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THE CHEMICAL STRUCTURE OF STEVIOL-GLYCOSIDES AS BASE OF BIOLOGICAL ACTIVITY

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Abstract

This article is dedicated to study of the peculiarities of influence of glycosides extracted from *Stevia Rebaudiana* plant, namely stevioside, steviolbioside, rebaudioside A and C as well as of commercial mixture of steviol-glycosides “Sweta” on growth as well as on physical and biochemical processes of wheat plants. Compounds with glycoside nature often demonstrate various types of biological activity. The chemical structure of stevia glycosides is similar to the well-known plant growth and development regulating chemicals, i.e. gibberellins and alongside with that these glycosides induce the same physiological reactions in plants as gibberellins do. This fact puts glycosides within the zone of intense interest from the point of view of phytophysiological investigations. Since the difference between the mentioned compounds lies in composition and structure of a carbohydrate branch of a glycoside molecule study of correlation between the structure and the biological influence of such molecules on plants is the question of major priority.

The article represents data indicative of direct relation between the chemical structure of a carbohydrate branch of a glycoside molecule and the impact on *Triticumaestivum* plants induced by it. There was demonstrated ambiguous nature of the studied compounds impact on the physiological and biochemical state of wheat plants.

Key words: steviol-glycosides, stevioside, rebaudioside, *Triticumaestivum*, plant growth and development regulating chemicals.

Introduction

Nowadays one can not imagine efficient functioning of the agroindustrial complex without innovative technologies which include among other plant growth and development regulating chemicals. Notably that it is natural compounds which are of the greatest interest for their cost-effectiveness, specific biological activity, influence on plants growth, development and productivity having human and environment-friendly nature.

In this regard diterpenoids is one of the groups of compounds which is of interest. It includes natural substances having different chemical structure and most commonly exhibiting a wide range of biological activity. In particular such compounds include ent-kaurene glycosides extracted from *Stevia Rebaudiana* plant. Stevia glycosides have sweet taste (approximately 300-fold sweeter as compared to sugar) [1],[2]. In regard of acute toxicity steviosides are graded as the IV class of hazard, i.e. as low-hazardous substances [3]. And since December 2008 according to FDA data this substance has been acknowledged as a non-toxic compound. Due to this stevioside became widely used both in the food and the pharmaceuticals industry as a natural sweetener. For example sweet stevia glycosides were demonstrated to have a therapeutic effect lying in antihyperglycemic action, hypotensive, anti-inflammatory, antitumoral, diuretic effects and immunomodulatory action [4]. According to the results of other experiments an extract of *S. rebaudiana* leaves has strong potential for use as a natural antioxidant agent [5].

It is well known that steviol is a common aglycone for all stevia glycosides [6]. It's also worth noting that up to the moment of an intermediate product generation (i.e. kaurene) synthesis of gibberellic acid and steviol in stevia plants proceeds in the similar way [7].

Therefore all steviol-glycosides are characterized by likeness of the chemical structure with the well-known phytohormones, namely gibberellins. Phytohormone gibberellin has a range of important functions in plants. Its most typical action consists in increase of dwarf plants footstalk growth or normal plants footstalk extension. It activates hydrolytic enzymes (for example, α -amylase) and their synthesis in graminaceous plants kernel which results in more intensive transformation of storage compounds and seed germination. Phytohormones also have other physiological effects of the same level of relevance.

Due to the above it is important to note that the literature contains data on steviol-induced promotion of growth of dwarf corn mutants which are sensitive only to gibberellins action [6]. Nevertheless isosteviol had absolutely no influence on the growth of this tested plant [6], since it differs from steviol and gibberellic acid by its specific ent-beyeran hydrocarbonic skeleton.

On the basis of the experiments which have been carried by our group earlier it could be concluded that stevioside apart of its growth-stimulating activity demonstrated protective action in relation to heavy metals (reduced their toxic action on lectins activity) [8] and enhanced cold resistance of winter wheat plants [9], [10], [11].

As was said earlier all steviol-glycosides have common aglycon similar to gibberellic acid. Differences in chemical structure of these molecules are conditioned by contained sugar residues which vary in nature and quantity. Until now

there have been no discussions in the literature as to which of structural peculiarities determines high physiological activity of these compounds in a plant.

Due to this we have taken comparative study of influence of different steviol derivatives varying in quantity and composition of carbohydrate residues (stevioside (has 3 glucose residues), rebaudioside A (4 glucose residues) and C (3 glucose residues and rhamnose residue), steviolbioside (2 glucose residues) and enzymic glycosylated commercial preparation “Sweta” used as a sweetener and constituting a mixture of stevia glycosides (rebaudioside A as a main component) by physiological and biochemical methods as a basis of our investigation. The studies showed that physiological effects of the tested compounds are determined to a large extent by their carbohydrate component, namely by nature and quantity of carbohydrate residues and the place of their adjoining.

