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EFFECTS OF A BIOSTIMULANT AND A MINERAL FERTILIZER ON THE ANTIOXIDATIVE DEFENCE SYSTEM OF CHILLING-EXPOSED MAIZE PLANTS

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Abstract

The aim of the study was to investigate the effects of the biostimulant *Terra-Sorb Foliar* and the mineral fertilizer Poly-Plant on the antioxidative defence system of chilling-exposed young maize plants (*Z. mays* L., hybrid *Kneza* 307). The plants were grown as a substrate-hydroponic culture in a controlled environment at 25±1°C/20±1°C (day/night) temperature. At the appearance of the third leaf, the maize plants were exposed to chilling stress (constant 10°C) for 14 days.

Control plants were grown at 25/20°C. Seven days after the beginning of the chilling treatment, a part of the chilled plants were sprayed with 1% water solution of the biostimulant *Terra-Sorb Foliar* and with 1% water solution of the mineral fertilizer *Poly-Plant*. The plants were left to grow for another 7-day period under the same conditions. At the end of the experimental period, the antioxidant activity, polyphenols and anthocyanins content, as well as the guaiacol peroxidase (GPOD) activity, were determined. The applied biostimulant and the mineral fertilizer ameliorated to some extent the negative effect of the chilling treatment of the young maize plants.

Keywords: *Zea mays* L., chilling, antioxidant activity, anthocyanin, polyphenols, biostimulant, leaf fertilizer.

INTRODUCTION

Chilling is one of the major factors limiting growth, distribution, and productivity of tropical plants, such as maize (*Zea mays* L). Chilling adversely affects the physiological processes in maize plants (Hund et al., 2007), especially when they are in early stage, during the transitional period from heterotrophic to autotrophic nutrition (Stamp, 1984). Temperatures below 15°C may cause chilling stress and induce different physiological disorders in young maize plants (Hola et al., 2007; Leipner, 2009).

During chilling stress, the production of reactive oxygen species (ROS) is accelerated, which may damage proteins, nucleic acids, lipids and other molecules (Aroca et al., 2003). Plants have several mechanisms to prevent or alleviate the damage from ROS. The mechanisms include non-enzymatic antioxidants, metabolites such as ascorbate, alfa-tocopherol, etc. and enzymatic antioxidants, such as SOD, CAT, POX, APX, etc. (Farooq et. al., 2008; Foyer et al., 1994; Pinhero et al., 1997).

Other secondary products of the metabolite processes, which are synthesized very intensively under chilling stress, such as anthocyanins, phenols, etc. possess a radical scavenging activity and act as antioxidants (Christie et al., 1994; Rivero et al., 2001).

Chilling temperatures may provoke decrease of water and mineral uptake (Aroca et al., 2003), stomatal conductance (Melkonian et al., 2004) and photosynthetic activity (Foyer et al., 2002). The negative effects have further consequences (Aroca et al., 2003; Foyer et al., 2002; Melkonian et al., 2004) leading to plant growth retardation (Leipner, 2009; Zaidi et al., 2010).

The application of different biostimulants and leaf fertilizers has been used to improve plant tolerance to environmental stresses, such as drought (Petrozza et al., 2014), low temperature (Botta, 2013), salinity (Ertani et al., 2013) and others. Surprisingly, there is a very little information about the effects of biostimulants and leaf fertilizers on the physiological status of maize plants exposed to chilling temperatures. Therefore, the aim of our study was to evaluate the effects of a biostimulant and a mineral fertilizer on an antioxidative defence system of young maize plants under chilling stress.

MATERIALS AND METHODS

Growth Conditions and Experimental Design

The experiments were carried out in a climatic room of the Department of Plant Physiology and Biochemistry at the Agricultural University of Plovdiv, Bulgaria. Maize plants, from hybrid “Kneza

307", were grown as a substrate-hydroponic culture, using ½ strength modified Hoagland nutrient solution, at controlled environment: photoperiod – 12 hours, PPFD (Photosynthetic photon flux density) 200 µmol m^{-2} s^{-1}, temperature – 25±1°C/20±1°C (day/night) and relative air humidity – 60±5%. At the appearance of the third leaf, the maize plants were exposed to chilling stress (constant 10°C) for 14 days. Control plants were grown at 25/20°C. Seven days after the beginning of the chilling treatment, a part of the chilled maize plants were sprayed with 1% water solution of the biostimulant Terra-Sorb Foliar and another part with 1% water solution of the mineral fertilizer Poly-Plant. The volume of leaf spraying was 1 ml per plant. The plants were left to grow for another 7 days under the same conditions. The product Terra-Sorb Foliar contains free amino acids and small peptides, while the product Poly-Plant contains only macro- and microelements. The experimental design included 4 treatments, namely: control 25°C, chilling 10°C, Terra-Sorb Foliar 10°C and Poly-Plant 10°C. Each treatment had 3 replications (pots) with 4 plants per pot. Analyses were conducted at the end of the experimental period. The experiment was performed twice.

