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Influence of stevioside and heavy metals on physiological and biochemical parameters of winter wheat

Julia Yurevna Nevmerzhitskaya*, Alexander Leonidovich Mikhailov, Anastasia Sergeyevna Strobykina, Olga Arnoldovna Timofeeva
Kazan (Volga region) Federal University, Kremlevskaya 18, 420008, Kazan, Russia.

***Corresponding author**

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Abstract
Diterpene stevioside glycoside ability to increase resistance to heavy metals influence on germinating seedlings of Mironovskaya 808 type winter wheat is studied. It is shown that $CdSO_4$, $CuSO_4$, and $ZnSO_4$ (10 μmol) inhibited the plant growth and caused accumulation of proline amino acid. Preliminary growing on stevioside solution (10^{-8} mole) for 5 days considerably decreased toxic effect of heavy metals on plant growth, amounts of heavy metals in roots and leaves of germinating seedlings and increased the proline level.

Keywords: Winter wheat; stevioside; heavy metals; frost resistance; growth; lectins activity.

Introduction

Secondary metabolites of plants constantly cause researchers' interest due to their various biological activity and ability to serve as base for creation of plants growth regulators. Among secondary metabolites, diterpene glycosides of *Stevia rebaudiana* Bertoni plants with steviol (13-hydroxy-*ent*-kaur-16-en-19-oic acid) as aglycon is of particular interest [1]. Previously, steviol was considered as precursor of gibberellic acid due to *cis*-coupling of B and C rings of tetracyclic hydrocarbon system, typical for gibberellins. However, it was discovered that *Gibberelle fujikuroi* fungus does not turn steviol into gibberellic acid, but metabolize it to gibberellins-like compound [2]. There is also data in literature about steviol glycoside derivatives that show gibberellins-like activity [3]. Earlier, it was shown that main stevia glycoside – stevioside – has antistress properties and increases frost resistance of wheat plants [4]. The aim of this study was to discover protective abilities of gibberellins-like diterpene stevioside glycoside on winter wheat plants under heavy metals influence.

Methods

Objects of the study were roots of winter wheat germinating seedlings (*Triticum aestivum* L., Mironovskaya 808 type). Diterpene stevioside glycoside was obtained from raw *Stevia rebaudiana* Bertoni plant in the Laboratory of phosphorus analogs of natural compounds (Head of the Laboratory is Doctor of Chemistry, Professor, the Corresponding Member of RAS Vladimir F. Mironov) in AE Arbuzov Institute of Organic and Physical Chemistry. Plants were grown in the laboratory in sample chambers with tap water under the conditions of light at 100 W/m^2 and 12-h photoperiod at temperature 23°C for 9 days. In experimental samples, the plants were grown on diterpene stevioside glycoside solution (10^{-8} mole) for 5 days. After 5 days, plants have been transferred to heavy metals solutions ($CdSO_4$, $CuSO_4$, and $ZnSO_4$) in 10 μmol concentration. Concentration of stevioside and heavy metals was chosen according to preliminary experiments. Amounts of heavy metals in wheat germinating seedlings were determined in mass spectrometer Elan DRC II (PerkinElmer, USA) [5]. Activity of ascorbate peroxidase was determined by the method described in Ref. [6]. Extraction and defining of proline were conducted by the method described in Ref. [7]. Experiments were performed in biological triplicate.

Results and Discussion

Growing of winter wheat plants on stevioside solution (10^{-8} mole) caused the increase the height of germinating seedlings leaves by 14% and length of the roots by 18% in comparison to the control samples (Table 1). It is reported in literature that some diterpenoids and glycosides of *ent*-Kaurane Glycosides boost the plants growth [3].

$CdSO_4$, $CuSO_4$, and $ZnSO_4$ inhibited the growth of germinating seedlings roots and leaves in different measures (Table 1). Decrease in growth speed is a common effect of heavy metals on plants, based on their direct influence on division and stretching of cells [8,9]. As it is shown in Table 2, a significant amount of Cd^{2+}, Cu^{2+}, Zn^{2+} has been accumulated in germinating seedlings roots and leaves under these conditions (Table 2). Modification of heavy metals influence on cultivated plants растения by applying different growth regulators is shown in Ref. [10]. In our experiments, stevioside decreased heavy metals influence on the plant growth (Table 1) and lowered heavy metals accumulation level in germinating seedlings (Table 2).

There is a number of data about heavy metals influence on active oxygen generation in literature [11-13]. Antioxidant enzymes, including a wide group of peroxidases, take part in detoxification of active oxygen. Ascorbate peroxidase is a main enzyme involved in detoxification of H_2O_2 in the cell, due to oxidation of ascorbic acid. Cd^{2+} and Zn^{2+} inhibited activity of this enzyme, but Cu^{2+} increased ascorbate peroxidase activity by 50% (Figure 1). Pretreatment of germinating seedlings with stevioside decreased the effect of Cu^{2+} on ascorbate peroxidase activity to the level of control plants, while ascorbate peroxidase activity increased by 38% and 56% under the influence of Cd^{2+} and Zn^{2+}, respectively, in comparison to control plants (Figure 1).

