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Stevioside-induced molecular heterogeneity and lectin activity at low positive temperatures

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ABSTRACT

This article describes the results of a study on the effect of pre-sowing treatment of *Triticum aestivum* L. seeds with stevioside (10^{-8} M) for 24 h on the activity and molecular heterogeneity of lectins in seedlings at low positive temperatures. Phytolectins take part in a wide range of physiological processes in plants, regulating not only growth, but also increasing resistance to salinity, heavy metals, and temperature stress. In this regard, the study of changes in the activity and heterogeneity of these molecules in the search for new regulators of plant growth and development is urgent. The observed stevioside-induced increase in the activity of lectins bound to the cell wall and changes in their qualitative and quantitative composition against the background of temperature stress may indicate an increase in the adaptive potential of wheat plants.

Keywords: Triticum aestivum, Lectins, Stevioside, Steviol glycoside, Wheat germ agglutinin.

INTRODUCTION

Natural compounds exhibiting biological activity on plants are currently one of the most interesting areas of science. This is primarily due to the polyfunctional action of these molecules. In addition to stimulating growth processes, treatment with these compounds can increase the resistance and adaptive potential of plants. Secondly, natural phytoregulators are distinguished by low consumption and lower production costs. Thirdly, as the source is natural raw materials, these compounds are environmentally friendly. These compounds include, in particular, sweet diterpene steviol glycosides isolated from *Stevia rebaudiana* B. (Brandle & Telmer 2007). The chemical structure of these molecules differs only in the amount and composition of carbohydrate residues combined with aglycone - steviol. In particular, stevioside contains three glucose residues, rebaudioside A — four, and rebaudioside C — two glucose residues and one rhamnose (Ceunen & Geuns 2013). A large number of commercial sweeteners are currently being produced based on a set of glycosides or stevioside, which since 2008 has been recognized by the FDA as a safe compound. Stevioside has been shown to exhibit various biological activities and have antimicrobial, hypotensive, hypoglycemic effects on the human body. However, the fact that steviol (aglycone) has a gibberellin-like chemical structure has left out. This is due to the peculiarities of the biosynthesis of these compounds, as the synthesis of steviol and gibberellins in the stevia plant to ent-kaurene follows the same path. This fact suggests the presence of some biological effects of stevioside, including as a regulator of plant growth and development. Earlier, our group showed the gibberellin-like activity of derivative steviol glycosides (Timofeeva *et al.* 2010) and also revealed that stevioside, which includes three glucose residues, has the greatest effect on wheat plants than a number of natural and synthetic steviol derivatives (Ogorodnova U *et al.* 2016). Plants are incapable of active movement, therefore they have specific responses to motion-induced stress and adaptation. Of great interest is a special class of compounds - phytolectins. These are glycoproteins (molecular weight from 36 kD to 265 kD) with the ability to recognize, highly specific and reversibly bind the carbohydrate

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moiety of glycoconjugates, without causing their chemical transformation (Ugit & Usukeeva 2015). It is known that the content of phytolectins is a very variable indicator, and the activity depends on abiotic and biotic factors of the environment (Markov & Khavkin 1983). These molecules in plants have a wide range of different functions. It is assumed that lectins take part in the processes of cell growth and differentiation (Jiang *et al.* 2010) can perform a storage and protective function in response to biotic and abiotic stress, participate in the transport of sugars and hormones, regulate plant development, and also participate in the formation of symbiosis (Shakirova & Bezrukova 2007; De Hoff *et al.* 2009; Kovalchuk *et al.* LI 2012). There is information about the participation of lectins in the processes of adaptation of plants to low and negative temperatures, as they play the role of positive-negative "effectors" in membrane processes (Komarova *et al.* 2000). Temperature is known as a limiting factor for growth processes, which, in turn, are directly related to plant productivity. In our work, we consider the effect of the natural regulator of plant growth and development, stevioside, on the activity of soluble and bound lectins, and also investigated the molecular composition of lectins in the cell wall of spring wheat plants of the Omskaya 33 variety under low positive temperatures.

