marfà et al., 2009; Botta, 2012), with the idea of protective or curative action.

Foliar fertilization is a traditional practice in plant nutrition, but it is also an effective method of improving plants physiological status under stress (Dimkpa and Bindraban, 2016). Information on the effect of biostimulants on maize plants exposed to low temperatures is currently limited. Biostimulants are an innovative group of products that support the growth and development of plants directly and / or affecting the rhizosphere. Biostimulants contain various bioactive natural substances, as: (1) humic and fulvic acids, (2) protein hydrolysates, (3) macroalgae extracts, (4) the beneficial element Si for plants, and (5) beneficial microorganisms.

Protein hydrolysates (PH) are one of the most interesting groups. They are “mixtures of free amino acids (AK), low molecular weight peptides and to a much lesser extent other organic substances” (Colla et al., 2017). They are obtained by chemical or biological hydrolysis of various animals (Cavani et al., 2006 ; Ertani et al., 2013; Grabowska et al. 2012) and plant sources (Schiavon et al., 2008; Ertani et al., 2009; De Lucia and Vecchietti, 2012).

PH has been found to have protective effects in plants exposed to salinity, drought, high temperatures and other types of stress (Nardi et al., 2016; Colla et al., 2017). Much less is known about the effects of PH on plants subjected to low temperature stress (Polo et al. 2006, Marfà et al., 2009).

The above motivates us to conduct research on the physiological response of modern maize hybrids to low temperature stress and to assess the healing potential of various biostimulants (protein hydrolysates) and foliar fertilizers.

### **III. AIM AND TASKS**

#### **AIM**

To study the influence of chronic low-temperature impact on the physiological status of young maize plants and the possibility of overcoming the functional disorders, by applying foliar fertilizers and biostimulants.

#### **TASKS**

A/ In connection with the influence of low positive temperatures on the physiological and biochemical parameters of young maize plants

1. To describe the visual changes in the growth of young maize plants that have experienced chronic low temperature exposure.
2. To monitor the physiological reaction of maize plants to the applied low temperature impact through electrophysiological, conductometric and biochemical indicators.
3. To study the influence of chronic low temperature impact on the mineral and water status of maize plants/
4. To characterize the photosynthetic activity of maize plants subjected to low temperature stress by analyzes on the leaf gas exchange, the content of photosynthetic pigments and the chlorophyll fluorescence parameters

B/ In connection with the effect of foliar products on physiological and biochemical parameters of chilled young maize plants

- 1.To study the effect of foliar biostimulant on the physiological status of young maize plants grown at optimum temperature and low temperature.
- 2.To study and compare the effects of foliar applied products on the physiological status of young maize plants, during low temperature stress as follows:
  - 2.1. Comparison between foliar fertilizer and biostimulant
  - 2.2. Comparison between biostimulant product and its organic and inorganic components
  - 2.3. Comparison between two foliar biostimulants

## **IV. MATERIAL AND METHODS**

### **1. MATERIAL**

#### **1.1. *Plant material***

Kneza 307 is a new maize hybrid, selected at the Institute of Maize - Kneza in 2014. The tolerance of the hybrid under stress conditions has not been studied.

P9528 is produced by Pioneer. It is characterized by excellent tolerance to stress conditions during germination.

#### **1.2 . *Biostimulants and fertilizers***

- ❖ Terra-Sorb Foliar® is PH. Contains free amino acids and low molecular weight peptides. Manufactured by Bioiberica S.A. (Spain).
- ❖ Naturamin WSP is PH. It contains up to 80% free amino acids, total nitrogen - 12.8% and organic nitrogen - 12.8%. Manufactured by Desarrollo Agrícola Y Mimero S. A. (DAYMSA), Spain

- ❖ Amino Expert® Impulse is a biostimulant containing a combination of amino acids, phytohormones, chelated elements, organic substances and vitamins, lactic acid, oligopeptides and natural wetting agents. Manufactured by Ecofol AD, Bulgaria.
- ❖ Polyplant 20/20/20 is a water-soluble granular fertilizer, contains macro- and microelements. Manufactured by Phytothreptiki S.A., Greece.

## 2.EXPERIMENTS

The experiments were performed in the laboratories of the Department of Plant Physiology and Biochemistry of the Agricultural University - Plovdiv and in the Laboratory of Artificial Climate- Moscow Agricultural Academy "K.A. Timiryazev ", Russia.

### 2.1. Experimental designs

Maize seeds are germinated in perlite and than transferred to light modules and are been grown as hydroponic (Photo 1) or hydroponic-substrate (Photo 2) culture under the following conditions: photoperiod - 14/10 hours (day / night) , illuminance (PHARE) - 170  $\mu\text{mol m}^{-2} \text{s}^{-1}$  and relative humidity - 55-60%.



**Photo 1.** General view of maize plants from the Kneza 307 hybrid and the P9528 hybrid, grown as an hydroponic culture on ½ modified Hogland nutrient solution.



**Photo 2.** General view of maize plants grown as a hydroponic-substrate culture.

### 2.3. Preliminary experimental designs

Preliminary experiments were performed with plants from the hybrid Kneza 307. The purpose of these experiments was to select the appropriate temperature and duration of the low temperature exposure, which can cause a chronic stress in the

young maize plants. Three constant growing temperatures were tested,  $5 \pm 1^\circ\text{C}$ ,  $10 \pm 1^\circ\text{C}$  and  $15 \pm 1^\circ\text{C}$ , with duration of the low-temperature exposure - 3, 7 and 10 days.

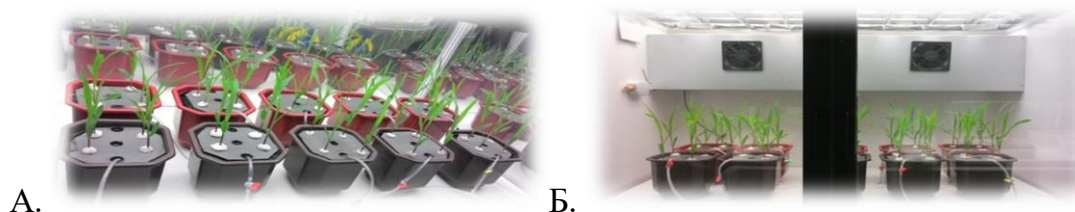
#### 2.4. Main experimental designs

##### ◆ *Experimental design 1*

Maize plants at the age of 3rd leaf were grown for 7 days at two different temperature regimes:  $25 \pm 1^\circ\text{C}$  /  $20 \pm 1^\circ\text{C}$  (day / night) and constant  $10 \pm 1^\circ\text{C}$  temperature. The experimental setup includes 4 variants, set in 3-fold repeatability (pot), with 4 plants in a pot, namely:

Var 1. Kneza 307 –  $25/20^\circ\text{C}$     Var 2. Kneza 307 –  $10^\circ\text{C}$

Var3. P9528 –  $25/20^\circ\text{C}$     Var 4. P9528 –  $10^\circ\text{C}$



**Photo 3. and Photo 4.** Appearance of maize plants grown at different temperature regime (A - regime  $25 \pm 1/20 \pm 1^\circ\text{C}$  day / night, B -  $10 \pm 1^\circ\text{C}$  constantly).

##### ◆ *Experimental design 2*

The experiment was derived with plants from the hybrid Kneza 307 as a hydroponic-substrate culture. Plants of 3rd leaf stage were used, which were developing for 14 days at two different temperature regimes (TP):  $25 \pm 1^\circ\text{C}$  /  $20 \pm 1^\circ\text{C}$  (day / night) and constant  $10 \pm 1^\circ\text{C}$  temperature). After 7 days, half of the plants were sprayed with 1% water solution of the biostimulant Terra-Sorb Foliar® at a dose of 1 ml / plant and were left to grow under the same conditions for another 7 days.

Var 1.  $25/20^\circ\text{C}$  ; without treatment    Var 2.  $10^\circ\text{C}$  , without treatment

Var 3.  $25/20^\circ\text{C}$  ; with treatment    Var 4.  $10^\circ\text{C}$ ; with treatment

##### ◆ *Experimental design 3*

The experiment was derived with plants from the hybrid Kneza 307 as a hydroponic-substrate culture. Plants of 3rd leaf stage were used, which were grown for 14 days at two different temperature regimes:  $25 \pm 1^\circ\text{C}$  /  $20 \pm 1^\circ\text{C}$  (day / night) and constant  $10 \pm 1^\circ\text{C}$  temperature. After 7 days, half of the plants were sprayed with 1% water solution of the biostimulant Terra-Sorb Foliar® - dose of 1 ml / plant and were left to grow under the same conditions for another 7 days.



**Photo 5.** General view of an experimental setting in which maize plants from the hybrid Kneza 307 are exposed to low temperature stress for a period of 14 days ( $10 \pm 1$  °C; constant) and sprayed medically with fertilizer products.

*Experiment 1.* The biostimulant Terra-Sorb Foliar® and the foliar fertilizer Polyplant were applied in 1% concentration of water solution. *Experiment 2.* The biostimulant Amino Expert Impuls® and its organic and inorganic components were sprayed in a concentration of 1%. *Experiment 3.* The biostimulant Terra-Sorb Foliar® was used at a concentration of 1% and Naturamin WSP at a concentration of 0.1%. The volume of treatment with the products was 1 ml / plant.

### 3.

## METHODS OF ANALYSIS

### 3.1. *Biometric parameters*

The analysis of fresh (FW) and dry (DW) mass of plants and their organs (roots and leaves) was determined by weight method. The dimensions of the height of the plants and the length of the roots - linear. Leaf area - with leaf area meter in cm<sup>2</sup> and the relative growth rate (RGR) according to Beadle, (1993).

### 3.2. *Content of mineral elements*

The content of total nitrogen was analyzed by a modified Keldal method, phosphorus (P<sub>2</sub>O<sub>5</sub>) - spectrophotometrically, and potassium (K<sub>2</sub>O) - flame photometer (Tomov et al., 1999). The content of the microelements Zn, Cu, Mn and Fe was determined by atomic emission spectrometry with inductively coupled plasma.

### 3.3. *Photosynthetic indicators*

#### *Photosynthetic pigments*

Photosynthetic pigments were determined spectrophotometrically by Lichtenthaler (1988).

#### *Leaf gas exchange*

Leaf gas exchange rates were determined with a portable photosynthetic system LCpro + [AnalyticalDevelopmentCompanyLtd., Hoddesdon, England].

#### *Chlorophyll fluorescence*

Chlorophyll fluorescence was determined with two equipments with different measurement principles - (1) pulse modulated fluorimeter MINI-PAM (H. Walz,



Effeltrich, Germany) and (2) fluorimeter for direct registration of chlorophyll fluorescence Handy-PEA (Hasatech instruments ., UK).

### ***3.4. Electrophysiological methods***

The bioelectrical reaction (BER) of leaf tissues was determined by an advanced method of Panichkin et al. (2009).

### ***3.5 Conductometric measurements***

Electrolyte leakage from plant tissues was determined conductometrically with a conductometer Expert - 002.

### ***3.6. Biochemical methods***

#### ***Guaiacol peroxidase (GPOD)***

GPOD activity (EC 1.11.1.7) was determined spectrophotometrically according to Bergmeyer et al., (1974).

#### ***Content of total phenols***

The total amount of phenolic compounds was determined by the method of Singleton and Rossi (1965), with slight modifications.

#### ***Anthocyanin content***

The amount of anthocyanins was measured by the method of Guisti and Wrolstad (2001).

#### ***Antiradical activity***

Antiradical activity was reported by the method of Shalaby et al., (2013).

#### ***Lipid peroxidation***

The degree of lipid peroxidation was determined by the method of Heath and Packer (1968).

### ***3.6. Statistical data processing***

Data are presented as means  $\pm$  SD. The results were processed statistically by one-way ANOVA analysis, followed by a Duncan test. The significance of the differences was determined at a significance level of 95% ( $P < 0.05$ ). The different letters (a, b, c or d) after the mean value show statistically significant differences between the compared variants.