Despite the important role of antioxidant enzymes in various types of stress, low-molecular organic antioxidants can protect metabolism from active oxygen more effectively in some cases [14]. One of these compounds is proline amino acid.

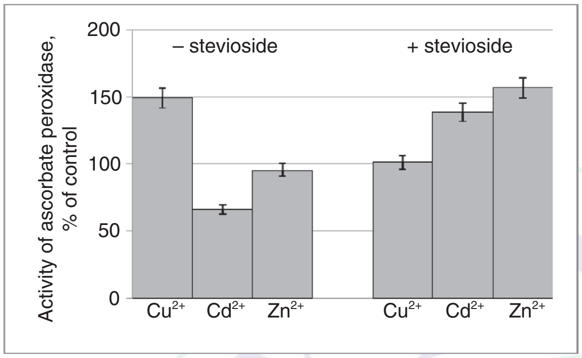
Table 1: Influence of stevioside on roots length and leaves height of 9-day-old germinating seedlings of Mironovskaya 808 winter wheat.

Sample	H_2O		Stevioside	
	Leaves length (mm)	Roots length (mm)	Leaves length (mm)	Roots length (mm)
Control	158.5 ± 2.0	90.3 ± 2.3	180 ± 2.1	107 ± 3.3
$CdSO_4$ (10 μmol)	129 ± 1.5	64 ± 2.5	134 ± 3.0	71 ± 1.7
$ZnSO_4$ (10 μmol)	157 ± 3.5	75.5 ± 1.5	172 ± 1.8	98 ± 3.1
$CuSO_4$ (10 μmol)	136 ± 1.9	51 ± 1.5	143 ± 1.5	71 ± 3.2

Table 2: Influence of stevioside on heavy metals content in 9-day-old germinating seedlings of Mironovskaya 808 winter wheat (μg/dry weight g).

Sample	H_2O		Stevioside	
	Control	Heavy metals	Control	Heavy metals
	Roots			
$CdSO_4$ (10 μmol)	14.36 ± 0.11	632.79 ± 0.78	22.36 ± 0.12	566.31 ± 0.81
$ZnSO_4$ (10 μmol)	43.53 ± 0.67	551.93 ± 0.62	81.90 ± 0.54	533.93 ± 0.63
$CuSO_4$ (10 μmol)	25.25 ± 0.08	68.11 ± 0.11	25.22 ± 0.14	45.71 ± 0.22
	Leaves			
$CdSO_4$ (10 μmol)	0.30 ± 0.01	8.50 ± 0.08	0.94 ± 0.01	7.27 ± 0.06
$ZnSO_4$ (10 μmol)	7.55 ± 0.08	16.48 ± 0.20	8.94 ± 0.08	11.54 ± 0.09
$CuSO_4$ (10 μmol)	2.24 ± 0.02	3.22 ± 0.05	2.25 ± 0.04	2.10 ± 0.04

Figure 1: Influence of heavy metals and stevioside on ascorbate peroxidase activity.



According to the obtained data, addition of heavy metals into plants growing medium significantly increases the proline level. Proline accumulates the most with Cu^{2+}. Some scientists connect heavy metals resistance with proline accumulation [15].

Stevioside stimulated proline accumulation in germinating seedlings roots up to 4 times in comparison to plants grown on water. We suggest that in this case the proline level may indicate the increase of adaptive potential of stevioside-treated plants. Proline concentration increased even higher in germinating seedlings treated with heavy metals and stevioside in comparison to treatment with pollutants only (Table 3).

At present, there is a very few data on stevioside mechanism of action, but experiments with animals have shown that this glycoside is a calcium channel blocking agent [16]. It can be supposed that stevioside changes adsorption and transportation of heavy metals in plant cells in the same way.

Thus, according to the performed research, stevioside (10^{-8} mol) decreased metals effect of plants growth and lowered their accumulation level in germinating seedlings and also caused a significant growth of proline level, which indicates its protective action on winter wheat plants under heavy metals caused stress.

Table 3: Proline content (mg/dry weight g) in roots of Kazanskaya 560 winter wheat, grown on water and on stevioside under influence of $CdSO_4$, $ZnSO_4$, and $CuSO_4$.

Samples	– Stevioside	+ Stevioside
Control	0.004 ± 0.001	0.016 ± 0.001
Cu (10 μmol)	0.039 ± 0.002	0.155 ± 0.002
Cd (10 μmol)	0.015 ± 0.001	0.063 ± 0.001
Zn (10 μmol)	0.009 ± 0.001	0.041 ± 0.001

Conclusions

1. It is determined that $CdSO_4$, $ZnSO_4$, and $CuSO_4$ induced proline accumulation, which indicates that the main protective function under oxidation stress caused by heavy metals is carried out by low-molecular no-enzymatic.
2. Stevioside lowered heavy metals accumulation level in roots and leaves of winter wheat germinating seedlings, decreased pollutants effects on plant growth, increased ascorbate peroxidase activity and proline content, which indicated its protective action on winter wheat plants under heavy metals caused stress.

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