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## ORIGINAL RESEARCH PAPER

# Photosynthetic performance of young maize (*Zea mays* L.) plants exposed to chilling stress can be improved by the application of protein hydrolysates

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**Abstract**

Biostimulants offer a novel approach for the regulation of crucial physiological processes in plants. Recently, it has been observed that the application of biostimulants on both seeds and plants may ameliorate to some extent the negative effects of abiotic stresses such as drought, heat, salinity, and others. In the climate conditions of Bulgaria, the early developmental stages of warm climate crops, like maize, often occur under suboptimal temperatures. Although the mitigation of abiotic stress is perhaps the most frequently cited benefit of biostimulant formulations, little is known about their influence on chilling-stressed plants. The aim of our study was to evaluate the effects of a biostimulant from the group of protein hydrolysates on both the growth and the photosynthetic performance of chilling-exposed young maize plants grown in controlled environment. Here, we report that application of a protein hydrolysate increased the performance of chilled maize plants, as demonstrated by leaf gas exchange, photosynthetic pigment content, and chlorophyll fluorescence, but did not affect their growth. Nevertheless, based on the better preserved photosynthetic performance of the biostimulant-treated maize plants exposed to chilling, we assume that under subsequent favorable conditions their growth would recover more quickly as compared to the untreated ones.

**Keywords**

stress; *Zea mays* L.; chilling; biostimulants; photosynthesis

**Introduction**

Biostimulants are increasingly being integrated into agriculture with the aim of modifying physiological processes in plants which ultimately optimize their performance. In recent years, they have received considerable attention by both the scientific community and the business [1–3]. The European Biostimulant Industry Council (EBIC) defines biostimulants “as substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to enhance/benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and crop quality” [4].

Biostimulants may include various components like plant hormones, amino acids, peptides, proteins, sugars, lipids, vitamins, etc. They are categorized in several groups, depending on their origin [3]. Widely used sources for biostimulant production are humate-based materials [5,6], different algae species [1,7], as well as protein hydrolysates of plant [8,9] and animal [10,11] origin.

Environmental stress conditions can strongly affect plant performance and productivity. Abiotic stresses are shown to be a significant cause of yield losses (60–70%) in agriculture [12]. Biostimulants can improve plant tolerance to abiotic stresses by influencing many morphological and physiological targets. For example, some of their

known beneficial effects include maintenance of cell membrane stability, accumulation of osmolytes and antioxidants, activation of stress-responsive enzymes, as well as modification of hormonal status [13]. However, due to the chemical complexity of these products, the molecular mechanisms through which they act on plants are still difficult to reveal [14]. It has been suggested that biostimulants do not act on plant metabolism directly, but rather indirectly, through their influence on plant-signaling cascades that trigger the mitigation of negative stress responses [15]. Therefore, understanding the mechanisms of biostimulant mode of action, through advances in plant biostimulant research, is crucial for agriculture.

Lately, significant attention in agronomic practice has been paid to the group of protein hydrolysates (PHs). They are a category of plant biostimulants defined as “mixtures of polypeptides, oligopeptides and amino acids that are manufactured from protein sources” [16]. Protein hydrolysates are produced after enzymatic, chemical, or thermal hydrolysis [10,17]. Most typical sources are animal collagen and elastine [18], alfalfa residue [19], algal proteins [20], etc.

Several studies report the use of a variety of PHs to stimulate both growth and stress tolerance of plants. For example, Cerdán et al. [21] described the application of PHs as a promoter of nutrient uptake, especially nitrogen and iron, finally leading to better growth of the observed plants. Foliar application of legume-derived PH “trainer” increased biomass, chlorophyll content, and leaf nitrogen concentration of young maize [22] and tomato plants [9]. The tolerance of lettuce to low temperature was enhanced with foliar applications of PHs [23].

However, still little is known about the influence of PHs on the performance of warm climate crops exposed to chilling stress. Chilling (exposure to low positive temperatures) may provoke disorders in mineral nutrition, water relations, photosynthesis, dark respiration, etc., ultimately leading to plant growth retardation [24,25]. For example, maize plants in the early developmental stages may be damaged by temperatures below 12°C [26]. In the climate conditions of Bulgaria, these early stages of maize development often occur under suboptimal temperatures. This motivated our research to assess the effect of PHs on the performance of young maize plants subjected to chilling stress, using as a marker the process of photosynthesis, which is very sensitive to temperature changes.