## V. RESULTS AND DISCUSSION

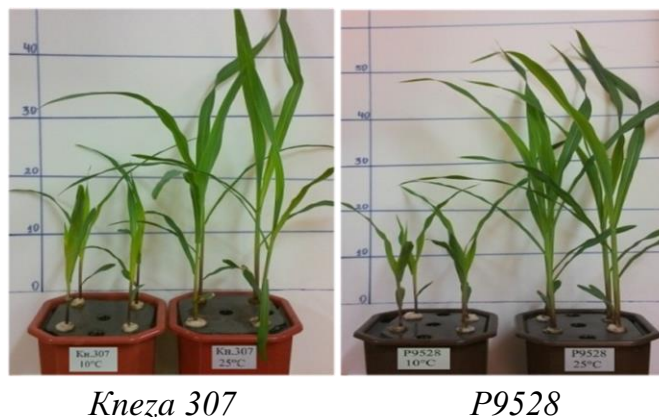
### 1. *Influence of the low positive temperature on the physiological and biochemical parameters of the young maize plants*

#### 1.1. *Visual symptoms and effects of low temperature stress on the young maize plants*

The selection of maize hybrids with improved low temperature tolerance would allow earlier sowing dates, avoiding the main limit factor of maize production - the drought stress. The research was carried out mainly with the new maize hybrid Knezha 307. Hybrid P9528, which is characterized by increased resistance to stress conditions during the germination period, was included also as a comparison variant.

Visual observations show that 7 days of exposure to low-temperature stress ( $10 \pm 1^\circ \text{C}$ ) maize plants from hybrid Knezha 307 and hybrid P9528 have a much lower growth rate than the control plants grown at optimum temperature ( $25 \pm 1^\circ \text{C}$ ). Growth can be seen on Photo 6A and 6B.

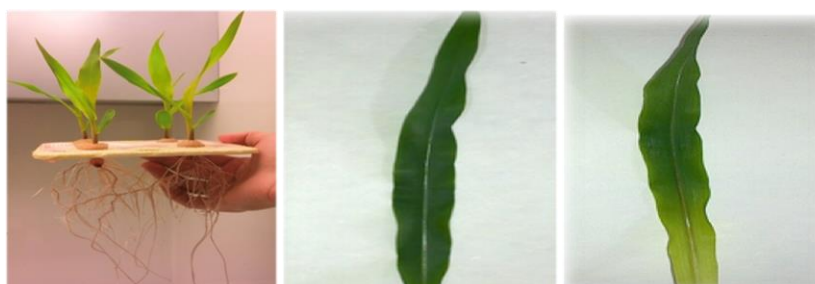
The results show that the height of the plants and the length of the roots decrease significantly, by 44% to 47%, in the hybrids Knezha 307 and P9528, respectively. Stressed maize plants do not form a new leaf, while control plants from both hybrids develop a fourth leaf. The fresh mass of stressed corn plants was reduced by 58% to 61% in the hybrids Knezha 307 and P9528, and the accumulation of dry mass was in the range of 47% - 48%. The leaf area differs significantly - 63% - 64% less.



**Photos 5 and 6.** Appearance of maize plants exposed to low temperature stress and plants grown at optimal temperature.

Also plants showed chlorotic symptoms (Photo 9), and the roots were characterized by less development and branching (Photo 7). The observed symptoms are identical to those described by Miedema (1982), Farooq et. al. (2009) and Riva-

Roveda et al. (2016) for the leaves and Kiel and Stamp (1992) for the roots of maize plants subjected to low temperatures.



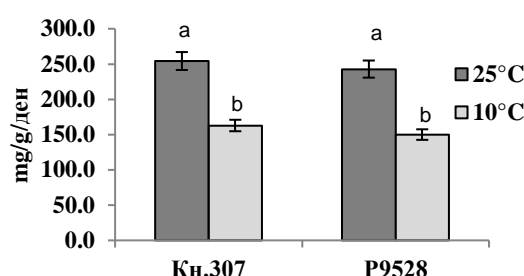
**Photo.7**

**Photo.8**

**Photo.9**

**Photos 7, 8 and 9.** Appearance of maize plants exposed to low temperature (Fig. 7), leaf of a plant at optimal temperature (Fig. 8) and leaf of a plant subjected to low temperature (Fig. 9).

The relative growth rate (RGR), gave good idea of the daily growth of the chilled plants. The data presented in Figure 1 show that the RGR in the control plants was in the range 240 - 250 mg / g fresh weight / day, while in the low- stressed plants, it was 36% to 38% lower.



**Fig 1.** Influence of 7-day low-temperature effect on the relative growth rate of young maize plants from the hybrid Kneza 307 and the hybrid P9528. The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The results of the performed biometric measurements and the observed visual symptoms show that the applied 7-day low temperature stress ( $10 \pm 1^{\circ}\text{C}$ ) has a significant inhibitory effect on the growth of maize plants from the hybrids Kneza 307 and P9528. Our research also confirm the fact that low positive temperatures strongly affects the biometric parameters, changes the root morphology and causes chlorotic changes in the leaves of maize plants, which are in line with the studies of a number of authors (Leipner, 2009; Hodges et al., 1997, Aroca et al., 2003; Ribas-Carbo et al., 2000; Reimer, 2010). Although in our studies there is no wilting of plants, infiltration of fluids, necrosis of leaves and other characteristic symptoms that have been described by Lukatkin et al. (2012). Our results support the opinion of Kaspar and

Bland (1992) that low temperature has a much stronger effect on the length of the roots than on their dry mass. This effect, together with the established stronger reduction of fresh compared to dry mass of plants, shows that low temperature stress disrupt the water status of maize plants (Ben-Haj-Salah and Tardieu, 1995).

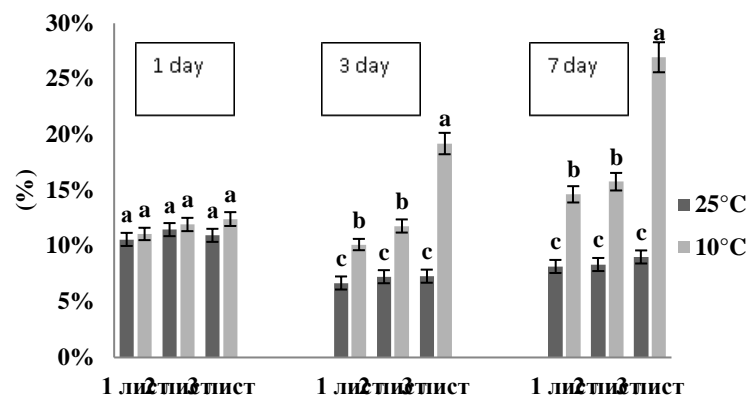
The physiological state of the chilled maize plants was monitored by analysis of their bioelectrical reaction (BER) and their redox status. The data show that the control and low- stressed maize plants differ in some electrophysiological parameters of BER (Table 1). One day low temperature stress increased the functional activity of the leaves, visible from the smaller RP, compared to the number of control plants (29.83 to 48.00 mV, but did not significantly affected them. However, the functional activity of the leaves is sufficiently reduced, 2 to 4 times, after 3 and 7 days of low temperature exposure. The lower functional activity of the 3- and 7-day low-temperature exposed maize plants, obviously speaks of the presence of various structural and functional facilities that modify these BER parameters. It should be note also that the negative result is not that sever after 3 days of low temperature stress, which is probably due to the process of acclimatization of maize plants.

**Table 1.** Influence of 7-day low-temperature effect on bioelectrical reaction of young maize plants from the hybrid Kneza 307.

Var.	Ex. RP (mV)	Amplitude of BER (mV)	Time depolarisation. (s)	Time repolarisation. (s)	Area of regulation. (cm <sup>2</sup> )
1 day					
Control 25°C	48,00±1,6 <sup>a</sup>	15,16±1,2 <sup>a</sup>	126,67±7,0 <sup>a</sup>	153,33±41,7 <sup>a</sup>	1025,56±128,8 <sup>a</sup>
Chilling 10°C	29,83±2,7 <sup>b</sup>	12,72±1,5 <sup>a</sup>	66,22±1,0 <sup>b</sup>	97,53±22,9 <sup>a</sup>	1240,72±264,7 <sup>a</sup>
3day					
Control 25°C	-16,50±5,9 <sup>b</sup>	10,92±5,9 <sup>a</sup>	115,83±2,5 <sup>a</sup>	126,66±20,1 <sup>a</sup>	1281,11±125,5 <sup>a</sup>
Chilling 10 °C	-52.33±7,4 <sup>a</sup>	23,00±3,0 <sup>b</sup>	120,00±20,2 <sup>b</sup>	212,50±51,1 <sup>b</sup>	4802,50±242,2 <sup>b</sup>
7 day					
Control 25°C	-28,17±8,5 <sup>a</sup>	10,33±1,1 <sup>a</sup>	131,67±1,6 <sup>a</sup>	150,00±20,0 <sup>a</sup>	1570,9±106,6 <sup>a</sup>
Chilling 10°C	-38,50±12,7 <sup>a</sup>	18,67±3,2 <sup>b</sup>	115,83±18,3 <sup>b</sup>	185,00±26,1 <sup>b</sup>	3525,56±358,3 <sup>b</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

Electrolyte leaking, which is an indicator of changes in cell membrane permeability, shows that the amount of released electrolytes of the chilled maize plants, depends on the age of the leaves exoped to chilling stress. (Figure 2).



**Fig 2.** Influence of 7-day low-temperature effect on the electrolyte leakage of young maize plants from the hybrid Kneza 307 and the hybrid P9528. The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

One-day exposure of maize plants at low temperatures did not have a detrimental effect on the functioning of the cell membranes of the 1st, 2nd and 3rd leaf of maize plants. Differences in leaf sensitivity appeared on the third day and more strongly on the seventh day. The highest degree of disruption of cell membranes is observed in the actively growing III leaf. The proportion of electrolytes released from it is almost 2 times higher than the other leaves and 2 times higher than the control variant.

Our results correspond to the data of Lukatkin (1997), Markowski and Skrudlik (1995) and Grzybowski et al. (2019), who observed a strong exoosmosis of electrolytes from the leaves of young maize plants subjected to low temperature stress. The higher damage of the third, youngest and actively growing leaf of the stressed maize plants in comparison with the first, older leaf does not correspond to the data of Panichkin et al. (2009) obtained in cucumber plants subjected to low temperature stress.

Low temperature exposure has been shown to cause oxidative stress in maize plants, with the degree of damage depending on the applied temperature and the duration of exposure (Fryer et al., 1998; Leipner et al., 1999; Pastori et al., 2000). In our studies, the activity of the antioxidant enzyme - guaiacol peroxidase (GPOD) after 7 days of exposure of maize plants at low temperatures, increased significantly only in the roots (Table 2). The data show that the activity of GPOD in the leaves is increased by 17% in the plants of the hybrid Kneza 307 and by 5% in the plants of the hybrid P9528. The GPOD values in the roots increased by 34% in the plants from Knezha 307 and by 32% in P9528.

**Table 2.** Influence of 7-day low-temperature effect on GPOD level ( $U\text{ mg g}^{-1}\text{FW}$ ) of young maize plants from the hybrid Kneza 307 and P9528.

Var.	GPOD	
	Leaves	Roots
Kneza 307		
Control 25°C	$1,56 \pm 0,29^a$	$7,79 \pm 0,40^b$
Chilling 10°C	$1,83 \pm 0,06^a$	$10,44 \pm 0,36^a$
P9528		
Control 25°C	$1,33 \pm 0,07^a$	$7,13 \pm 0,48^b$
Chilling 10°C	$1,40 \pm 0,09^a$	$9,42 \pm 0,79^a$

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

When the antioxidant defense system is unable to control the reactive oxygen species (ROS) at a safe level, oxidative damage to macromolecules occurs. Among the most characteristic negative effects of ROS is lipid peroxidation. The analysis of lipid peroxidation confirmed the presence of oxidative stress in the chilled maize plants (Table 3). Statistically significant differences with control plants on this indicator appear only after 7 days of exposure.