Polyphenols content

The total amount of phenolic compounds in the plant extracts was determined with the reagent of Folin-Ciocalteu (Waterman et al., 1994) following the methodology of Singleton and Rossi (1965), with slight modifications. The samples (1 g of fresh leaf material) were ground with quartz sand and 10 ml 60% acidic methanol, and submerged in an ultrasound bath for 15 min.

The homogenized material was then transferred to suitable tubes, which were carefully sealed and left for 15 hours in the dark at room temperature for extraction.

During the incubation period, the tubes were periodically stirred. Afterwards, the tubes were centrifuged and the supernatant, which was used for the measurement of total phenolics, anthocyanins, and antioxidant activity, was carefully collected in new clean tubes. For the determination of total phenolics were mixed 40 µl of extract, 3160 µl distilled water, 200 µl Folin-Ciocalteu reagent and after a minute was added 600 µl 20% NaCO3.

The test tubes were left for 2 hours at room temperature for the reaction to occur. After that, the extinction at 765 nm wavelength was measured. Total phenolics were calculated as gallic acid equivalents (GAE) using a standard curve and are presented as mg/g fresh weight. The standard curve was prepared with gallic acid (Sigma-Aldrich, St. Louis, MO) in the range 0–500 mg/l.

Anthocyanins content

The measurement of the number of monomeric anthocyanins was performed with the pH - differential method (Guisti & Wrolstad, 2001). The plant extracts with acidic methanol (as described in the procedure for phenolics measurement) were diluted with a buffer (0.025 mol/l potassium chloride), adjusted to pH = 1 with HCl, and another buffer (0.4 mol/l sodium acetate) with pH = 4.5. Each sample was diluted to a certain extent with the first buffer with pH=1 (which gives the value DF) and the absorption was measured at 520 nm and 700 nm. A second aliquot of each sample was diluted to the same extent with the second buffer with pH = 4.5 and again the extinction at 520 nm and 700 nm was measured. To calculate total anthocyanins content, the absorption values were used in the following formula:

$A = (A\lambda 520 - A\lambda 700)pH1.0 - (A\lambda 520 - A\lambda 700)pH4.5$

$TA = (A*MW*DF*1000)/ \varepsilon*1$

where: the molar extinction coefficient (ε) for cyanidin-3-glucoside = 26900 (M^{-1} cm^{-1}), the molecular weight (MW) of cyanidin-3-glucoside = 449.2 g/mol and the dilution factor (DF) demonstrates the times' dilution of the sample. The results (TA – total anthocyanins) are expressed as mg cyanidin-3-glucoside chloride/100 g fresh plant material used.

Antioxidant activity

This parameter was measured with the preformed radical monocation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS*+) (Shalaby et al. 2013) in extracts obtained in the way described for total phenolics.

The incubation mixture contained 300 µl extract and 3.0 ml ABTS (with 0.7 abs at 734 nm wavelength). After 6 min at room temperature, the absorbance was measured at 734 nm. For reference (blank sample) ABTS + 300 µl 80% MeOH was used. The antiradical activity is expressed as % total antioxidant activity and is equal to:

TAA% (Total antioxidant activity) = (Abs(plant sample) – Abs(blank)/Abs(plant sample))*100

GPOD enzyme activity

To obtain the enzyme extract 1 gram of fresh plant material was homogenized in 5 ml ice-cold extraction 0,1 M Tris-HCl (pH = 7,8) buffer containing 1 mM DTT and 1mM EDTA. After that, the samples were centrifuged at 13 500 g (4°C for 10 min).

The supernatant was used to determine the enzymatic activity spectrophotometrically on a UV/VIS spectrophotometer Pharo 300, according to

the methodology of Mocquot et al. (1996). The activity of guaiacol peroxidase (GPOD) (EC 1.11.1.7) was measured at 436 nm according to Bergmeyer (1974). The reaction mixture in the cuvette contained: 2,3 ml 0,1 M KH2PO4 (pH 7,0), 300 µl 5 mM H2O2, 300 µl guaiacol and 100 µl enzyme extract.