## Material and methods

### Growth conditions and experimental design

The maize plants were cultivated in a climatic room at the Department of Plant Physiology and Biochemistry at the Agricultural University of Plovdiv, Bulgaria. The plants of the hybrid Kneza 307 were grown as a substrate-hydroponic culture in 1/2 strength modified Hoagland nutrient solution, in a controlled environment: photoperiod – 12 hours, photosynthetic photon flux density (PPFD) – 200  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (cool-white fluorescent lamps; Osram Dulux L 80W/840, Italy), temperature – 25  $\pm$ 1°C / 20  $\pm$ 1°C (day/night), and relative air humidity – 60  $\pm$ 5%. Uniform plants in the three-leaf stage were used for the study. The experimental design included four treatments (variants), namely: (1) plants, grown at 25°C day temperature (control); (2) plants grown at 10°C; (3) plants grown at 25°C and sprayed with 1% biostimulant water solution; and (4) plants grown at 10°C and sprayed with 1% biostimulant water solution. Each treatment had three replications (pots) with four plants per pot. The biostimulant was applied once, 7 days after the beginning of the experiment, as a therapeutic agent. The whole experiment lasted 14 days and was performed twice. The applied biostimulant was Terra-Sorb Foliar, which is a protein hydrolysate containing free amino acids and small peptides.

### Plant growth analysis

The plants were harvested at the end of the treatment period and fresh weight (FW), shoot height (SH), root length (RL), and leaf area (LA) were measured.

### Leaf gas exchange analysis

Leaf gas exchange ( $A$  – net photosynthetic rate;  $E$  – transpiration rate;  $g_s$  – stomatal conductance) was measured by an open photosynthetic system LCpro+ (ADC, England) on the upper fully developed leaf of the plants. Before the measurement plants from all treatments were adapted for 1 hour at PPFD of  $450 \mu\text{mol m}^{-2} \text{s}^{-1}$  and  $25 \pm 1^\circ\text{C}$ . The measurements were done under the same conditions.

### Photosynthetic pigments content and ratios

Photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, and total carotenoids) were extracted in 80% acetone, measured spectrophotometrically, and calculated according to the formula of [27].

### Chlorophyll fluorescence analysis

Chlorophyll fluorescence measurements were performed with a pulse modulation fluorometer (MINI-PAM, Heinz Walz, Germany) on the same plants used for gas exchange measurements. After dark adaptation (30 min), the minimal ( $F_0$ ) and maximal level of fluorescence ( $F_m$ ) were measured and the maximal quantum yield of PSII ( $F_v/F_m$ ) was calculated as  $F_v = F_m - F_0$ . After light adaptation (30 min), the apparent electron transport rate (ETR) was determined [ $ETR = Y \times PAR \times 0.5 \times 0.84$  [28], where  $Y = (F'_m - F)/F'_m$ ], as well as photochemical [ $qP = (F'_m - F)/(F'_m - F_0)$ ] and nonphotochemical quenching [ $qN = (F_m - F'_m)/(F_m - F_0)$ ], where  $qP$  and  $qN$  were calculated according to Schreiber [29].

### Statistical analysis

Statistical analysis was performed using one-way ANOVA (for  $p < 0.05$ ).

## Results

The data presented in (Tab. 1) demonstrate that the chilling conditions ( $10 \pm 1^\circ\text{C}$ ; Variant 2) significantly decreased the growth parameters of young maize plants. Visual chlorotic symptoms appeared in the base of the leaf lamina (data not shown). All growth parameters of the chilling-exposed plants were more than 50% lower than the respective values of the control ones. The application of the biostimulant Terra-Sorb Foliar did not result in a significant difference in the assessed biometric parameters of chilling-stressed plants (Variant 4), neither in the plants grown at optimal temperature regime (Variant 3).

The chilling treatment significantly decreased the leaf gas exchange parameters (Tab. 2). The net photosynthetic rate ( $A$ ) was reduced by 43%, the transpiration rate ( $E$ ) – by 42%, and the stomatal conductance ( $g_s$ ) – by 43%. The almost equal repression of these parameters indicates that stomatal limitation could be one of the leading factors for photosynthesis inhibition. However, a slight improvement (over 20%) in the values of these parameters was observed in the chilled plants which were treated with the biostimulant Terra-Sorb Foliar. In the case of the  $A$  and  $E$  components, this rescue effect was significant, while for the stomatal conductance values it was not. The application of the biostimulant on plants grown at optimal temperature did not result in changes in leaf gas exchange.