MATERIALS AND METHODS

Research object: roots of Omskaya 33 spring wheat (*Triticum aestivum* L.). Before planting, seeds were sterilized and grown *in vitro* in cuvettes at an illumination of 100 W/m^2, a 12-hour photoperiod and at a temperature of 23°C. The plants of the experimental group were soaked for 24 hours in a solution of stevioside isolated from the leaves of stevia (*Stevia rebaudiana* B.) at A.E. Arbuzov Institute of Organic and Physical Chemistry in the laboratory of phosphorus analogues of natural compounds under the guidance of RAS Corresponding Member V.F. Mironov. We used the active concentration of this substance, which was determined by our research group earlier and amounted to 10^{-8}M. Control plants were soaked in water. All plants were grown for 7 days. To determine the effect of low temperatures, wheat seedlings were placed in a refrigerator (+4°C) for the last 3 hours of the seventh day. The roots of 7-day-old wheat seedlings were used to isolate and determine the activity of lectins, as well as for gel filtration separation of proteins with lectin activity. Lectin activity was determined by the hemagglutination reaction with blood group A erythrocytes (Roopashere *et al.* 2006).

For gel filtration separation of cell wall lectins, a chromatographic system with a 26 cm × 1.4 cm column packed with SephadexG-150f was used. The supernatant was loaded onto a column pre-equilibrated with a buffer mixture (0.9% NaCl; 50 mM K-phosphate buffer). The flow rate of the column with Sephadex G-150 f is 0.48 mL/min. The volume of collected fractions is 0.5 ml. In the collected fractions, the protein content and lectin activity were determined. The column was calibrated using standard proteins: bovine serum albumin (67 kDa) and cytochrome B (44 kDa).

RESULTS AND DISCUSSION

In the first series of experiments, the effect of stevioside on the activity of soluble (SL) and cell wall-bound (CWL) lectins in the roots of spring wheat seedlings under stress by low positive temperatures was determined. As Figure 1 shows, stress induced an increase in SL activity by 60% compared to control. It is known that, along with the synthesis of stress proteins under the action of unfavorable factors, there is an increase in the synthesis of some proteins inherent in plants under normal conditions. WGA also belongs to such proteins. According to literature, the accumulation of this lectin in wheat plants occurs in an ABA-mediated way in response to drought, osmotic shock, salinity, and hyperthermia (Shakirova 2001). Based on this, we can assume the presence of protective functions of WGA from unfavorable environmental factors of an abiotic nature. In addition, growth-stimulating hormones such as gibberellic acid (GA), indoleacetic acid (IAA), cytokinin (CK), 6-benzylaminopurine, 24-epibrassinolide (EB) are inducers of WGA synthesis (Shakirova *et al.* 2001, Ogorodnova *et al.* 2020). This fact may indicate the participation of WGA in the regulation of growth processes. Pretreatment of spring wheat seeds with stevioside (10^{-8} M, 24 h) did not change the SL activity both in control and under stress conditions, although the activity of this protein fraction increased in control plants. We have previously shown that under the action of low positive temperatures on winter wheat varieties, stevioside increases the SL activity. At the same time, there is a change in the hormonal status of plants, in particular, the content of IAA, CK and ABA (Ogorodnova *et al.* 2020) phytohormones that control the content of lectins, increases.

However, spring wheat had no stevioside-induced changes in SL activity. We assume that the effect of stevioside on soluble lectins under low temperature conditions is associated with the varietal characteristics of winter and

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spring wheat plants. As shown in Fig. 3, the activity of cell wall lectins (CWL) under control conditions is the same, and against the background of low positive temperatures, an increase in this indicator is observed, and stevioside induces an even greater increase in activity.