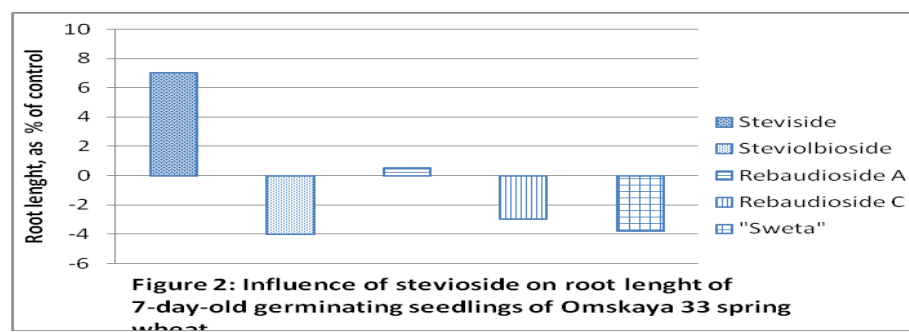
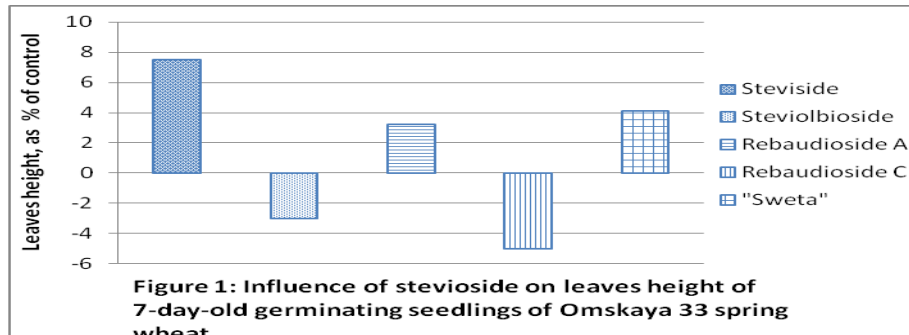
Methods

Omskaya 33 winter wheat germs served as a subject of study. The plants were grown under laboratory conditions in the cells with use of solutions containing steviol derivatives (stevioside, steviolbioside, rebaudioside A, rebaudioside C) and enzymic glycosylated preparation “Sweta” under the illumination intensity of 100 W/sq.m, the photoperiod of 12 hours and the temperature of 23°C. Seeds before planting were sterilized by 2% potassium permanganate (within 15 minutes), rinsed with distilled water and sown in the cells. Active concentrations of the tested compounds were determined by our science team earlier, namely stevioside – 10^{-8} M, rebaudioside A – 10^{-8} M, rebaudioside C – 10^{-8} M, steviolbioside – 10^{-8} M, mixture of stevia glycosides “Sweta” – 10^{-8} M. Controls were represented by the plants grown under the same conditions but with use of tap water. Leaves and roots length was measured by the 7th day of the plants growing. In order to determine activity of amylolytic enzymes in one-day-old seedlings grown with use of the tested glycosides mixtures and of tap water a test portion of the plant material was ground with 1% NaCl solution, incubated within 1.5 hour under the cold and periodical stirring conditions. Supernatant after sedimentation was used for study of amylases activity according to the method offered in [12]. Protein was determined according to the Bradford method. Soluble lectins were extracted with 0.05% HCl normal solution, cell wall lectins by 0.9% NaCl and 0.05% Triton X-100 solution [13]. Lectins activity was determined by the hemagglutination reaction with red blood cells (blood group 0) [14]. Experiments involved 3 biological replications.