As a next step, the effect of chilling on the photosynthetic pigment content and ratios in the maize plants was evaluated. The results presented in Tab. 3 show that the total chlorophyll and carotenoid contents diminished by 43% and 20%, respectively, compared to those of the control plants. The less affected carotenoid content by the chilling treatment can be related to the essential role of carotenoids as defensive compounds.

**Tab. 1** Influence of chilling and the biostimulant Terra-Sorb Foliar on growth parameters of young maize plants.

Variants	Plant growth parameters			
	Fresh weight (g plant <sup>-1</sup> )	Height (cm)	Root length (cm)	Leaf area (cm <sup>2</sup> )
(1) 25°C (control)	9.23 ±0.71 <sup>a</sup>	63.00 ±1.75 <sup>a</sup>	18.00 ±0.74 <sup>a</sup>	150.40 ±3.10 <sup>a</sup>
(2) 10°C	2.50 ±0.09 <sup>b</sup>	29.00 ±0.48 <sup>b</sup>	11.00 ±0.74 <sup>b</sup>	54.20 ±0.34 <sup>b</sup>
(3) 25°C + Terra-Sorb	9.91 ±0.32 <sup>a</sup>	64.00 ±1.83 <sup>a</sup>	17.50 ±0.83 <sup>a</sup>	154.80 ±2.80 <sup>a</sup>
(4) 10°C + Terra-Sorb	2.65 ±0.05 <sup>b</sup>	30.00 ±0.63 <sup>b</sup>	11.50 ±0.47 <sup>b</sup>	55.00 ±0.23 <sup>b</sup>

The data presented are sample means ±SD. Different letters (a and b) following the SD values indicate significant differences at  $p < 0.05$ .

**Tab. 2** Influence of chilling and the biostimulant Terra-Sorb Foliar on leaf gas exchange in young maize plants. A – net photosynthetic rate (mol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>); E – transpiration rate (mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup>); g<sub>s</sub> – stomatal conductance (mol m<sup>-2</sup> s<sup>-1</sup>).

Variants	Leaf gas exchange parameters		
	A	E	g <sub>s</sub>
(1) 25°C (control)	17.39 ±0.97 <sup>a</sup>	1.16 ±0.28 <sup>a</sup>	0.07 ±0.01 <sup>a</sup>
(2) 10°C	9.94 ±0.02 <sup>c</sup>	0.67 ±0.00 <sup>c</sup>	0.04 ±0.00 <sup>b</sup>
(3) 25°C + Terra-Sorb	17.88 ±0.54 <sup>a</sup>	1.21 ±0.13 <sup>a</sup>	0.07 ±0.00 <sup>a</sup>
(4) 10°C + Terra-Sorb	12.29 ±0.19 <sup>b</sup>	0.82 ±0.07 <sup>b</sup>	0.05 ±0.01 <sup>b</sup>

The data presented are sample means ±SD. Different letters (a, b, and c) following the SD values indicate significant differences at  $p < 0.05$ .

**Tab. 3** Influence of chilling and the biostimulant Terra-Sorb Foliar on photosynthetic pigment content (mg g<sup>-1</sup> fresh weight) and ratio in young maize plants.

Variants	Photosynthetic pigment content and ratio (mg g <sup>-1</sup> )					
	Chl a	Chl b	Chl (a+b)	Car	Chl a/b	Chl (a+b)/Car
(1) 25°C (control)	3.25 ±0.07 <sup>a</sup>	0.83 ±0.02 <sup>a</sup>	4.08 ±0.08 <sup>a</sup>	1.04 ±0.11 <sup>a</sup>	3.91 ±0.41 <sup>b</sup>	3.92 ±0.09 <sup>a</sup>
(2) 10°C	1.87 ±0.01 <sup>b</sup>	0.44 ±0.02 <sup>b</sup>	2.31 ±0.02 <sup>b</sup>	0.83 ±0.00 <sup>b</sup>	4.25 ±0.18 <sup>a</sup>	2.78 ±0.03 <sup>b</sup>
(3) 25°C + Terra-Sorb	3.39 ±0.04 <sup>a</sup>	0.90 ±0.01 <sup>a</sup>	4.29 ±0.04 <sup>a</sup>	1.10 ±0.21 <sup>a</sup>	3.77 ±0.13 <sup>b</sup>	3.90 ±0.07 <sup>a</sup>
(4) 10°C + Terra-Sorb	2.08 ±0.09 <sup>c</sup>	0.59 ±0.03 <sup>c</sup>	2.67 ±0.12 <sup>c</sup>	0.95 ±0.01 <sup>a</sup>	3.53 ±0.05 <sup>c</sup>	2.81 ±0.14 <sup>b</sup>

The data presented are sample means ±SD. Different letters (a, b, and c) following the SD values indicate significant differences at  $p < 0.05$ .