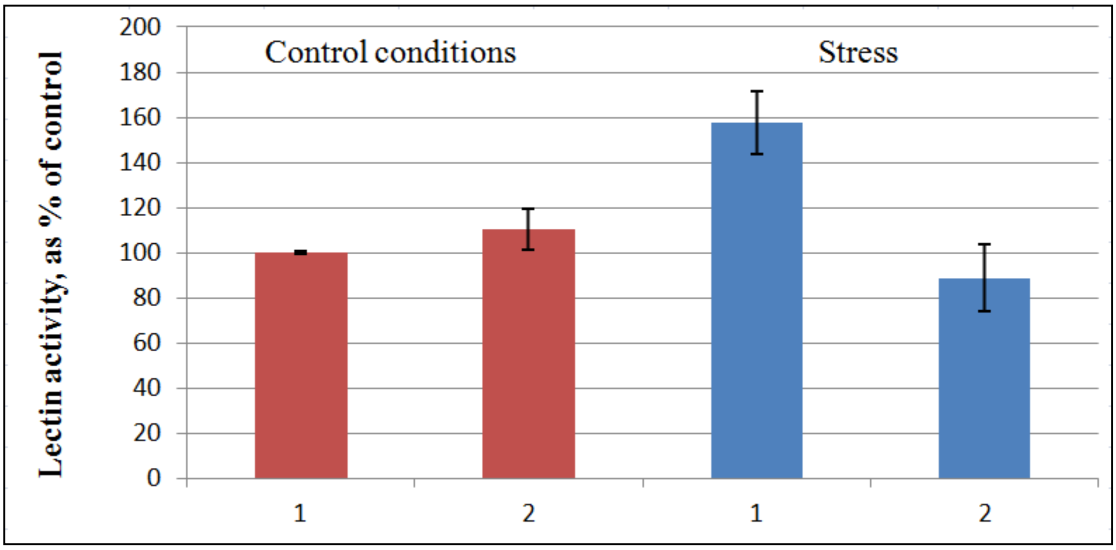

Fig. 1. Activity of soluble spring wheat lectins at stress of low positive temperatures: 1-control (H_2O), 2- stevioside
When measuring the activity of soluble lectins, we primarily record a change in the activity of wheat germ agglutinin (WGA), which is present in the organs and tissues of wheat in different phases of ontogenesis (Timofeeva *et al*. 2010) and is a classic lectin of cereals.

The action of abiotic factors (water deficit and hyperthermia) is known to cause a significant increase (by a factor of 2-5) in the activity of lectins in the cell walls of seedlings of drought-resistant varieties and lines of grain crops, and in weakly drought-resistant varieties - a decrease or preservation of their activity at the level of control plants. Which is explained by the different rates of mobilization of storage lectin mRNAs for the biosynthesis of these proteins (Molodchenkova & Adamovskaia 2014).

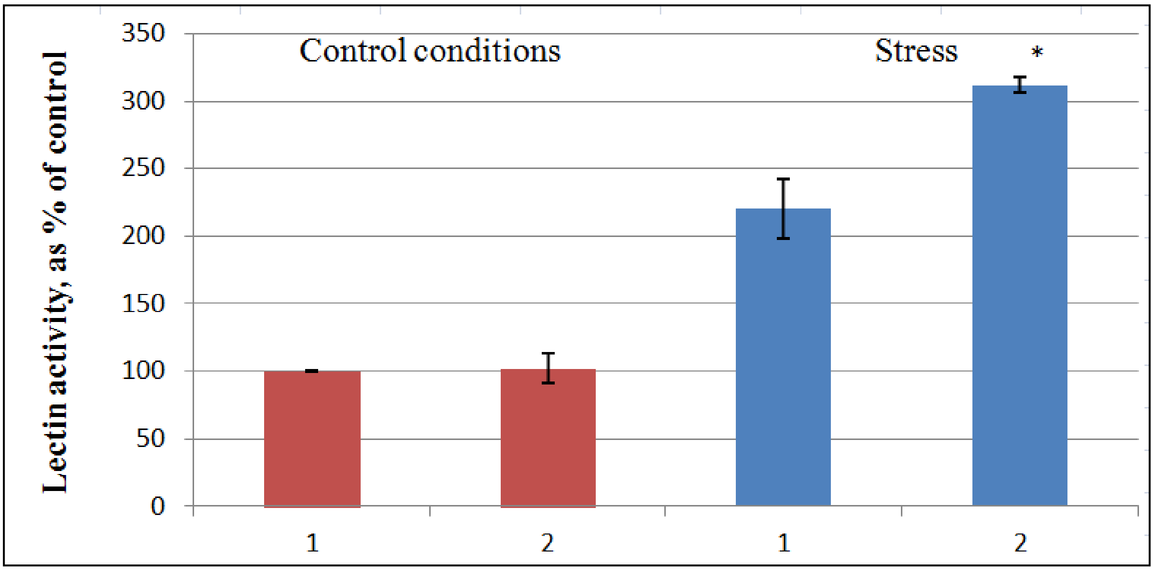

Fig. 2. Activity of cell wall spring wheat lectins at stress of low positive temperatures: 1-control (H_2O), 2- stevioside.