The content of MDA in the leaves and roots of stressed Kneza 307 is increased by 55% and 36%, respectively, and in those of P9528 by 30% and 34%.

**Table 3.** Influence of 7-day low-temperature effect on MDA level ( $\text{nmol MDA g}^{-1}\text{FW}$ ) of young maize plants from the hybrid Kneza 307 and P9528.

Var.	MDA	
	Leaf	Roots
Kneza 307		
Control 25°C	$18,12 \pm 1,44^b$	$8,96 \pm 0,05^b$
Chilling 10 °C	$28,00 \pm 0,89^a$	$12,19 \pm 1,44^a$
P9528		
Control 25°C	$21,70 \pm 1,01^b$	$9,51 \pm 1,27^b$
Chilling 10 °C	$28,21 \pm 0,25^a$	$12,71 \pm 0,51^a$

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The high sensitivity of photosynthesis to low positive temperatures in C-4 type plants, such as maize, has long been researched (Stamp, 1984; Long et al., 1994). The main structural and functional disorders in the photosynthetic process that low temperatures cause in maize plants are well documented, but there are also

incompletely clarified issues that keep the problem relevant, both theoretically and practically.

The photosynthetic response of maize plants to low temperature stress was studied by analyzes on foliar gas exchange, content and relationships between photosynthetic pigments and chlorophyll fluorescence. Most of the research was conducted with the new Bulgarian maize hybrid Kneza 307. In some of the research we included the hybrid P9528, which is characterized by increased tolerance to stress factors during germination. The obtained information serves to compare the actual photosynthetic activity of maize plants from the two variants. The data presented in Table 4 clearly show the negative effect of low temperature impact on the potential foliar gas exchange of maize plants. The applied low temperature ( $10 \pm 1^\circ\text{C}$ ) reduces the net photosynthetic rate (A) by 67% in the plants from Kneza 307 and by 44% in those from P9528. The intensity of transpiration (E) decreased significantly - by 66% and by 39% in the plants of the hybrid Kneza 307 and P9528, respectively. The changes in the stomata conductivity ( $g_s$ ) follow a similar trend, as again the decrease in Kneza 307 is bigger (80%) than in P9528 (50%). The opposite trend is observed in the intercellular concentration of  $\text{CO}_2$  ( $c_i$ ). The values in the stressed plants increased - by 39% in Kneza 307 and by 48% in P9528. The applied chilling had a strong negative effect on the integral photosynthetic process (A) in maize plants. According to Long and Spence (2013) and Salesse-Smith et al. (2019) carbon assimilation in maize under chronic low temperature ( $10\text{-}14^\circ\text{C}$ ) effects can be reduced by 50-60%, which fully corresponds to our results.

**Table 4.** Influence of 7-day low-temperature effect on the parameters of leaf gas exchange in the leaves of young maize plants from the hybrid Kneza 307 and the hybrid P9528. A - rate of net photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), E - intensity of transpiration ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ ),  $g_s$  - stomata conductivity ( $\text{mol m}^{-2} \text{ s}^{-1}$ ) and  $c_i$  - intercellular  $\text{CO}_2$  concentration (vpm).

Var.	Leaf gas exchange			
	A	E	$g_s$	$c_i$
Кнежа 307				
Control $25^\circ\text{C}$	$16,69 \pm 0,11^a$	$0,71 \pm 0,04^b$	$0,05 \pm 0,01^b$	$121 \pm 6^b$
Chilling $10^\circ\text{C}$	$5,43 \pm 0,01^c$	$0,24 \pm 0,08^c$	$0,01 \pm 0,00^c$	$168 \pm 2^a$
P9528				
Control $25^\circ\text{C}$	$18,09 \pm 0,10^a$	$1,08 \pm 0,07^a$	$0,08 \pm 0,01^a$	$61 \pm 8^d$
Chilling $10^\circ\text{C}$	$10,07 \pm 0,15^b$	$0,66 \pm 0,09^b$	$0,04 \pm 0,01^b$	$90 \pm 9^c$

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The significant difference in the inhibition of A in the plants of the two maize hybrids (over 20%) demonstrates the presence of genotypic specificity in the photosynthetic response to the applied low temperature stress, also found by Aroca et al. (2001) and Wijewardana et al. (2016).

The difference in the degree of inhibition of carbon assimilation of the two hybrids to the applied 7-day low temperature exposure may be due to different photosynthetic sensitivity (Grzybowski et al., 2019 ). It is known that photosynthesis can be limited by stomata and mesophilic factors. Considering the decrease of the values of A, E and  $g_s$  in stressed plants and the increases of the intercellular concentration of CO<sub>2</sub> (ci), we can assume that mesophilic limiting factors predominate in the inhibition of A.

Chlorotic changes show that the content of photosynthetic pigments in the low-temperature maize plants is lower than in the control plants. The results obtained, presented in Table 5, confirm the observed visual chlorotic symptoms. The total content of chlorophyll in the stressed plants was significantly reduced compared to the level in the control plants - by 46% and 21% in Kneza 307 and P9528, respectively.

**Table 5.** Influence of 7-day low-temperature effect on the content and relationships between photosynthetic pigments in the leaves of young maize plants from the hybrid Kneza 307 and the hybrid P9528.

Var.	Photosynthetic pigments				
	Chl. (a)	Chl.( b)	Car.	Chl. (a+b)	Car./ Chl.(a+b)
Kneza 307					
Control 25°C	3,12±0,29 <sup>a</sup>	1,00±0,08 <sup>a</sup>	0,85±0,06 <sup>a</sup>	4,12±0,12 <sup>a</sup>	0,21 ±0,01 <sup>b</sup>
Chilling 10°C	1,76±0,04 <sup>d</sup>	0,48±0,05 <sup>d</sup>	0,65±0,05 <sup>c</sup>	2,24±0,07 <sup>c</sup>	0,29 ±0,03 <sup>a</sup>
P9528					
Control 25°C	2,35±0,19 <sup>b</sup>	0,87±0,08 <sup>b</sup>	0,73±0,09 <sup>b</sup>	3,22±0,07 <sup>b</sup>	0,23± 0,00 <sup>b</sup>
Chilling 10°C	1,94±0,10 <sup>c</sup>	0,59±0,07 <sup>c</sup>	0,70±0,12 <sup>b</sup>	2,53±0,14 <sup>c</sup>	0,28± 0,02 <sup>a</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

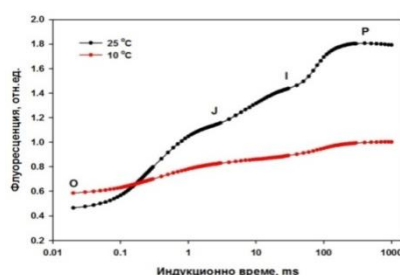
Low temperature had a lower effect on carotenoid content than chlorophyll. The negative effect on these pigments is 24% in the plants of the Kneza 307 and significantly less - 4% in those of P9528. The changes affect not only the content of total chlorophyll and carotenoids, but also their ratio. The carotenoid / chlorophyll ratio is 38% higher in chilled Kneza 307 plants and 22% higher in the chilled P9528.



Our results are in accordance with the data of a number of authors who studied the content of photosynthetic pigments in maize plants under conditions close to our experimental design (Kosova et al., 2005; Leipner, 2009; Riva-Roveda et al., 2016). . Decreased chlorophyll content may result from both inhibited biosynthesis and accelerated chlorophyll degradation (Riva-Roveda et al., 2016; Leipner et al., 2009). The lower inhibition of carotenoids compared to chlorophyll in stressed maize plants, leading to an increased carotenoid / chlorophyll ratio, can be explained by the protective role of the carotenoid pigments - the protection of chlorophyll from reactive oxygen species (ROS). In the studies of Kosova et al. (2005), Leipner et al. (2009) and Riva-Roveda et al. (2016) found that the content of total carotenoids in stressed plants does not change significantly, which support our observations.

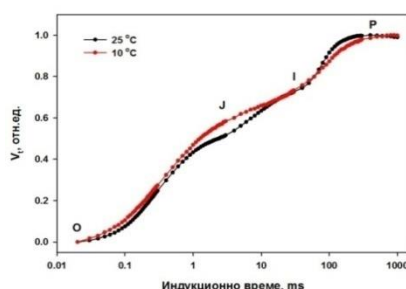
Objective information about light-dependent photosynthetic reactions can be obtained by analysis of chlorophyll fluorescence (CF) (Goltsev et al., 2014). In our studies with maize plants, both types of CF measuring instruments were used. The induction kinetics of CF was determined with Handy-PEA, and the quantum yield (Y), the photochemical (qP) and non-photochemical (qN) quenching, etc. were determined with MINI-PAM.

The results presented in Figure 3A, showing that point O is located higher and point P is significantly lower in stressed plants than in control plants. In essence, these points indicate the initial (F<sub>0</sub>) and maximum (F<sub>m</sub>) values of chlorophyll fluorescence. The increase in F<sub>0</sub> indicates that the low temperature effect reduces the quantum efficiency of FS2, and the decrease in F<sub>m</sub> is probably the result of a complex of reasons, among which is the reduced chlorophyll content of the leaves in the stressed plants.



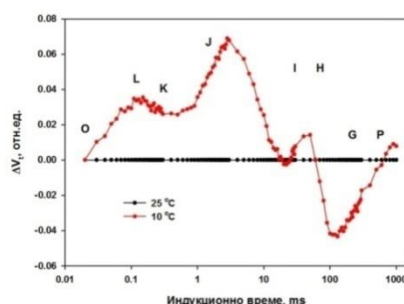
**Fig 3A.** Chlorophyll fluorescence (F) induction curves recorded at 3500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PHAR for 1 s in dark adapted leaves of 14-day-old maize plants (*Zea mays*): grown at ( $25 \pm 1^\circ\text{C}$ ) (control plants, black) ) and at ( $10 \pm 1^\circ\text{C}$ ) (red). The characteristic points of the curves - O, J, I and P, occurring at 0.02, 2, 30 and  $\sim 300$  ms after the beginning of the measurement, are marked. Each point corresponds to a certain level of reduction of transporters in the ETR of thylakoid membranes - from 0% at O to 100% at P.

The results of Figure 3B using the JIP test of Strasser et. al. (2004), show us an increased J peak in stressed plants, which means reduced quantum efficiency of electronic transport from the reaction centers of PH2 to electronic carriers from the plastoquinone (PH) pool. An increase in peak J in low -stressed maize plants was also found by Franic et al. (2020), but the authors indicate that peak I also increases, which differs from our results.



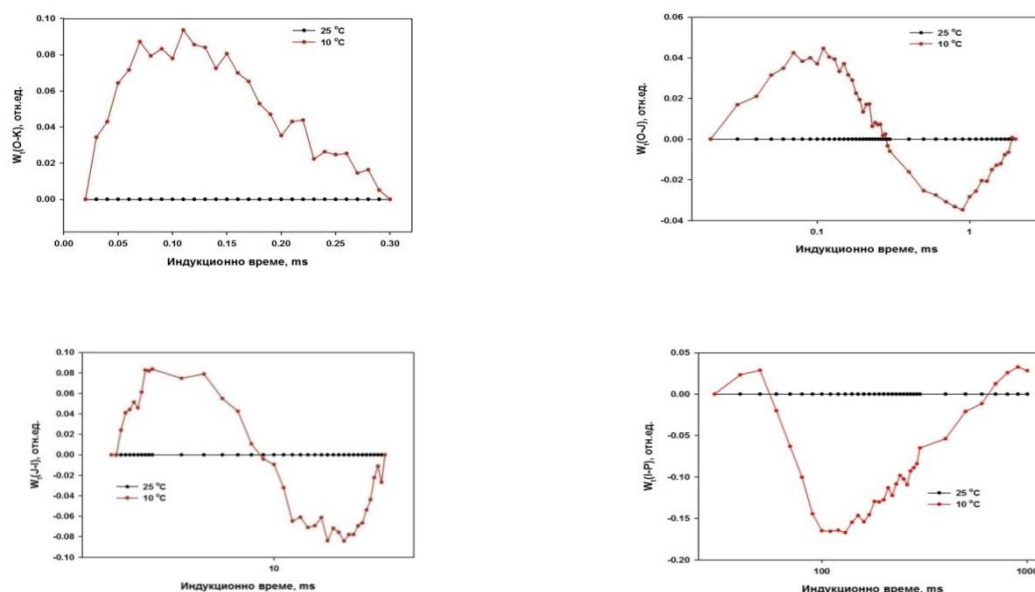
**Fig 3B.** Induction curves of the relative variable chlorophyll fluorescence ( $V_t$ ) obtained after double normalization of the values from A to the values of O and P for each curve.