According to some researchers, for example Haldimann [30], carotenoids may perform the role of protectors of the photosynthetic apparatus against oxidative damage during chilling stress. The application of Terra-Sorb Foliar significantly ameliorated the negative impact of chilling on both the chlorophyll and carotenoid contents by 16% and 14%, respectively, while it did not affect the amounts of pigments in the control plants.

The chilling exposure disturbed the photochemical processes in maize plants, as indicated by the chlorophyll fluorescence parameters in both dark- and light-adapted leaves (Tab. 4). The maximal quantum yield of PSII (Fv/Fm) decreased by 10%, but it was out of the range for healthy plants – 0.75–0.83 [31]. The lower Fv/Fm can be explained as a result of photoinhibition, induced by the chilling temperatures, as was described in the study of Bilská and Sowiński [32]. The application of Terra-Sorb Foliar tended to preserve the Fv/Fm value, which was close to normal.

**Tab. 4** Influence of chilling and the biostimulant Terra-Sorb Foliar on selected chlorophyll fluorescence parameters in young maize plants. Fv/Fm – maximal quantum yield of PSII; ETR – apparent electron transport rate (mol quanta m<sup>-2</sup> s<sup>-1</sup>); qP – photochemical quenching; qN – nonphotochemical quenching.

Variants	Chlorophyll fluorescence parameters			
	Fv/Fm	ETR	qP	qN
(1) 25°C (control)	0.752 ± 0.27 <sup>a</sup>	37.6 ± 1.02 <sup>a</sup>	0.594 ± 0.06 <sup>a</sup>	0.206 ± 0.00 <sup>d</sup>
(2) 10°C	0.676 ± 0.12 <sup>b</sup>	24.9 ± 0.94 <sup>b</sup>	0.535 ± 0.10 <sup>b</sup>	0.486 ± 0.08 <sup>a</sup>
(3) 25°C + Terra-Sorb	0.759 ± 0.34 <sup>a</sup>	40.4 ± 2.17 <sup>a</sup>	0.596 ± 0.03 <sup>a</sup>	0.290 ± 0.01 <sup>c</sup>
(4) 10°C + Terra-Sorb	0.715 ± 0.44 <sup>a</sup>	26.0 ± 1.95 <sup>c</sup>	0.547 ± 0.16 <sup>b</sup>	0.403 ± 0.04 <sup>b</sup>

The data presented are sample means ±SD. Different letters (a, b, c, and d) following the SD values indicate significant differences at  $p < 0.05$ .

The fluorescence parameters of light-adapted leaves were affected by chilling, as well. The apparent electron transport rate (ETR) was reduced by 34%, the photochemical quenching (qP) by 10%, while the nonphotochemical quenching (qN) was increased more than twice. As qP indicates the proportion of open reactive centers of PSII, while qN – heat dissipation, the applied chilling decreased the primary photochemistry and stimulated the loss of the excited energy as heat. It is important to note that the observed qP value should be considered only as a first approximation, due to the lack of possibility of correct measurement of  $F'_0$  (minimal fluorescence in a light-adapted sample) with the used device.

In these conditions, the application of Terra-Sorb Foliar had a small but significant protective effect on the ETR, qP, and qN parameters. In addition, the biostimulant seemed to have a detectable positive impact on the ETR in the plants grown at optimum temperature.

## Discussion

The unstable climate conditions and increased risks of crop exposition to strong abiotic stress factors, coupled with the constantly growing demand for food quantity and quality, make the topic of plant tolerance to unfavorable conditions of serious interest to both researchers and the industry.

In our study, the applied chilling stress (10°C, during 14 days) provoked visual chlorotic symptoms and growth retardation in young maize plants. The observed growth inhibition was accompanied by a lower content of photosynthetic pigments, reduced photosynthetic and transpiration rates, and suppression of photochemical processes, as was described in our previous studies [33] and by other authors as well [34–36].