Alterations in carbohydrate specificity (Komarova 1987) and activity of CWL in seedlings of grain crops under the action of biotic and abiotic factors can be associated with an increase in the content of carbohydrates, which accumulate in excess during hydrolysis of polysaccharides of plant cell walls (when infected with fungal

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pathogens, under conditions deficiency of moisture and hyperthermia), and on the other hand, probably with conformational transformations of proteins, changing the availability of sugars to carbohydrate-binding sites, or to the appearance of lectin isoforms with different affinity for carbohydrates. Changes in the activity and carbohydrate specificity of lectins upon infection and exposure to abiotic factors can serve as a signal for triggering other protective reactions and the formation of a multicomponent biochemical response of a plant to unfavorable growing conditions (Molodchenkova & Adamovskaia 2014). Thus, the combined effect of low positive temperatures and stevioside (10^{-8} M, 24 h) causes a change only in the activity of CWL. Stevia glycoside stimulates an increase in the activity of lectin proteins of this fraction by 40% under stress conditions compared to control plants. In the next part of the research, we carried out the gel filtration separation of cell wall proteins in the roots of Omskaya 33 spring wheat seedlings treated with stevioside and exposed to low positive temperatures.

Among the obtained protein fractions in control plants (water), lectin activity was found in fractions containing proteins with molecular weights of 96, 64, 56, 36, 16 kDa (Fig. 3). A protein with a molecular weight of 36 kDa can presumably be a classic lectin of wheat plants - WGA, which can be located in the space between the cytosolic membrane and the cell wall. In plants exposed to stress by low positive temperatures, the spectrum of CWL slightly differed from the control plants: proteins with molecular weight of 96 and 36 kDa remained and proteins of 116 and 24 kDa appeared (Fig. 4), and the protein content in this variant increased (Table 1).

Table1. Changes in the content of cell wall proteins in the roots of spring wheat seeding under the action of hypothermia and stevioside µg mL^{-1}.

Variant	Proteins obtained by gel chromatography	Glucose eluted protein fractions
Control	30.6 ±1.53	31.7 ± 1.58
Hypothermia (3h)	36.7 ±1.84	38.3 ± 1.91
Hypothermia (3h)+ stevioside (24h)	17.8 ± 0.89	23.7 ± 1.19

Earlier, when studying the mechanisms of action of low-temperature stress, a protein with molecular weight of 24 kDa was detected in the protein spectrum of seedlings of Mironovskaya 808 winter wheat after 1 h and 3 h of hypothermia (Timofeeva *et al.* 2010). Prolonged exposure to temperature stress (low temperatures and cold hardening) is known to cause fluctuations in this indicator, which are accompanied by an increase in plant frost resistance (Garaeva *et al*. 2006). Despite the decrease in the amount of CWL fractions in the roots of spring wheat under stress, we observe an increase in the activity of this fraction of lectins. It is necessary to understand that the content of lectin proteins and their activity are not always directly related. This may be due to the presence of a pool of storage lectin mRNAs in the plant or conformational changes in the molecule. Stevioside, against the background of low positive temperatures, also changed the composition of the CWL in Omskaya 33 plants. Lectin activity was found in fractions containing proteins with molecular weights of 116, 96, 64, 44, 36, 16 kDa (Fig. 5). Against the background of pretreatment with stevioside, the protein content in the cell wall significantly decreased in comparison with control plants and plants subjected to hypothermia (Table 1). It is interesting that our earlier studies detected a 44 kDa protein in plants treated with stevioside (10^{-8}M), in Mironovskaya 808 winter wheat, which was accompanied by increased growth, increased frost resistance and plant resistance to heavy metals (Mikhailov *et al.* 2018).

SUMMARY

Thus, gel chromatography of cell wall proteins showed that short-term exposure to low positive temperatures led to qualitative and quantitative changes in cell wall proteins. At the same time, pretreatment with stevioside caused the appearance of a lectin protein with molecular weight of 44 kDa in the cell wall of wheat plants. We assume that the appearance of this lectin in a spring cultivar under the influence of stevioside may contribute to an increase in its frost resistance.