Figure 3C shows more detailed analysis of the processes occurring in certain time intervals during the induction transition, we can consider individual parts of the IC (O - K, O - J, J - I and I - P).



**Fig 3C.** Curve of the difference  $\Delta V_t$  between  $V_t$  for the stressed and control plants, obtained by subtracting the values of the stressed plants from the respective values of the control plants. In addition to the characteristic points O, J, I and P, the intermediate bands L, K, H and G, which are differentiated in the differential curves at approximately 0.1, 0.3, 50 and 100 ms, are also marked.

This approach allows the identification of intermediate bands (local minimal and maximal) by induction time, marked as L, K, H and G. The information they provide is described by Strasser et al. (2004) and Stirbet and Govindjee (2012) and Samborska et al. (2019). In the available literature there is no data on changes in the fluorescent bands L, K, H and G in plants subjected to low temperature stress, so the results are compared with data from studies with other stressors - heavy metals (Paunov et al., 2018), mineral deficiencies (Samborska et al., 2019) and high temperatures (Dimitrova et al., 2020).



**Fig 4.** Differential curves of the relative variable chlorophyll fluorescence ( $W$ ), double normalized in the induction intervals: from  $O$  to  $K$  (A), from  $O$  to  $J$  (B), from  $J$  to  $I$  (C) and from  $I$  to  $P$  (D), obtained when subtracting the values of the stressed plants from the corresponding values for the control plants.

The L peak (Figure 4A) shows that the connection between the reaction center and the antenna complexes of PS2 (Strasser and Stirbet, 2001; Stirbet, 2013) decreased in plants subjected to low temperature stress, which was in accordance with the higher  $O$  level of the curve in Figure 3A. An identical increase in the L peak was noted by Paunov et al. (2018) in wheat plants exposed to heavy metals. The authors associated this effect with decreased excitatory energy transfer between adjacent photosynthetic units and impaired stacking of thylakoid membranes. Those facts are also observed in maize plants subjected to low temperature stress (Grzybowski et al., 2019).

The K peak reflects the function of the donor part of PS2 and the stability of the oxygen-releasing system (ORS) (Strasser et al., 1996). In our measurements, the K peak was not detected because the differential curve (Figure 4B) showed a sigmoidal course (positive peak at the beginning and negative peak at the end). Its first part overlapped with the course of the curve for L, i.e. the positive peak registered at the beginning was in fact a strong L peak. The lack of a positive K peak indicated that the low temperature stress did not cause serious disturbances in the oxygen-releasing system (ORS) of the stressed maize plants. In the studies of Franic et al. (2020) with maize plants was established K peak, which is probably due to the stronger damage of the photosynthetic apparatus from the lower applied temperature (5°C).

The H band shows the relative size of the free plastochin pool (Strasser and Tsimilli-Michael 1998), i. the number of plastochin molecules available for reduction

compared to their number in the control. In stressed maize plants, no H peak was observed (Figure 4C) due to the sigmoidal nature of the differential curve. This result can be interpreted as preserving the relative volume of the electronic carrier pool between the two photosystems.

Strip G gives an idea of the efficiency of electronic transport to the final acceptors of PS1 (Strasser et al., 2004) and its relative content (Ceppi et al., 2012). It can be assumed that G mainly reflects the relative size of the NADP pool, which is available for reduction. In the case of a positive band, it means it is smaller, and in the case of a negative band, it is larger compared to the control. In our studies, a well-defined negative G peak was observed (Figure 4D), which means that at low temperatures the number of available NADP molecules increases. Similar results were obtained by Dimitrova et al. (2020) in studies with leaves of the Italian plane tree ecotype (*Platanus orientalis*) subjected to high temperature stress.

Measurements with MINI-PAM in dark and light-adapted leaves provide additional information about the condition of the photosynthetic apparatus in the low-temperature maize plants from the Kneza 307 and P9528 hybrids.

The results presented in Table 6 show that the values of the maximum photochemical activity of PS2 ( $F_v / F_m$ ) in the control plants was in the range of 0.75 - 0.83, typical for healthy leaves (Bolhar-Nordenkamp and Oquist, 1993). In stressed plants,  $F_v / F_m$  decreased, and this effect was more pronounced in Kneza 307 plants (by 15%) and less pronounced in P9528 (by 8%). The decrease in  $F_v / F_m$  in low-temperature stressed plants is due to an increase in  $F_0$  and a decrease in  $F_m$ . The values of  $F_0$  and  $F_m$  are not presented, but the indicated changes in these parameters are reflected in the induction fluorescence kinetics presented in Figure 3A.

Decreases in  $F_v / F_m$  in low stressed maize plants have been found by a number of authors (Kosova et al., 2005; Yang et al., 2009; Riva-Roveda et al., 2016; Grzybowski et al., 2019; Salesse-Smith et al., 2019). The degree of  $F_v / F_m$  inhibition varies from very low (Yang et al., 2009) to moderate (Riva-Roveda et al., 2016; Grzybowski et al., 2019; Long et al., 1994) depending on the strength of the applied effect. and hybrid tolerance.

**Table 6.** Influence of 7-day low-temperature effect on chlorophyll fluorescence in dark and light-adapted leaves in young maize plants from the Knezha 307 hybrid and the P9528 hybrid. *F<sub>0</sub>* - minimal fluorescence; *F<sub>m</sub>* - maximum fluorescence; *F<sub>v</sub> / F<sub>m</sub>* - potential photochemical activity of FSII; *ETR* - speed of photosynthetic electronic transport; *qP* - photochemical quenching and *qN* - non-photochemical quenching of chlorophyll fluorescence.

Var.	Chlorophyll fluorescence			
	<i>F<sub>v</sub>/F<sub>m</sub></i>	<i>ETR</i>	<i>qP</i>	<i>qN</i>
Kneza 307				
Control 25°C	0,78±0,03 <sup>a</sup>	42,2±3,2 <sup>a</sup>	0,477±0,11 <sup>a</sup>	0,293±0,15 <sup>c</sup>
Chilling 10°C	0,66±0,02 <sup>c</sup>	23.8±5.1 <sup>b</sup>	0,446±0,06 <sup>b</sup>	0,577±0,10 <sup>a</sup>
P9528				
Control 25°C	0,78±0,03 <sup>a</sup>	38,1±3,0 <sup>a</sup>	0,420±0,20 <sup>c</sup>	0,241±0,08 <sup>d</sup>
Chilling 10°C	0,72±0,01 <sup>b</sup>	28,3±4,2 <sup>b</sup>	0,397±0,14 <sup>c</sup>	0,415±0,12 <sup>b</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

Table 6 presents the values of the rate of photosynthetic electronic transport (*ETR*), photochemical (*qP*) and non-photochemical (*qN*) quenching of chlorophyll fluorescence in the analyzed maize plants. The data show that the chilling significantly reduced *ETR* (by 44%) and less affected *qP* (by 6%) in the plants of the Kneza 307 hybrid. The corresponding values for P9528 were 26% and 5%. At the same time, the values of *qN* increase significantly - by 97% in Kneza 307 and by 72% in P9528. The obtained results showed that *ETR* in the stressed plants of the two hybrids is suppressed to a significantly higher degree compared to *F<sub>v</sub>/F<sub>m</sub>*. On the other hand, the main part of the absorbed light energy is dissipated as heat, an indication of which is the sharp increase in *qN*. The observed trends of change in the considered fluorescent parameters correspond to the data of Riva-Roveda et al. (2016), Grzybowski et al (2019) and Salesse-Smith et al. (2019) for maize plants subjected under low temperature stress.

## 2. Comparative study of effects of foliar applied products on physiological and biochemical parameters of maize plants

The effects of foliar fertilizers and biostimulants in chilled plants have been relatively poorly studied (Polo et al., 2006; Marfà et al., 2009; Botta, 2013), and studies with maize plants are almost non-existent. In the following trials, we studied the effects of foliar applied biostimulants and fertilizers on maize plants from the hybrid Kneza 307, subjected to low temperature stress. The studies included 3

biostimulant products (Terra-Sorb Foliar, Naturamin WSP, Amino Expert Impuls) and one foliar fertilizer (Polyplant).

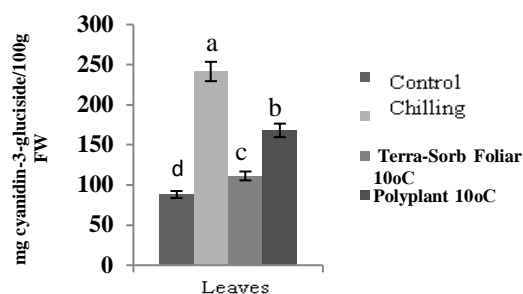
In order to test the hypothesis of a possible positive effect of foliar products on the redox status of stressed maize plants, comparative studies were performed between the biostimulant Terra-Sorb Foliar and the fertilizer Polyplant. The low temperature effect the antioxidant defense system of maize plants (Table 7). The total antioxidant activity (OAA) of stressed maize plants was increased by 134% in the leaves and by 14% in the roots compared to the control plants.

**Table 7.** Effects of biostimulant Terra-Sorb Foliar and foliar fertilizer Polyplant on the total antioxidant activity (%) in the organs of maize plants exposed to low temperature stress.

Var.	(OAA;%)	
	Leaves	Roots
Control 25°C	27,1±0,6 <sup>c</sup>	11,7±0,1 <sup>b</sup>
Chilling 10°C	63,5±0,3 <sup>a</sup>	13,7 ±1,1 <sup>a</sup>
Terra-Sorb Foliar 10°C	47,3±0,2 <sup>b</sup>	10,3±0,5 <sup>c</sup>
Polyplant 10°C	59,2 ±1,2 <sup>a</sup>	10,0 ±0,1 <sup>c</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The leaf application of the products Terra-Sorb Foliar and Polyplant reduced OAA. OAA levels in Terra-Sorb Foliar-treated plants were reduced by 25% in roots and leaves, and in Polyplant by 27% and 7%, respectively. These results show that the applied foliar products alleviate the stress effects of low temperatures, as a result of which the OAA is normalized. The low temperature effect increased the content of anthocyanins in the leaves and total phenols in the leaves and roots of maize plants, which corresponds to their increased OAA level. Specifically, the content of anthocyanins in the leaves of stressed plants was increased more than 2 times, and in total phenols - by 75% in the leaves and by 42% in the roots. The results obtained for the accumulation of anthocyanins and phenols correspond to the results of Zhou et al. (2018) in low-temperature tobacco plants, as well as those summarized by Sharma et al. (2019).



**Figure 5.** Effects of the biostimulant Terra-Sorb Foliar and the foliar fertilizer Polyplant on the content of anthocyanins (mg cyanidin-3-glucoside / 100g FW) in the leaves of maize plants exposed to low temperature stress. The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The application of Terra-Sorb Foliar and Polyplant lowers the levels of anthocyanins and total phenols (Table 8) in stressed maize plants, which is probably the reason for their reduced OAA. The content of anthocyanins in the leaves of plants treated with Terra-Sorb Foliar is 54%, and in those with Polyplant 30% lower than in untreated plants.