The need for amelioration of the negative effects of chilling during the early stages of maize development stimulates the introduction of new approaches of cultivation such as supplementation with different biostimulants. Our research showed that the application of the protein hydrolysate Terra-Sorb Foliar exerted a positive impact on young maize plants subjected to chilling stress. In fact, all of the assessed plant photosynthetic parameters were improved to a different extent – leaf gas exchange by 22–23%, around 15% for photosynthetic pigment content, and a bit less for the photochemical indicators. It is well known that photosynthesis is a very sensitive process to different abiotic stresses. In this regard, our results confirm the potential of PHs to ameliorate the negative effect of chilling, as indicated by the improved photosynthetic parameters.

The observed protective action of the biostimulant Terra-Sorb Foliar is in line with the data of Botta [23] who found that the cold tolerance of lettuce had been improved by the treatment with PHs. Several authors proposed that the supplementation with products that contain free amino acids and small peptides supports the recovering process of stressed plants by their involvement in the metabolic pathways and stimulation of the secondary plant metabolism [5,37]. Recently, it has been shown that a significant part of the positive effects of PHs on plants could be attributed to the antioxidative

properties of important individual amino acids, applied to both seeds and leaves [38]. Evidence was documented that glutamate, cysteine, phenyl alanine, and glycine can act as signaling amino acids, since small doses were enough to increase the activity of the antioxidant enzymes, such as superoxide dismutase, catalase, and peroxidase, in soybean plants. It is widely accepted that the negative impact of chilling on plants is partly related to oxidative damages on photosynthetic pigments and electron transport processes, and therefore we may speculate that the observed improvement of the photosynthetic performance of the maize plants supplemented by Terra-Sorb Foliar could be due to the enhanced antioxidant network. This viewpoint is in line with the recent data of Ertani et al. [39] who performed transcriptome – wide identification of differentially expressed genes in tomato plants treated with PHs. The authors concluded that PHs have a potential to upregulate stress-related responses as well as genes involved in primary carbon and nitrogen metabolism, photosynthesis, nutrient uptake, and developmental processes.

In summary, the applied protein hydrolysate Terra-Sorb foliar is able to partially improve the photosynthetic performance of maize plants exposed to chilling stress. At the same time, it needs to be noted that despite its clear rescue effect, most of the assessed plant performance indicators were still lower than in the controls cultivated at normal temperature. Nevertheless, based on the better preserved function of the photosynthetic apparatus of the maize plants treated with Terra Sorb Foliar, we may assume that under subsequent favorable conditions their growth will recover more quickly as compared with untreated ones.

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### Wpływ hydrolizatów białkowych na wydajność fotosyntetyczną młodych roślin kukurydzy (*Zea mays* L.) poddanych stresowi chłodu

#### Streszczenie

Zastosowanie biostymulantów jest nowatorskim sposobem regulacji procesów fizjologicznych w roślinach. W ostatnich latach dowiedziono również, że ich aplikacja, zarówno na nasiona jak i części wegetatywne może w pewnym zakresie niwelować negatywne skutki stresów abiotycznych, między innymi suszy, zasolenia czy zbyt wysokiej temperatury. W warunkach klimatycznych Bułgarii wczesne fazy rozwoju roślin uprawnych pochodzących z cieplejszych stref klimatycznych, na przykład kukurydzy, odbywają się w okresach panowania temperatur suboptymalnych. Mimo, że łagodzenie negatywnych skutków stresu jest jednym z najczęściej podawanych korzystnych efektów działania biostymulantów, wiedza na temat ich wpływu na rośliny poddane stresowi chłodu jest wciąż niewystarczająca. Celem badań była ocena działania biostymulantów z grupy hydrolizatów białkowych na wzrost i przebieg procesu fotosyntezy kukurydzy uprawianej w warunkach obniżonej temperatury. Aplikacja hydrolizatów białkowych miała pozytywny wpływ na wymianę gazową, zawartość barwników fotosyntetycznych i sprawność aparatu fotosyntetycznego mierzoną parametrami fluorescencji chlorofilu *a*, ale nie wpłynęła na wzrost roślin. Na podstawie uzyskanych wyników można przypuszczać, że ze względu na sprawniejszy przebieg procesu fotosyntezy, rośliny kukurydzy poddane działaniu zbyt niskiej temperatury we wczesnych etapach rozwoju i potraktowane biostymulantami będą rosły lepiej po ustąpieniu suboptymalnych temperatur w porównaniu z roślinami nie poddanymi działaniu biostymulantów.