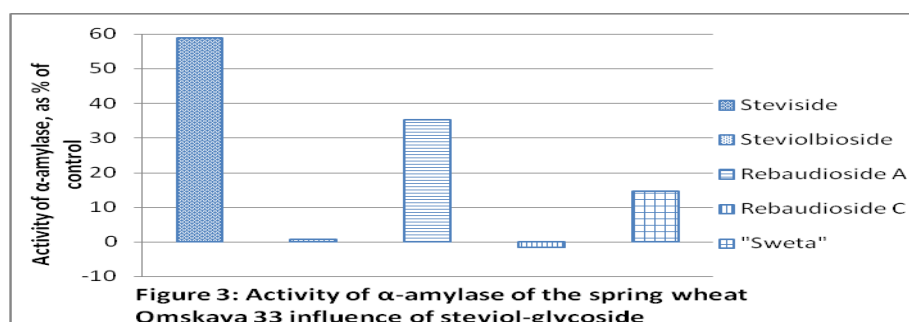
Results

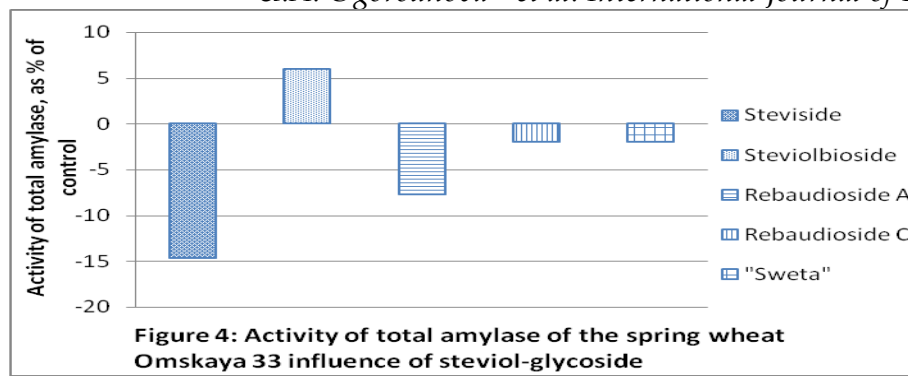
Morphometric studies showed that the influence of steviolbioside and rebaudioside C was expressed in general decrease of the height of elevated and the length of underground parts of the seedlings as compared to the controls

(plants grown with use of water). Preparation «Sweta» consisting of the mixture of stevia glycosides showed stimulating influence on the leaves but suppressed root system development as well as the two previous compounds. Only stevioside and rebaudioside A induced general increase of the seedlings height. Notably that the effect of stevioside was considerably more intensive (Figures 1, 2).

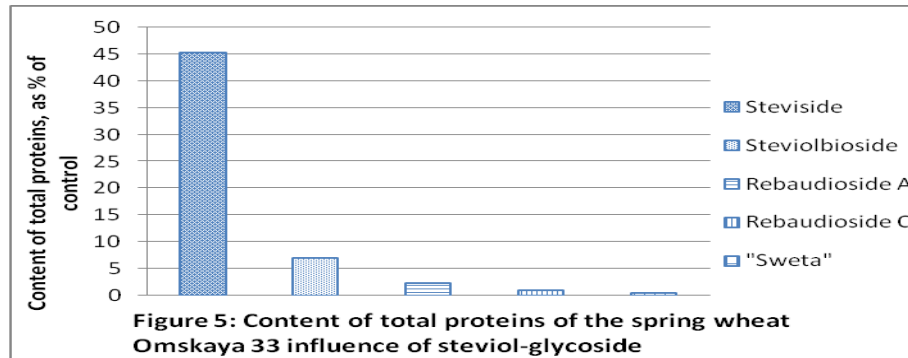


As was said earlier, glycosides of *Stevia rebaudiana* Bertoni plant are synthesized through ent-kaurene which is a precursor for gibberellic acid as well. Due to this steviol-glycosides have such similar chemical structure to gibberellins. These phytohormones are traditionally known for intensification of seeds germination (particularly through α -amylase activation); this property is also used for determination of a nature of influence of gibberellins-like substances. Change of amylases activity accordingly may serve one of the possible evidences of gibberellin-like action of the studied compounds. For example steviolbioside and rebaudioside C almost didn't change α -amylase activity while stevioside, rebaudioside A and «Sweta» showed significant increase of the mentioned factor as compared to the controls. Moreover stevioside increased the enzyme activity by several times more than two other compounds. At the same time all of the studied compounds except for steviolbioside inhibited activity of total amylase (Figures 3,4).

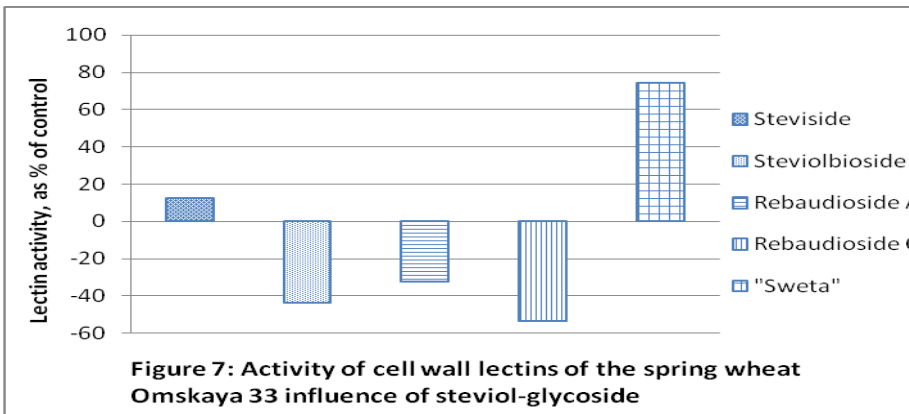
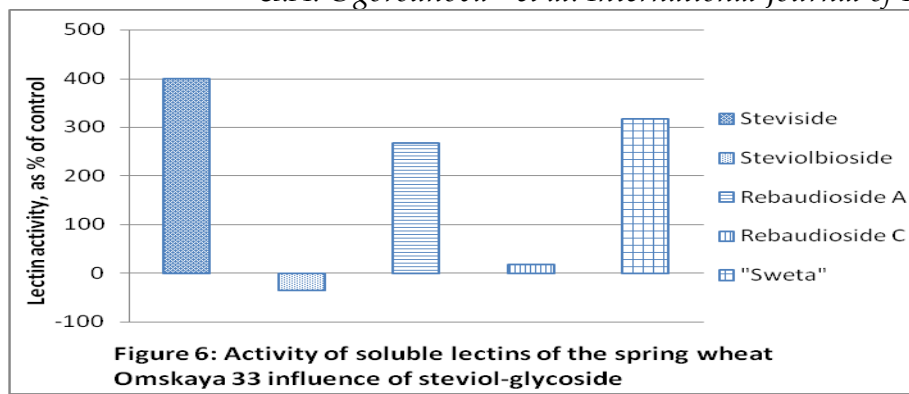