CONCLUSION

Of course, there are different opinions about the period of frost exposure that is most dangerous for wheat plants, however, all authors assert the very negative effect of this factor, even of a small force, which, among other things, affects the productivity of the plant. And the possibility of increasing the adaptive potential of a plant, including with the use of regulators of plant growth and development, is extremely significant because wheat is the most important food crop in the world. Its wide geographical distribution is due to its high general ontogenetic adaptability (Andreeva 2001).

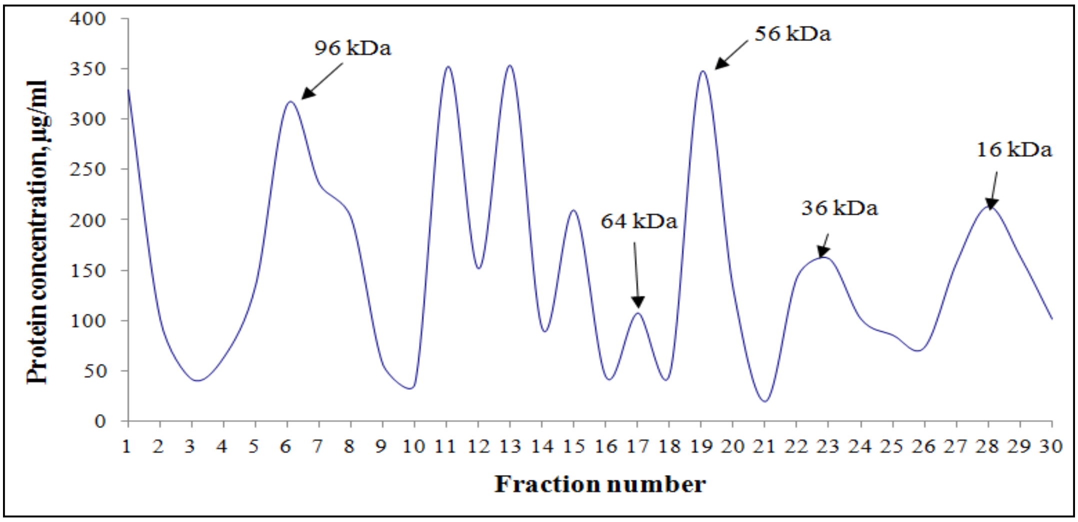

Fig. 3. Elution profile by gel-penetrating chromatography of the cell wall proteins of the spring wheat germs of control conditions (H_2O).

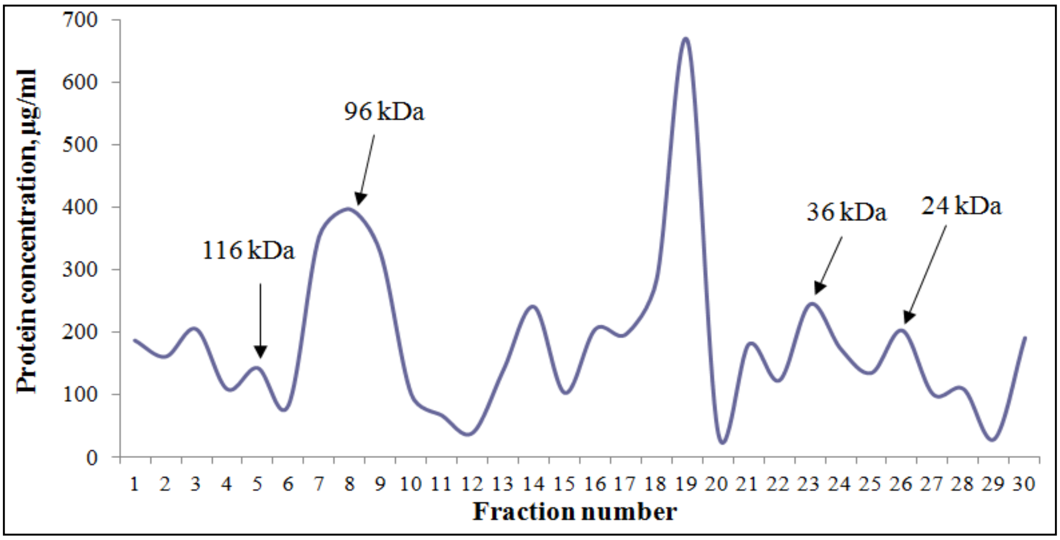

Fig. 4. Elution profile by gel-penetrating chromatography of the cell wall proteins of the spring wheat germs of control conditions (H_2O) at stress of low positive temperatures (3h).