Statistical Analysis

Statistical analysis was performed using one-way ANOVA (for $P < 0.05$). Based on ANOVA results, a Duncan test for mean comparison was performed, for a 95% confidence level, to test for significant differences among treatments. In the figures and the tables, different letters (a, b, c and d) express significant differences at the $P < 0.05$.

RESULTS AND DISCUSSION

The young maize plants subjected to 14 days chilling had higher total antioxidant activity (TAA) in both (leaves and roots) compared to the control plants. The TAA was increased by 134% in the leaves and by 17% in the roots of the chilled maize plants (Table 1). The absence of stress conditions in the control plants kept the value of the total antioxidant activity low.

The application of the biostimulant Terra-Sorb Foliar and the mineral fertilizer Poly-Plant changed the TAA of the chilled maize plants. The TAA values of the plants from these treatments were diminished by 26% – leaves and 25% – roots for Terra-Sorb Foliar-treated chilled plants and 7% - leaves and 27% – roots for Poly-Plant–treated chilled plants, respectively, as compared to untreated chilled plants.

The chilling induced changes in anthocyanins and polyphenols content (Table 2 and Table 3) of maize plants, which is in accordance with the higher value of the total antioxidant activity. The value of anthocyanins content in leaves of chilled plants raised more than twice compared to that of the control plants and the polyphenols content was enhanced by 75% in leaves and by 42% in roots compared to that of the control plants, respectively.

The application of Terra-Sorb Foliar and Poly-Plant on chilled maize plants reduced the appearance of significant changes in the anthocyanins content. The anthocyanins level in the leaves of Terra-Sorb Foliar-treated chilled plants was by 54% lower compared to the untreated chilled plants and by 30% in the leaves of Poly-Plant-treated chilled plants.

The same tendency is observed in polyphenols content. The polyphenols content in the leaves of Terra-Sorb Foliar-treated chilled plants was diminished by 9% and 30% of the leaves and roots, respectively, as compared to the untreated chilled plants. In Poly-Plant-treated chilled plants, the respective values were lowered by 11 and 2%.

The applied 14 days chilling treatment (10^{o}C) increased the activity of guaiacol peroxidase (GPOD) in the leaves and roots of young maize plants from the hybrid Kneza 307. The chilled maize plants enhanced their GPOD activity by 26% in leaves and 76% in the roots as compared to the control plants (Table 4).

The leaf application of both Terra-Sorb Foliar and Poly-Plant lowered the GPOD activity in The leaf application of both Terra-Sorb Foliar and Poly-Plant lowered the GPOD activity in the chilled plants.

The GPOD activity of Terra-Sorb-treated chilled plants was lowered by 29% in the leaves and by 34% in the roots.

The respective values in Poly-Plant-treated chilled plants were diminished by 21% in both roots and leaves.

Table 1. Effects of a biostimulant and a mineral fertilizer on total antioxidant activity (%) in leaves and roots of chilling-exposed young maize plants (10±1˚C)

TAA (%)		
Treatment	Leaves	Roots
Control 25°C	27.1±0.6c	11.7±0.1b
Chilling 10°C	63.5±0.3a	13.7±1.1a
Terra-Sorb Foliar 10°C	47.3±0.2b	10.3±0.5c
Poly-Plant 10°C	59.2±1.2a	10.0±0.1c

The data presented is an average ± SD. Different letters (a, b, c and d) following the mean values indicate significant differences at $P < 0.05$.

Table 2. Effects of a biostimulant and a mineral fertilizer on leaf anthocyanins content (mg cyanidin-3-gluciside/100g FW) of chilling-exposed young maize plants (10±1°C)

Anthocyanins content	
Treatment	Leaves
Control 25°C	88,1±3.6d
Chilling 10°C	241,5±12.1a
Terra-Sorb Foliar 10°C	111,3±5.8c
Poly-Plant 10°C	168,1 ±10.2b

The data presented is an average ± SD. Different letters (a, b, c and d) following the mean values indicate significant differences at P<0.05.

Table 3. Effects of a biostimulant and a mineral fertilizer on polyphenols content (mg GAE/g FW) in leaves and roots of chilling-exposed young maize plants (10±1°C)

Polyphenols		
Treatment	Leaves	Roots
Control 25°C	6.1±0.8b	4.3± 0.5b
Chilling 10°C	10.7±0.2a	6.1±1.5a
Terra-Sorb Foliar 10°C	9.7±1.1a	4.3±0.3b
Poly-Plant 10°C	9.5±1.0a	6.0±0.5a

The data presented is an average ± SD. Different letters (a, b, c and d) following the mean values indicate significant differences at P<0.05.