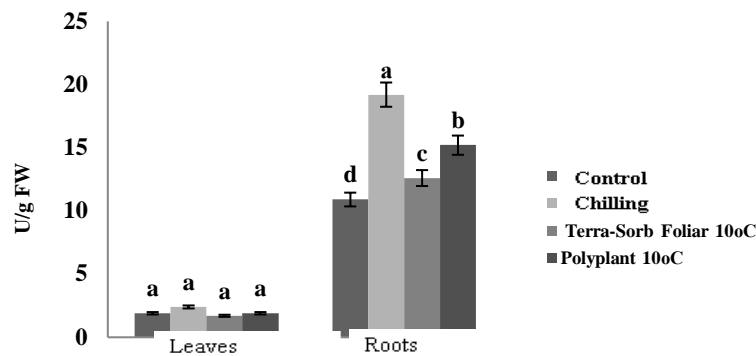
**Table 8.** Effects of the biostimulant Terra-Sorb Foliar and the foliar fertilizer Polyplant on the content of total phenols (mg GAE / g FW) in the organs of maize plants exposed to low temperature stress.

Var.	Total phenols	
	Leaves	Roots
Control 25 °C	6,1±0,8 <sup>b</sup>	4,3±0,5 <sup>b</sup>
Chilling 10 °C	10,7±0,2 <sup>a</sup>	6,1 ±1,5 <sup>a</sup>
Terra-Sorb Foliar 10°C	9,7±1,1 <sup>a</sup>	4,3±0,3 <sup>b</sup>
Polyplant 10°C	9,5±1,0 <sup>a</sup>	6,0±0,5 <sup>a</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The applied foliar products reduced the total phenols in the plant organs, in the leaves of treated by Terra-Sorb Foliar (9%) and by Polyplant (11%). The decrease of phenols in the roots of plants treated with Terra-Sorb Foliar is more pronounced and statistically proven (30%), and by the fertilizer is insignificant (2%).

Exposure to low positive temperatures increased guaiacol peroxidase (GPOD) activity in maize by 26% in leaves and 76% in roots (Figure 6).



**Figure 6.** Effects of the biostimulant Terra-Sorb Foliar and the foliar fertilizer Polyplant on the activity of the enzyme guaiacol peroxidase-GPOD (U / g FW) in the organs of maize plants exposed to low temperature stress. The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

In the roots of Terra-Sorb Foliar-treated plants, GPOD activity was reduced by 34% compared to the untreated plants. GPOD values in the organs of Polyplant were reduced to an identical degree, by 21%. The observed changes in GPOD activity are consistent with changes in the content of non-enzymatic antioxidants. Activation of antioxidant enzymes, including GPOD, under stress is a well-known fact, cited many times, including with low positive temperatures in maize plants (Fryer et al., 1998; Leipner et al., 1999; Khorshidi and Moafi ; 2014). The results obtained, in general, confirm the hypothesis of a positive effect of the applied foliar products on the physiological status of stressed maize plants, as their antioxidant potential is been normalized and their photosynthetic activity is been increased. Obviously, the applied foliar products are involved in the processes of acclimatization of plants to stress. Several possibilities are discussed, concerning the exact mechanisms of action of this effect. The main components with antioxidant potential in the applied foliar products are the free amino acids in the biostimulant Terra-Sorb Foliar and the microelements Zn, Mn, Cu in the water-soluble fertilizer Polyplant. Sourì (2016) and Teixeira et al. (2017) believe that the application of amino acids lowers the level of reactive oxygen species (ROS), as a result the oxidative stress in plants is reduced. Nahed et al (2010) show that foliar application of amino acids increases the content of total hydrocarbons and polysaccharides in stressed plants, which increases the possibilities of fast regenerating processes. Sadak et al. (2015) and Sadak and Abdelhamid (2015) accept that exogenous amino acids are included in the composition of important proteins that are involved in coping with stress.



On the other hand, Dimkpa and Bindraban (2016) point out the important role of micronutrients in protecting plants from stressors. The improved physiological status of the corn plants stressed by the low temperatures, as a result of the applied foliar products, is a prerequisite for faster recovery and growth in the post-stress period. This opinion corresponds to the opinion of Riva-Roveda et al. (2016) that more temperature-tolerant maize hybrids have a better physiological status and have been prepared for rapid growth (“ready to grow”) when the appropriate temperature conditions are restored.

The effect of Amino Expert Impuls and its components and the comparison of PH (Terra-Sorb and Naturamin WSP) on maize plants were evaluated by analysis of photosynthetic parameters.

The results obtained from the influence of Amino Expert Impuls and its components are presented in Tables 9, 10 and 11.

**Table 9.** Comparative effects of the biostimulant Amino Expert Impuls and its components on the parameters of leaf gas exchange in young maize plants from the hybrid Kneza 307. A - rate of net photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); E - intensity of transpiration ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ );  $g_s$  – stomata conductivity ( $\text{mol m}^{-2} \text{ s}^{-1}$ ). BS - biostimulant, OC - organic component, MC - mineral component.

Var.	Leaf gas exchange		
	A	E	$g_s$
Control 25°C	17,39±1,14 <sup>a</sup>	1,16±0,42 <sup>a</sup>	0,07±0,00 <sup>a</sup>
Chilling 10°C	11,94±0,10 <sup>c</sup>	0,65±0,19 <sup>b</sup>	0,04±0,00 <sup>b</sup>
BS 10°C	14,00±0,18 <sup>b</sup>	0,87±0,15 <sup>b</sup>	0,04±0,00 <sup>b</sup>
OC 10°C	14,04±0,18 <sup>b</sup>	0,82±0,15 <sup>b</sup>	0,04±0,00 <sup>b</sup>
MC 10°C	13,78±0,16 <sup>b</sup>	0,71±0,36 <sup>b</sup>	0,04±0,0 <sup>b</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The low temperature stress lowered the parameters of leaf gas exchange in maize plants (Table 9). The application of the biostimulant Amino Expert Impuls and its organic and inorganic component restored, to some extent, the rate of photosynthesis (A) in stressed plants, by 17%, 18% and 15%, respectively, but without reaching the value of control, unstressed plants. .

Table 10 presents results reflecting the effects of low temperature exposure and applied foliar products on photosynthetic pigments in maize plants. The data shows that the content of chlorophyll (a + b) in stressed plants is

lower by 36% and the content of carotenoids by 24%, which again corresponds to our previous studies.

**Table 10.** Effects of the biostimulant Amino Expert Impuls and its components on the content and relationship between photosynthetic pigments in the leaves of young maize plants. BS - biostimulant, OC - organic component, MC - mineral component.

Var.	Photosynthetic pigments				
	Chl.(a)	Chl. (b)	Chl. (a+b)	Car.	Chl.(a+b)/ Car.
Control 25°C	3,59±0,33 <sup>a</sup>	0,86±0,05 <sup>a</sup>	4,45±0,25 <sup>a</sup>	1,36±0,12 <sup>a</sup>	3,27±0,14 <sup>a</sup>
Chilling 10°C	2,16±0,02 <sup>b</sup>	0,66±0,06 <sup>b</sup>	2,82±0,04 <sup>b</sup>	1,04±0,00 <sup>b</sup>	2,71±0,05 <sup>b</sup>
BS 10°C	2,36±0,12 <sup>b</sup>	0,75±0,04 <sup>b</sup>	3,11±0,09 <sup>b</sup>	1,23±0,07 <sup>b</sup>	2,53±0,09 <sup>b</sup>
OC 10°C	2,30±0,04 <sup>b</sup>	0,70±0,01 <sup>b</sup>	3,00±0,06 <sup>b</sup>	1,14±0,00 <sup>b</sup>	2,63±0,00 <sup>b</sup>
MC10°C	2,24±0,00 <sup>b</sup>	0,69±0,03 <sup>b</sup>	2,93±0,10 <sup>b</sup>	1,11±0,05 <sup>b</sup>	2,64±0,03 <sup>b</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The application of Amino Expert Impuls and its components had a weaker healing effect on the photosynthetic pigments in the treated plants. There is a tendency to increase their quantity in the range from 4% to 10%.

Table 10 shows results reflecting changes in photochemical processes in stressed maize plants. The low temperature reduced  $F_v / F_m$ ,  $qP$  and ETR, but the biostimulant and its organic component increased the ETR by 19% and 22%, however, the inorganic component had no significant effect.

**Table 11.** Effects of the biostimulant Amino Expert Impuls and its components on chlorophyll fluorescence parameters in light and dark adapted leaves of young maize plants.  $F_v / F_m$  - potential photochemical activity of FS2; ETR - electron transport rate ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ );  $qP$  - photochemical quenching;  $qN$  - non-photochemical quenching of chlorophyll fluorescence. BS - biostimulant, OC - organic component, MC - mineral component.

Var.	Chlorophyll fluorescence			
	$F_v/F_m$	ETR	$qP$	$qN$
Control 25°C	0,773±0,25 <sup>a</sup>	48,4±4,02 <sup>a</sup>	0,789±0,45 <sup>a</sup>	0,397±0,25 <sup>a</sup>
Chilling 10°C	0,676±0,49 <sup>c</sup>	25,9±2,16 <sup>c</sup>	0,542±0,65 <sup>b</sup>	0,680±0,34 <sup>b</sup>
BS 10°C	0,739±0,62 <sup>b</sup>	30,8±2,23 <sup>b</sup>	0,568±0,36 <sup>b</sup>	0,633±0,16 <sup>b</sup>
OC 10°C	0,719±0,44 <sup>b</sup>	31,5±2,40 <sup>b</sup>	0,579±0,14 <sup>b</sup>	0,518±0,36 <sup>b</sup>
MC 10°C	0,715±0,37 <sup>b</sup>	25,0±2,25 <sup>c</sup>	0,543±0,78 <sup>b</sup>	0,612±0,24 <sup>b</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The results obtained so far show that the biostimulant Amino Expert Impuls improved the photosynthetic activity of maize plants subjected to low temperature stress, as the stimulating effect on photosynthesis is probably an integral result of the action of many biomolecules in the biostimulant – amino acids, hormones, vitamins. substances.

Obviously, the retention effect of low temperatures on cell division and elongation in the meristematic zones of maize plants (Ben-Haj-Salah and Tardieu, 1995; Verheul et al., 1996) cannot be overcome by biostimulants and foliar fertilizers. At the same time, the improved photosynthetic activity probably supports the recovery and repair processes in maize plants. Our results correspond to the observations of other authors (Botta, 2013; Farooq et al., 2008a, 2008b; Ertani et al. 2013; Liu et al., 2008; Petrozza et al., 2014) and confirm the potential of foliar biostimulants. to improve the physiological condition of plants under stressful conditions. The data do not give grounds to assume that there is a significant synergistic interaction between the organic and mineral components of Amino Expert Impuls. This finding does not correspond to the opinion of Souri (2016), who believes that foliar products containing chelated microelements have a higher effect than "pure" mineral products. The reason for the lack of significant synergy in the action of the two main components of the product, in our opinion, is due to the high mineral status of corn plants in the conditions of hydroponic cultivation, which reduces the need for micronutrients.

Biostimulants from the PH group differ in the content of organic nitrogen, the amount of free amino acids, the composition of the amino acids and also the amount of the other substances presented in the composition of the products. In the specific experiments, the two biostimulants were applied in the same volume to the plants (about 1 ml per plant), but with different concentrations (1% for Terra Sorb Foliar and 0.1% for Naturamin - WSP. The lower recommended concentration of Naturamin - WSP is due to the high concentration of free amino acids in the product, which can induce the phenomenon of "amino acid inhibition" - suppression of nitrogen uptake by the roots (Ruiz et al., 2000).

Table 12 presents the results for the effects of both products on the parameters of foliar gas exchange in maize plants. It can be seen that the

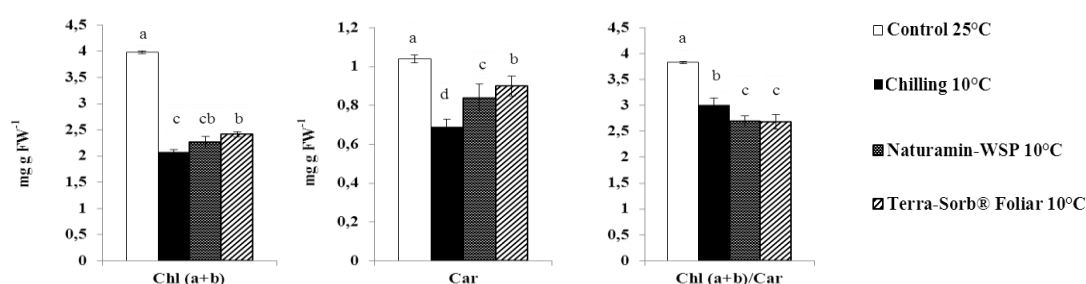
inhibitory effect of low temperatures on foliar gas exchange is identical to that found in the other experiments.