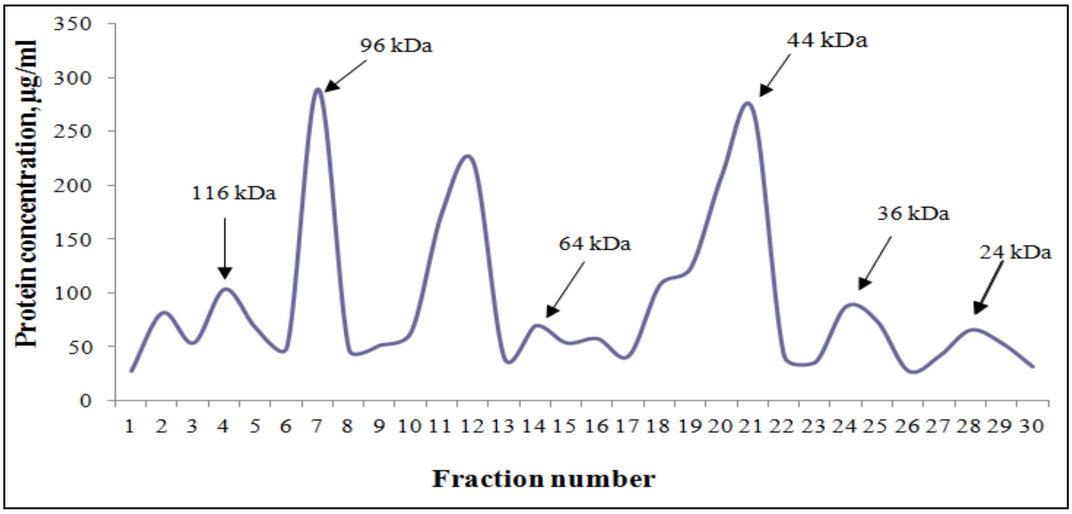

Fig. 5. Elution profile by gel-penetrating chromatography of the cell wall proteins of the spring wheat germs of control conditions (H_2O) at stress of low positive temperatures (3h).

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The results indicate that the stevioside-induced increase in CWL activity, changes in the qualitative and quantitative composition of lectin proteins in this compartment can serve as evidence of an increase in the adaptive potential of spring wheat plants in response to short-term stress by low positive temperatures. Due to the polyfunctionality of lectins and their involvement in various vital processes, it is possible for the plant to restore growth processes after the action of the stressor in a shorter period and reduce its negative effect on productivity.

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CONFLICTS OF INTERESTS

There is no conflicts of interests.

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ناهمگنی و عملکرد لکتین مولکولی ناشی از استیووزاید در دمای پایین مثبت

اولیانا الکساندرروونا گالیخانوا*، اولگا آرنولدوفنا تیموفیوا

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چکیده

این مقاله نتایج یک مطالعه در مورد اثر تیمار قبل از کاشت بذرهای Triticum aestivum L. با استویوزید (۱۰-۸ میلی متر) به مدت ۲۴ ساعت بر فعالیت و ناهمگنی مولکولی لکتین‌ها در نهال‌ها در دمای پایین مثبت را توصیف می‌کند. فیتولکتین‌ها در طیف گسترده‌ای از فرایندهای فیزیولوژیکی گیاهان شرکت می‌کنند ، نه تنها رشد ، بلکه مقاومت در برابر شوری ، فلزات سنگین و تنش دما را نیز تنظیم می‌کنند. در این راستا، بررسی تغییرات در فعالیت و ناهمگنی این مولکول‌ها در جستجوی تنظیم کننده‌های جدید رشد و نمو گیاه ضروری است. افزایش مشاهده شده ناشی از استویوزاید در فعالیت لکتین‌های متصل به دیواره سلول و تغییر در ترکیب کیفی و کمی آنها در برابر پس زمینه تنش دمایی ، ممکن است نشان دهنده افزایش پتانسیل سازش گیاهان گندم باشد.

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