Table 4. Effects of a biostimulant and a mineral fertilizer on guaiacol peroxidase activity (U/g FW) in leaves and roots of chilling exposed young maize plants (10±1°C)

Guaiacol peroxidase		
Treatment	Leaves	Roots
Control 25°C	1.9±0.3b	10.9 ± 0.6c
Chilling 10°C	2.4±0.4a	19.2±1.6a
Terra-Sorb Foliar 10°C	1.7±0.1b	12.6±0.8c
Poly-Plant 10°C	1.9 ±0.2b	15.2±1.3b

The data presented is an average ± SD. Different letters (a, b, c and d) following the mean values indicate significant differences at P<0.05.

The detected responses of both enzymatic and non-enzymatic components of the plant antioxidant system are a result of the negative effects of chilling temperature. Considering the effects of both biostimulants, Terra-Sorb Foliar and Poly-Plant on the different components of the antioxidative defence system of the chilled maize plants, we may conclude that they had ameliorating influence.

The observed effects of chilling on young maize plants correspond to the results of other authors' research work, concerning exposure of warm climate plants to chilling temperatures. Sarkar et al. (2009) reported that the total antioxidant activity (ABTS assay) increased in turfgrass species with gradual reduction of temperature. Singh et al. (2014) also observed that the decrease in temperature enhanced the total phenolic compounds in maize plants.

The increased levels of the total phenolic compounds have been observed in chill-stressed tomato and melon (Rivero et al., 2001), pepino

plants (Sivaci et al., 2014), in lettuce and leaf basil (Kalisz et al., 2016).

Enhanced levels of anthocyanins and peroxidases in warm climate plants subjected to chilling stress have been reported by a number of authors. Pietrini et al. (2002) observed enhanced anthocyanins content in maize plants exposed to low temperatures. Leipner (2009) also found a higher anthocyanins level in chilled-stressed maize plants.

Khorshidi & Moafi (2014) and Kang & Saltveit (2002) reported for enhanced activity of guaiacol peroxidase in maize plants exposed to chilling temperatures. Increased activity of peroxidases, including guaiacol peroxidase, has been reported by Lee & Lee (2000) in the response of cucumber to chilling stress.

The leaf application of the biostimulant Terra-Sorb Foliar and the mineral fertilizer Poly-Plant ameliorated the negative effects of chilling temperature. The components they contain may be easily taken from the leaves and therefore may be involved in different metabolic pathways. The biostimulant Terra-Sorb Foliar contains free amino acids and small peptides.

According to observations of Matsumiya and Kubo (2011) amino acids and small peptides can be easily absorbed by the leaves. The leaf uptake of mineral nutrients is a common practice in agriculture, especially when the root mineral nutrition is disturbed. In our previous study (Cholakova & Vassilev, 2015) we found that chilling diminished the uptake of macroelements in maize plants, consequently, the leaf application of the fertilizer Poly-Plant caused a positive effect of these plants.

The observed positive effects of both biostimulants Terra-Sorb Foliar and foliar fertilizer Poly-Plant on chilled maize plants are in a good correspondence with the results of other authors' research work connected with the influence of similar products on stress-exposed plants. For example, Ertani et al. (2013) used biostimulants to ameliorate the negative effect of salt stress on the physiological status of maize plants.

Petrozza et al. (2014) also used biostimulants to improved drought resistance of tomato plants and Botta (2013) – cold tolerance of lettuce plants. It was also shown that the application of protein hydrolysates increased nitrogen assimilation and shoot biomass in hydroponically-grown maize (Schiavon et al., 2008) and secondary plant metabolism (Ertani et al., 2011; Schiavion et al., 2010). Hu et al. (2008) also conducted some analyses to evaluate the effect of foliar fertilization on the growth and mineral nutrient content of maize seedlings under drought and salinity. These reports gave some evidence that leaf-applied amino acids, small peptides, and mineral elements may get into metabolic pathways and support the recovering process in the stressed plants.

CONCLUSIONS

In conclusion, the chilling temperatures caused chilling stress and induced changes in the antioxidant defence system of young maize plants. The negative effects have been slightly ameliorated when the plants were sprayed by the biostimulant Terra-Sorb Foliar as well as by the mineral fertilizer Poly-Plant.

Further studies are being in progress to clarify the nature of the detected positive effects of the used biostimulant and mineral leaf fertilizer on the physiological status of chilling–exposed maize plants.

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