**Table 12.** Test of influence of low temperature influence ( $10 \pm 1^{\circ}\text{C}$ ) and application of biostimulants on the parameters of leaf gas exchange of young maize plants from the Knezha hybrid 307. A - rate of net photosynthesis ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ); E - transpiration intensity ( $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ );  $g_s$  - oral conductivity ( $\text{mol m}^{-2} \text{ s}^{-1}$ )

Var.	Leaf gas exchange		
	A	E	$g_s$
Control 25°C	16,9±0,14 <sup>a</sup>	1,06±0,03 <sup>a</sup>	0,08±0,00 <sup>a</sup>
Chilling 10°C	11,9±0,09 <sup>c</sup>	0,61±0,00 <sup>c</sup>	0,04±0,00 <sup>b</sup>
Naturamin WSP 10°C	12,9±0,10 <sup>b</sup>	0,78±0,01 <sup>b</sup>	0,05±0,00 <sup>b</sup>
Terra-Sorb Foliar 10°C	13,6±0,17 <sup>b</sup>	0,89±0,04 <sup>b</sup>	0,05±0,00 <sup>b</sup>

The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

The data show that the applied products have a positive effect on the parameters of the leaf gas exchange. The biostimulant Terra-Sorb Foliar increased the value of A by 14%, and Naturamin WSP - by 8%. The two products increased  $g_s$  identically, by 25%, and the effect on E is even more significant. (with 28% and 46%). The application of both products increased the content of photosynthetic pigments in stressed corn plants (Figure 7). The positive effect on the total content of chlorophylls is in the range of 10-14%, and on the total carotenoids - 14-24%. The effect of the biostimulant Terra-Sorb Foliar on the content of total carotenoids is higher than that of Naturamin WSP.



**Fig 7.** Effects of biostimulants Naturamin - WSP and Terra-Sorb Foliar on photosynthetic pigments in the leaves of young maize plants subjected to low temperature exposure. The different letters (a, b, c) after the mean values show significant differences at  $P < 0.05\%$ .

Biostimulants also had positive effect on light-dependent processes of photosynthesis (Table 13). The maximum efficiency of PH2 ( $F_v / F_m$ ) in the plants treated with them increased by 7%, but did not reach the level in the control plants. Leaf treatment significantly increased ETR (by 21-25%) and had a weak positive

effect on qP. The results of the study show that the biostimulants Naturamin - WSP and Terra-Sorb Foliar improved the photosynthetic activity of maize plants exposed to low temperatures. The effect of Terra-Sorb Foliar had a slight advantage over this of Naturamin - WSP, but the differences were small and did not allow the formulation of a definite conclusion.

**Table 13.** Effects of biostimulants Naturamin - WSP and Terra-Sorb Foliar on the parameters of chlorophyll fluorescence in the leaves of young maize plants subjected to low temperature stress. *F<sub>v</sub> / F<sub>m</sub>* - potential photochemical activity of PS2; *ETR* - electron transport rate; *qP* - photochemical quenching; *qN* - non-photochemical quenching.

Var.	Chlorophyll fluorescence			
	<i>F<sub>v</sub>/F<sub>m</sub></i>	<i>ETR</i>	<i>qP</i>	<i>qN</i>
Control 25°C	0,77±0,10 <sup>a</sup>	48,4±3,19 <sup>a</sup>	0,499±0,02 <sup>a</sup>	0,197±0,02 <sup>c</sup>
Chilling 10°C	0,67±0,04 <sup>b</sup>	35,5±1,00 <sup>c</sup>	0,454±0,00 <sup>b</sup>	0,431±0,09 <sup>a</sup>
Naturamin-WSP 10°C	0,72±0,07 <sup>a</sup>	42,9±2,20 <sup>b</sup>	0,475±0,06 <sup>a</sup>	0,409±0,10 <sup>b</sup>
Terra-Sorb Foliar 10°C	0,72±0,11 <sup>a</sup>	44,2±3,10 <sup>b</sup>	0,489±0,15 <sup>a</sup>	0,425±0,04 <sup>a</sup>

The different letters (a, b, c) after the mean values show significant differences at *P* < 0.05%.

The active components in the compositions of both products are free amino acids, low molecular weight peptides and trace elements (only in Terra-Sorb Foliar). In order to have a physiological effect, these exogenous substances must be absorbed by plant tissues. The uptake of micronutrients and free amino acids by the leaves has been proven (Fernández et al., 2013), but how the peptides penetrate is not well understood. It is possible that some of them are transformed from phyllospheric microorganisms to amino acids, as suggested by Colla et al. (2017).

At present, the prevailing opinion is that the action of PH is mainly related to free amino acids, but low molecular weight peptides also have physiological effects. For example, Matsumiya and Kubo (2011) show that a low molecular weight peptide that induces root hair induction in rapeseed plants has been identified in plant-derived PH.

Trace elements and other substances (amino acids, peptides) can be absorbed by the leaves, but do not show a physiological effect if they are immobilized in the apoplastic space (Fernández et al., 2013). Radiometric tests give a clearer picture of the activity of the substances introduced into the leaves, but at present the information is limited. Foliar isotope-labeled glutamic acid (15N, 13C) has been shown to be involved in the biosynthesis of GABA (β-aminobutyric acid) and proline. Both AK (GABA and proline) play a significant role in protecting plants from stress (Seifikalhor et al., 2019; Teixeira et al., 2017; Ali et al., 2019).

The stimulation of the photosynthetic activity of stressed maize plants in the absence of effects on their growth can be explained by the use of the created photoassimilates in other parallel processes. Such can be various reactions aimed at repair, restoration of damaged structures. In our studies, we found that 7 days after the application of foliar products, the total antioxidant activity in low-temperature maize plants decreased. These results give grounds to assume that the substances imported with biostimulants are used in a number of repair processes in plants. At the present stage of research, there is no evidence of positive effects of specific AKs on plants stressed by low temperatures, but such have been reported against other types of stress. Ali et al. (2019) summarize that exogenous administration of glutamic acid (50  $\mu$ M) increases sunflower tolerance to osmotic and salt stress. The same authors point out that exogenous application of proline improves the growth, photosynthesis and antioxidant activity of wheat in drought conditions.

The foliar application of the biostimulants Terra-Sorb Foliar, Amino Expert Impuls and Naturamin - WSP and the foliar fertilizer Polyplant had a positive effect on the physiological status of maize plants exposed to low temperatures, but did not improve their growth parameters (data not presented).

The applied foliar products improved the photosynthetic activity of maize plants grown under conditions of low temperature stress. Their positive effect was clearly expressed on the leaf gas exchange, photosynthetic pigments and the speed of photosynthetic electronic transport ETR. In particular, the biostimulant Terra-Sorb Foliar had a positive effect on the antioxidant potential of maize plants.

There are differences in the degree of positive impact depending on the type of product (foliar fertilizer and biostimulant), the nature of the components in the composition (organic and inorganic component), as well as the individual composition of the specific representative of the group of protein hydrolysates, but differences in the effects are relatively small and do not allow the formation of a general conclusion.

The improved physiological status of low-stressed maize plants gives us a prerequisite to allow the idea of faster recovery in the post-stressed period. The effect of the applied products in the recovery period of maize plants is not the subject of this study. However, the above hypothesis can be supported on the basis of our observations on the visual changes in the appearance of plants in the first days after the removal of the low-temperatures (Photo 10). It can be seen that in maize-treated maize plants there are no anthocyan color on the leaves, which are observed in untreated but stressed plants. In addition, the leaves of biostimulant-treated plants are larger and have better turgor.



**Photo 10.** Appearance of maize plants (3 days) after the stress period (from low temperature impact - 7 days). The plants were treated with biostimulants during low temperature stress, with the exception of Variant 10°C (control). The plants treated with biostimulants were larger and with better turgor.

## VI. CONCLUSIONS

1. The applied low temperature stress (7 days, 10°C) 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 °C; 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 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.

## VII. 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.

## VIII. LITERATURE

1. Воденеев, В. А., 2009. Механизмы генерации и функциональная роль потенциалов возбуждения у высших растений. Автореферат докторской диссертации.
2. Гольцев, В. Н., М. Каладжи, М.А. Кузманова, С. И. Аллахвердиев, 2014. Переменная и замедленная флуоресценция хлорофилла а – теоретические основы и практическое приложение в исследовании растений. М.-Ижевск: Институт компьютерных исследований, 220 с.
3. Паничкин, Л. А., Г. А. Прудников, М. С. Красавина, 2009. Усовершенствования метода электрофизиологической диагностики холодоустойчивости растений. Известия ТСХА, 4, 133-137.
4. Славова Г. 2015. Производство на царевица в Европа, България и света, възможности и тенденции на развитие. Изв. СУ-Варна. 57-64.
5. Томов, Т., Г. Рачовски, Св. Костадинова, Ив. Манолов., 1999. Ръководство за упражнения по агрохимия, Пловдив: Академично издателство на

BCI, 1999

6. Aguilera C, Stirling CM, Long SP., 1999. Genotypic variation within *Zea mays* L. for susceptibility to and rate of recovery from chillinduced photoinhibition of photosynthesis. *Plant Physiology* 106, 429–436. doi: 10.1034/j.1399-3054.1999.106411.x
7. Ali, Q., H. Athar, M. Haider, S. Shahid, N. Aslam, F. Shehzad, J. Naseem, R. Ashraf, A. Ali, S. Hussain, 2019. Role of Amino Acids in Improving Abiotic Stress Tolerance to Plants, 175-202. In: *Plant Tolerance to Environmental Stress* (Eds., M. Hasanuzzaman, M. Fujita, H. Oku, M. Islam) CRC Press, <https://doi.org/10.1201/9780203705315>
8. Aroca R., P. Vernieri, J.J. Irigoyen, M. Sanchez-Diaz, F. Tognoni, A. Pardossi, 2003. Involvement of abscisic acid in leaf and root of maize (*Zea mays* L.) in avoiding chilling-induced water stress. *Plant Sci.* 165: 671-679
9. Aroca, R., G. Amodeo, S. Fernandez-Illescas, E. Herman, F. Chaumont, M. Chrispeels, 2005. The Role of Aquaporins and Membrane Damage in Chilling and Hydrogen Peroxide Induced Changes in the Hydraulic Conductance of Maize Roots. *Plant Physiology*, 137, 341–353
10. Aroca, R., J. Irigoyen, M. Sanchez-Diaz, 2001. Photosynthetic characteristics and protective mechanisms against oxidative stress during chilling and subsequent recovery in two maize varieties differing in chilling sensitivity. *Plant Science* 161, 719–726
11. Ben-Haj-Salah H, Tardieu F., 1995. Temperature affects expansion rate of maize leaves without change in spatial distribution of cell length. *Plant Physiology* 109, 861–870.
12. Bergmeyer H. U. & Bernt, E., 1974. *Methods of Enzymatic Analysis* (Bergmeyer, H. U., Ed.) Vol. 3, 1205-1215, Academic Press, New York, London
13. Bolhar-Nordenkamp, H.R., & Oquist, G., 1993. Chlorophyll fluorescence as a tool in photosynthesis research. In: *Photosynthesis and production in a changing environment: a field and laboratory manual* (Eds. D. O. Hall, J. M. O. Scurlock, h. R.
14. Botta, A., 2012. Enhancing plant tolerance to temperature stress with amino acids: an approach to their mode of action. *Acta Hort.* 1009, 29–35.
15. Bradačova, K., N. Weber, N. Moradtalab, M. Asim, M. Imran, M. Weinmann, G. Neumann, 2016. Micronutrients (Zn/Mn), seaweed extracts, and plant growth-promoting bacteria as cold-stress protectants in maize. *Chem. Biol. Technol. Agric.*, 3:19. DOI 10.1186/s40538-016-0069-1
16. Cavani, L.; Halle, A.T.; Richard, C.; Ciavatta, C., 2006. Photosensitizing properties of protein hydrolysate-based fertilizers. *Journal of Agricultural and Food Chemistry* 54: 9160 -9167.
17. Ceppi M.G., A. Oukarroum, N. Çiçek, R. J. Strasser, G. Schansker, 2012. The IP amplitude of the fluorescence rise OJIP is sensitive to changes in the photosystem I content of leaves: a study on plants exposed to magnesium and sulfate deficiencies, drought stress and salt stress. *Physiol. Plantarum*, 144, 277-288
18. Colla, G., L. Hoagland, M. Ruzzi, M. Cardarelli, P. Bonini, R. Canaguier, Y. Rouphael, 2017. Biostimulant Action of Protein Hydrolysates: Unraveling Their Effects on Plant Physiology and Microbiome. *Front. Plant Sci.* 8:2202. doi: 10.3389/fpls.2017.02202
19. De Lucia, B., and Vecchietti, L., 2012. Type of bio-stimulant and application method effects on stem quality and root system growth in L.A. Lily. *Europ. J. Hort. Sci.* 77, 10–15. Available online at: <http://www.jstor.org/stable/24126519>
20. Demmig-Adams B., 1990. Carotenoids and photoprotection in plants. A role for the xanthophyll zeaxanthin. *Biochim. Biophys. Acta* 1020: 1-24