The following set of experiments was aimed at determination of change of total protein in wheat plants grown with use of solutions of the studied steviol-glycosides (Figure 5).



Protein content in a plant may serve as one of the factors of growth processes intensification. Therefore stevioside-induced almost two-fold increase of the studied factor as compared to the controls correlates with the morphometry data. The mixture of steviol-glycosides "Sweta" didn't have any effect on total protein content in plants. While steviolbioside, rebaudiosides A and C induced insignificant factor increase. Since plant lectins besides carbohydrate-fixing sites have centers of hydrophobia fixing with molecules having non-carbohydrate nature it is supposed that such lectins interact with phytohormones. And as a consequence these glycoproteins are involved in the system of hormonal regulation, therefore lectins probably are involved in wide range of physiological response of an organism (growth, development, differentiation etc.). [15],[16]. It is possible that the complex "lectins-phytohormones" takes part in accumulation of hormones and regulation of plant growth [17]. Due to this the problem of the influence of the studied compounds (having structural and physiological similarity to gibberellins) on such polyfunctional molecules as phytolectins is of the utmost interest. We've demonstrated that stevioside, rebaudioside A and the mixture of steviol-glycosides "Sweta" (with rebaudioside A as a basic component) induced severalfold increase of activity of soluble lectins in germinating seedlings of wheat but had ambiguous influence on activity of phytohemagglutinins fixed to a cell wall. Action of steviolbioside, rebaudiosides A and C was expressed in total activity decrease of lectins of this fraction. Stevioside and "Sweta" promoted activity of cell-wall lectins (Figures 6 and 7).



Findings

Analysis of physiological and biochemical parameters of Omskaya 33 winter wheat plants which underwent treatment by the tested diterpenoids showed ambiguous nature of influence of the named compounds. Stevioside and rebaudioside A containing 3 and 4 glucose residues correspondingly induced overall elongation of germinating seedlings while steviolbioside (containing 2 glucose residues) and rebaudioside C (containing 3 glucose residues and rhamnose) vice versa suppressed their growth processes.

The mixture of stevia glycosides "Sweta" promoted growth of leaves but inhibited development of the root system as compared to the controls. It is interesting to note that treatment of seeds with only stevioside, rebaudioside A and "Sweta" preparation resulted in considerable growth of α -amylase activity. Use of the rest of compounds resulted in reduction of the enzyme activity to a different degree.

All steviol-glycosides promoted growth of total protein content however stevioside (containing 3 glucose residues) induced the most significant changes of the studied factor (i.e. induced almost 2-fold protein content increase as compared to the controls). The investigated compounds had various effects on soluble lectins and those fixed to a cell wall. At that under the action of stevioside, rebaudioside A and stevia glycosides mixture "Sweta" like in the previous experiments (morphometry and amylase activity) activity of the soluble lectines increased in a greater degree versus both the controls and the rest of the experimental variants.

Conclusion

Hence the comparative analysis of influence of steviol-glycosides showed that steviolglycosides with 3 and 4 glucose residues (stevioside and rebaudioside A correspondingly) induced positive dynamics of changes in the main physiological factors and lectins activity in the wheat plants. Nevertheless stevioside containing 3 glucose residues (in the established active concentration of 10^{-8} M) demonstrated the most intensive influence among two these compounds, namely it increased growth both of an overground part and an underground part of germinating seedlings, induced considerable increase of total protein content and α -amylase activity as well as gave rise to intensification of activity of soluble lectins at time when activity of agglutinins fixed to a cell wall remained almost unaffected.

Therefore we can certainly state that the chemical structure of molecules of glycosides, i.e. structure and composition of carbohydrate part of steviol-glycosides plays a key role in the changes of physiological and biochemical status of germinating seedlings of wheat as detected by us.

Acknowledgements

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