21. Dimitrova, A., M. Paunov, V. Pawlova, K. Dankov, M. Kouzmanova, V. Velikova, Ts. Tsonev, H. Kalaji, V. Goltsev, 2020. Analysis of photosynthetic efficiency in leaves of two ecotypes *Platanus orientalis* L. at room temperature and after treatment at moderately high temperature. *Photosynthetica* (in press)
22. Dimkpa, C., P. Bindraban, 2016. Fortification of micronutrients for efficient agronomic production: a review. *Agronomy for Sustainable Development*, Springer Verlag/EDP Sciences/INRA, 36 (1), pp.7. [ff10.1007/s13593-015-0346-6](https://doi.org/10.1007/s13593-015-0346-6). [ffhal-01532372f](https://doi.org/10.1532372f)
23. Ertani, A., M. Schiavon and S.Nardi, 2017. Transcriptome-Wide Identification of Differentially Expressed Genes in *Solanum lycopersicon* L. In Response to an Alfalfa-Protein Hydrolysate Using Microarrays. *Front. Plant Sci.* 8, 1159, doi: 10.3389/fpls.2017.01159
24. Ertani, A., Schiavon, M., Muscolo, A., and Nardi, S., 2013. Alfalfa plant-derived biostimulant stimulate short-term growth of salt stressed *Zea mays* L. plants. *Plant Soil* 364, 145–158. doi: 10.1007/s11104-012-1335-z
25. Farooq M, Aziz T, Hussain M, Rehman H, Jabran K, Khan MB ., 2008b. Glycinebetaine improves chilling tolerance in hybrid maize. *Journal of Agronomy and Crop Science* 194, 152–160. doi: 10.1111/j.1439- 037X.2008.00295.x
26. Farooq M., T. Aziz, Z. A. Cheema, M. Hussain and A. Khaliq 2008a. Activation of Antioxidant System by KCl Improves the Chilling Tolerance in Hybrid Maize; *Journal of Agronomy and Crop Science*; Volume 194, Issue 6, pages 438–448,
27. Farooq, M., T. Aziz, A. Wahid, D-J. Lee, K. Siddique, 2009. Chilling tolerance in maize: agronomic and physiological approaches. *Crop & Pasture Science*, 2009, 60, 501–516
28. Farooq, M., T. Aziz, S. Basra, M. Cheema, H.Rehman, 2008c.Chilling Tolerance in Hybrid Maize Induced by Seed Priming with Salicylic Acid. *Journal of Agronomy and Crop Science*, 194, 2, 161 – 168. [10.1111/j.1439-037X.2008.00300.x](https://doi.org/10.1111/j.1439-037X.2008.00300.x)
29. Farquhar, G.D.; S. Von Caemmerer, J. A. Berry, 1980. A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta*, 149, 78–90
30. Fernández, V., and Brown, P. H., 2013. From plant surface to plant metabolism: the uncertain fate of foliar-applied nutrients. *Front. Plant Sci.* 4:289. doi: 10.3389/fpls.2013.00289
31. Fryer M, Andrews J, Oxborough K, Blowers D, Baker N. 1998. Relationship between CO<sub>2</sub> assimilation, photosynthetic electron transport, and active O<sub>2</sub> metabolism in leaves of maize in the field during periods of low temperature. *Plant Physiology* 116: 571–580.
32. Giusti, M.M. and Wrolstad, R.E., 2001. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. In: Giusti, M.M. and Wrolstad, R.E., Eds., *Current Protocols in Food Analytical Chemistry*, John Wiley and Sons, Inc., Hoboken, F1.2.1.-F1.2.13.
33. Grabowska A, Kunicki E, Sełkara A, Kalisz A, Wojciechowska R. 2012. The effect of cultivar and biostimulant treatment on the carrot yield and its quality. *Veg Crop Res Bull.* 77:37– 48.
34. Grzybowski, M., J. Adamczyk, M. Jończyk, A. Sobkowiak, J. Szczepanik, K. Frankiewicz, J. Fronk, P. Sowiński, 2019. Increased photosensitivity at early growth as a possible mechanism of maize adaptation to cold springs. *Journal of Experimental Botany*, Vol. 70, No. 10 pp. 2887–290
35. Guan,Y., Z. Li, F. He, Y. Huang, W. Song, J. Hu, 2015. “On-Off” Thermoresponsive Coating Agent Containing Salicylic Acid Applied to Maize Seeds for Chilling Tolerance. *PLoS ONE* 10 (3), e0120695.doi:10.1371/journal.pone.0120695

36. Hacısalihoglu, G., S. Kantanka, N. Miller, J. Gustin, A. Settles, 2018. Modulation of early maize seedling performance via priming under sub-optimal temperatures. *PLoS ONE*, 13, (11): e0206861. <https://doi.org/10.1371/journal.pone.0206861>
37. Haldimann P., 1999. How do changes in temperature during growth affect leaf pigment composition and photosynthesis in *Zea mays* genotypes differing in sensitivity to low temperature? *Journal of Experimental Botany*, Vol. 50, No. 333, pp. 543–550
38. Haldimann P., 1997. Chilling-induced changes to carotenoid composition, photosynthesis and the maximum quantum yield of photosystem II photochemistry in two maize genotypes differing in tolerance to low temperature. *J Plant Physiol*;151:610–9.
39. Haldimann, P., 1998. Low growth temperature-induced changes to pigment composition and photosynthesis in *Zea mays* L. genotypes differing in chilling sensitivity. *Plant, Cell and Environment* 21,200–208. doi:10.1046/j.1365-3040.1998.00260.x
40. Heath, R. L. and Packer, L., 1968. Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch Biochem Biophys* 125 189-198.
41. Hodges, D. M., Ch. Andrews, D. A. Jonson, R. Hamilton, 1997. Antioxidant compound responses to chilling stress in differentially sensitive inbred maize lines. *Physiologia Plantarum*, 98 (4), 685-692.
42. Huang, J., J. Zhang, W. Li, W. Hu, L. Duan, Y. Feng, F. Qiu, B. Yue, 2013. Genome-wide Association Analysis of Ten Chilling Tolerance Indices at the Germination and Seedling Stages in Maize. *J Integrative Plant Biol.*
43. Kaspar TC, Bland WL, 1992. Soil temperature and root growth. *Soil Science* 154, 290–299. doi: 10.1097/00010694-199210000-00005
44. Kiel C, Stamp P., 1992. Internal root anatomy of maize seedlings (*Zea mays* L.) as influenced by temperature and genotype. *Annals of Botany* 70, 125–128.
45. Kosová, K., D. Haisel, I. Tichá, 2005. Photosynthetic performance of two maize genotypes as affected by chilling stress. *Plant Soil Environ.*, 51, 5, 206–212
46. Leipner J, Fracheboud Y, Stamp P. 1999. Effect of growing season on the photosynthetic apparatus and leaf antioxidative defenses in two maize genotypes of different chilling
47. Leipner, J. 2009. Chilling stress in maize: from physiology to genetics and molecular mechanisms. Habilitation thesis. ETH Zurich, 124 pages. <https://doi.org/10.3929/ethz-a-005794605>
48. Lichtenthaler, H.K., 1988. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods enzymol.*, 148, 350-382
49. Liu, Rui-Xian, Zhou, Z., Guo, W., Chen, B., & Oosterhuis, D., 2008. Effects of N fertilization on root development and activity of water-stressed cotton (*Gossypium hirsutum* L.) plants. *Agricultural Water Management*, vol. 95, issue 11, 1261-1270.
50. Lobell, D., G. Asner, 2003. Climate and management contributions to recent trends in US agricultural yields. *Science*, 299: 1032–1032. doi:10.1126/science.1077838.
51. Long, S.P., S. Humphries, P. G. Falkowski, 1994. Photoinhibition of photosynthesis in nature. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45, 633–662.
52. Long, S.P., A. K. Spence, 2013. Toward cool C4 crops. *Annu. Rev. Plant Biol.* 64, 701–722
53. Lukatkin A. S., Isaikina E. E., 1997. Calcium status and chilling injury in maize seedlings. *Russian Journal of Plant Physiology*, 44, 3, 339–342.

54. Lukatkin, A. S., 2003. Contribution of Oxidative Stress to the Development of Cold-Induced Damage to Leaves of Chilling-Sensitive Plants: 3. Injury of Cell Membranes by Chilling Temperatures. *Russian Journal of Plant Physiology*, Vol. 50, No. 2, 2003, pp. 243–246
55. Lutatkin, A. S., A. Brazaityte, Č. Bobinas, P. Duchovskis, 2012. Chilling injury in chilling-sensitive plants: a review. *Agriculture*, 99, 2, 111–124.
56. Marfà O., Cáceres R., Polo J., Ródenas J., 2009. Animal Protein Hydrolysate as a Biostimulant for Transplanted Strawberry Plants Subjected to Cold Stress. *Acta Hort.* 842, 315–318.
57. Markowski, A. G. Skrudlik, 1995. Electrolyte Leakage, ATP Content in Leaves and Intensity of Net Photosynthesis in Maize Seedlings at Permanent or Different Daily Exposure to Low Temperatures. *Journal of Agronomy and Crop Science*, 175, 2, 109–117.
58. Matsumiya Y., Kubo M., 2011. Soybean peptide: novel plant growth promoting peptide from soybean, in *Soybean and Nutrition*, ed. El-Shemy H. (Rijeka: InTech Europe Publisher; ), 215–230 10.5772/19132 [CrossRef] [Google Scholar]
59. Miedema P., 1982. The effects of low temperature on *Zea mays*. *Advances in Agronomy* 35, 93–128. doi: 10.1016/S0065-2113(08)60322-3
60. Miedema, P., J. Sinnaeve, 1980. Photosynthesis and Respiration of Maize Seedlings at Suboptimal Temperatures. *Journal of Experimental Botany*, 31, 3, Pages 813–819. <https://doi.org/10.1093/jxb/31.3.813>
61. Moradtalab, N., M. Weinmann, F. Walker, B. Höglinger, U. Ludewig, G. Neumann, 2018. Silicon Improves Chilling Tolerance During Early Growth of Maize by Effects on Micronutrient Homeostasis and Hormonal Balances. *Front. Plant Sci.* 9, 420. doi: 10.3389/fpls.2018.00420
62. Nahed, G., AbdelAziz, A.A., Mazher, M., Farahat, M.M., 2010. Response of vegetative growth and chemical constituents of *Thuja orientalis* L. plant to foliar application of different aminoacids at Nubaria. *Journal of American Science* 6, 3, 295–301
63. Nardi, S., D. Pizzeghello, M. Schiavon, A. Ertani, 2016. Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism. *Sci. Agric.* 73, 1, 18–23
64. Pastori G, Foyer CH, Mullineaux P. 2000. Low temperature-induced changes in the distribution of H<sub>2</sub>O<sub>2</sub> and antioxidants between the bundle sheath and mesophyll cells of maize leaves. *Journal of Experimental Botany* 51: 107–113.
65. Paunov, M., L. Koleva, A. Vassilev, J. Vangronsveld, V. Goltsev, 2018. Effects of different metals on photosynthesis: cadmium and zinc affect chlorophyll fluorescence in durum wheat. *Int. J. Mol. Sci.*, 19 (3), 787; doi:10.3390/ijms19030787
66. Petrozza, A., Santaniello, A., Summerer, S., Di Tommaso, G., Di Tommaso, D., Paparelli, E., 2014. Physiological responses to Megafol® treatments in tomato plants under drought stress: a phenomic and molecular approach. *Sci. Hort.* 174: 185–192.
67. Pietrini F, Ianneli MA, Battistelli A, Moscatello S, Loreto F, Massacci A (1999) Effects on photosynthesis, carbohydrate accumulation and regrowth increase in maize genotypes with different sensitivity to low temperature. *Australian Journal of Plant Physiology* 26, 367–373.
68. Polo J., Barroso R., Ródenas J., 2006. Porcine Hemoglobin Hydrolysate as a Biostimulant for Lettuce Plants Subjected to Conditions of Thermal Stress. *HortTechnology* 16, 483–487.
69. Reimer, R., 2010. Responses of maize (*Zea mays* L.) seed-lings to low and high temperature: association mapping of root growth and photosynthesis-related traits, dissertation ETH No. 18807. PhD thesis, Swiss Federal Institute of Technology,

Zurich, Switzerland.

70. Ribas-Carbo M1, Aroca R, González-Meler MA, Irigoyen JJ, Sánchez-Díaz M. 2000. The electron partitioning between the cytochrome and alternative respiratory pathways during chilling recovery in two cultivars of maize differing in chilling sensitivity. *Plant Physiol.* 2000 Jan;122(1):199-204

71. Riva-Roveda, L., B. Escale, C. Giauffret, C. Périlleux, 2016. Maize plants can enter a standby mode to cope with chilling stress. *BMC Plant Biology*, 16, 212; DOI 10.1186/s12870-016-0909-y

72. Rodríguez, V., A. Butrón, G. Sandoya, A. Ordás, P. Revilla, 2007. Combining Maize Base Germplasm for Cold Tolerance Breeding, *Crop Science*, 47, 4, 1467-1474

73. Ruiz J. M., N. Castilla, I. Romero, 2000. Nitrogen metabolism in pepper plants applied with different bioregulators. *J. Agric. Food Chem.* 48 2925–2929 10.1021/jf990394h

74. Sadak, M., M. Abdelhamid, 2015. Influence of aminoacids mixture application on some biochemical aspects, antioxidant enzymes and endogenous polyamines of *Vicia faba* plant grown under sea water salinity stress. *Gesunde Pflanze*, 67, 119–

75. Salaby EA, Shanab SMM., 2013. Comparison of DPPH and ABTS assays for determining of antioxidant of water and methanol extracts of *Spirulina platensis*. *Indian J Geo-Marine Sci* 42:556-564

76. Salesse-Smith, C. E., R. E. Sharwood, F. A. Busch, D. B. Stern, 2019. Increased Rubisco content in maize mitigates chilling stress and speeds recovery, *Plant Biotechnology Journal*, 1–12.

77. Samborska, I., H. M. Kalaji, L. Sieczko, W. Borucki, R. Mazure, M. Kouzmanova, V. Goltsev, 2019. Can just one-second measurement of chlorophyll a fluorescence be used to predict sulphur deficiency in radish (*Raphanus sativus* L. sativus) plants? *Current Plant Biol* 19: 100096, 2019.

78. Schiavon, M., Ertani, A., Nardi, S., 2008. Effects of an alpha-alpha protein hydrolysate on the gene expression and activity of enzymes of TCA cycle and N metabolism in *Zea mays* L. *J. Agric. Food Chem.* 56, 11800–1180

79. Seifikalhor, M., S. Aliniaieifard, B. Hassani, V. Niknam, O. Lastochkina, 2019. Diverse role of  $\gamma$ -aminobutyric acid in dynamic plant cell responses. *Plant Cell Rep.*, 38, 8, 847-867. doi: 10.1007/s00299-019-02396-z.

80. Sharma, A., B. Shahzad, A. Rehman, R. Bhardwaj, M. Landi, B. Zheng, 2019. Response of Phenylpropanoid Pathway and the Role of Polyphenols in Plants under Abiotic Stress, *Molecules*, 24, 2452; doi:10.3390/molecules24132452

81. Sharma, P., A. Jha, R. Dubey, M. Pessarakli, 2012. Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. *Journal of Botany*, ID 217037, doi:10.1155/2012/217037

82. Singleton VL, Rossi JA 1965. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Viticult* 16: 144-158.

83. Sobkowiak, A., M. Jończyk, J. Adamczyk, J. Szczepanik, D. Solecka, I. Kuciara, K. Hetmańczyk, m. J. Trzcinska-Danielewicz, M. Grzybowski, M Skoneczny, J. Fronk, P. Sowiński, 2016. Molecular foundations of chilling-tolerance of modern maize. *BMC Genomics* 17, 125, <https://doi.org/10.1186/s12864-016-2453-4>

84. Souri, M., 2016. Amino chelate fertilizers: The new approach to the old problem; A review. *Open Agriculture* 1, 1, 118–123 Folli-Pereira, M., A. Ramos, G. Canton, J. Conceição, S.

85. Stamp P., 1984. Chilling tolerance of young plants demonstrated on the example of maize (*Zea mays* L.). In ‘Advances in agriculture and crop science Vol. 7’.

(Ed. G Geisler) pp. 1–84. (Paul Parey: Berlin)

86. Stirbet A., Govindjee, 2012. Chlorophyll a fluorescence induction: a personal perspective of the thermal phase, the J-I-P rise. – *Photosynth. Res.* 113: 15-61, 2012.

87. Stirbet, A., 2013. Excitonic connectivity between photosystem II units: what is it, and how to measure it? *Photosynth. Res.* 116, 189-214, 2013.

88. Strasser R.J., A. D. Stirbet A.D., 2001. Estimation of the energetic connectivity of PS II centres in plants using the fluorescence rise O–J–I–P: Fitting of experimental data to three different PS II models. *Math. Comp. Sim.*, 56, 451-462, 2001.

89. Strasser R.J., M. Tsimilli-Michael, 1998. Activity and heterogeneity of PSII probed in vitro by the chlorophyll a fluorescence rise O-(K)-JIP. – In: G. Garab (ed) *Photosynthesis: mechanisms and effects* .

90. Strasser R.J., P. Eggenberg P., B. J. Strasser, 1996. How to work without stress but with fluorescence. *Bull. Royal Soc. Liege* 65, 330-349, 1996

91. Strasser R.J., Tsimilli-Michael M., Srivastava A., 2004. Analysis of the chlorophyll a fluorescence transient. – In: Papageorgiou G., Govindjee (ed.): *Chlorophyll a Fluorescence: a Signature of Photosynthesis*. pp. 321-362. Springer. Dordrecht, The Netherlands 2004

92. Teixeira WF, Fagan EB, Soares LH, Umburanas RC, Reichardt K, Dourado-Neto D., 2017. Foliar and seed application of amino acids affects the antioxidant metabolism of the soybean crop. *Front Plant Sci* 8:1–14

93. Verheul MJ, Picatto C, Stamp P., 1996. Growth and development of maize (*Zea mays* L.) seedlings under chilling conditions in the field. *European Journal of Agronomy* 5, 31–43. doi: 10.1016/S1161-0301(96)02007-2

94. Verheul MJ, Hasselt PRV, Stamp P., 1995. Comparison of maize inbred lines differing in low temperature tolerance effect of acclimation at suboptimal temperature on chloroplast functioning. *Annals of Botany* 76, 7–14. doi: 10.1006/anbo.1995.1072

95. Wang, L-J., P. Zhang, R-N. Wang, P. Wang, S-B. Huang, 2018. Effects of variety and chemical regulators on cold tolerance during maize germination. *Journal of Integrative Agriculture*, 17(12): 2662–2669

96. Wijewardana, C., M. Hock, B. Henry, K. R. Reddy, 2015. Screening Corn Hybrids for Cold Tolerance using Morphological Traits for Early-Season Seeding. *Crop Sci.* 55:851–867. doi: 10.2135/cropsci2014.07.0487

97. Wijewardana, C., W. B. Henry, M. W. Hock, and K. Raja Reddy, 2016. Growth and physiological trait variation among corn hybrids for cold tolerance. *Can. J Plant Sci.*, 96, 639-656.

98. Yang, J., Q. Kong, C. Xiang, 2009. Effects of low night temperature on pigments, chl a fluorescence and energy allocation in two bitter melon (*Momordica charantia* L.) genotypes. *Acta Physiol Plant*, 31:285–293

99. Zaidi, P.H., M. Yadav, P. Maniselvan, R. Khan, T.V. Shadakshari, R.P. Singh, D. Pal, 2010. Morpho-physiological traits associated with cold stress tolerance in tropical maize (*Zea mays* L.). *Maydica*. 55: 201-208.

100. Zhou, P., Q Li, G. Liu, N. Xu, Y. Yang, W. Zeng, A. Chen, S. Wang, 2018. Integrated analysis of transcriptomic and metabolomic data reveals critical metabolic pathways involved in polyphenol biosynthesis in *Nicotiana tabacum* under chilling stress. *Funct. PlantBiol.* 2018, 46, 30–43.



### **Publications:**

1. Cholakova-Bimbalova R., A. Vassilev, 2015. Influence of low temperatures on the growth and macronutrient content in young maize plants. Scientific Works, Agricultural University – Plovdiv, vol. LIX, book 2, 87-94.

2. Cholakova-Bimbalova R., A. Vassilev, 2017. Effect of chilling stress on the photosynthetic performance of young plants from two maize (*Zea mays* L.) hybrids. CBU International Conference Proceedings, vol. 2017, 1118-1123. March 22-24, Prague, Czech Republic

3. Rositsa Cholakova-Bimbalova, 2017. Influence of foliar fertilizer and its components on the tolerance of young corn plants experienced low temperatures. Scientific works of the Institute of Agriculture Karnobat (in press)

4. Cholakova-Bimbalova R., L. Koleva, A. Vassilev, 2018. Effects of a biostimulant and a mineral fertilizer on the antioxidative defence system of chilling-exposed maize plants. Agricultural Sciences, 33-39.

5. Cholakova-Bimbalova R, V. Petrov, A. Vassilev, 2019. Photosynthetic performance of young maize (*Zea mays* L.) plants exposed to chilling stress can be improved by the application of protein hydrolysates. Acta Agrobotanica, 72 (2):1769. <https://doi.org/10.5586/aa.1769>

### **Conferences:**

1. Jubilee Scientific Conference with International Participation Traditions and Challenges of Agricultural Education, Science and Business, 2017, 29-31, October. Agricultural University Plovdiv (статья 1)

2. CBU International Conference Proceedings, 2017, March 22-24, Prague, Czech Republic (статья 2)

3. Proceedings conference of agronomy students with international participation, Cacak, 2017, 23-25 August. Cholakova-Bimbalova R. and A. Vassilev, 2017. Доклад: Influence of biostimulants on growth and photosynthetic performance of young maize plants exposed to chilling stress

4. International Scientific Conference "Sustainable Development of Agriculture - a priority of modern agricultural science", Institute of Agriculture - Karnobat ", 2017

5. International scientific conference of young scientists and specialists, dedicated to the 150th anniversary of the birth of VP Goryachkina, Moscow, 2018, June 6-7. Poster: Evaluation of the effect of biostimulant use for amelioration of chilling stress in young maize plants (*Zea mays* L.)