Effects of Chemical Treatments (Iron, Zinc and Salicylic Acid) and Soil Water Potential on Steviol Glycosides of Stevia (*Stevia rebaudiana* Bertoni)

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ABSTRACT: *The most important characteristic of stevia is its high sweetness with zero calories which is due to the presence of Steviol glycosides (SVglys). This research aims to address the effect of Salicylic Acid (SA) and microelements viz. iron (Fe) and zinc (Zn) under different soil water potentials (-0.5, -3.5, -6.5 and -10 atm) on the production of SVglys and total sugar content in the leaves of stevia. The obtained results indicated that the soil water content and the exogenous application of SA and microelements significantly changed the accumulation of these sweet chemicals in the stevia leaves. The highest values of Stevioside (Stev), Rebaudioside C (Reb C), total SVglys and SVglys yield were obtained in SA + Fe + Zn treatment under the potential of -3.5 atm (76. 82, 2.82, 116.71 mg/g DW and 0.836 mg/g plant, respectively). Also, the HPLC results indicated that the highest rates of Rebaudioside A (Reb A) and the Reb A/Stev ratio (sweetness quality) belonged to SA + Zn treatment under the potential of -3.5 atm (28.63mg g^{-1} DW and 0.433). The application of SA + Fe + Zn was the most effective in terms of Rebaudioside B (Reb B), Dulcoside A (Dulc A), and total sugar (2.31, 5.73 and 335.8 mg/g DW, respectively). In general, our results suggest that it can be possible to improve the rate of secondary metabolites (SVglys) and hence the sweetness property in stevia leaves by applying SA, Fe, and Zn and particularly by the integrated application of these three agents.*

KEYWORDS: *Chemical treatments; Microelements; Soil water potential; Total sugar; Stevioside; Rebaudioside.*

INTRODUCTION

Stevia (*Stevia rebaudiana* Bert.), an anti-diabetic medicinal plant belonging to the Compositae (Asteraceae) family, is a multi-purpose plant and native to the northeast of Paraguay (South America) [1, 2]. Stevia is well-known for its sweet-tasting leaves and currently, the production of this herb has widely spread in many

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countries throughout the world such as Canada, Australia, China, Japan, Korea, Malaysia, and etc. [3]. The sweetness of stevia is related to diterpenoid glycosides of ent-kaurene type, which mainly present in the leaves of this plant [4]. Over 60 types of steviol glycosides (SVglys) have been identified in *S. rebaudiana* [5]. The SVglys mainly include Stev, Reb A, B, C, and Dul A [3]. SVglys found in stevia are a form of secondary metabolites [6] and all of them contain a similar chemical structure (steviol backbone), but differ by the number and type of carbohydrate residues at the C13 and C19 positions [7, 8]. Stev and Reb A are the most abundant SVglys in the leaves of stevia (5-10 % and 2-4 % of leaf dry weight, respectively) [9]. The concentration of these metabolites in stevia depends on the genotype and the environmental and growth conditions of the plant [10]. Stev and Reb A are differed only by the presence of one glucose moiety. Stev is formed by three molecules of glucose attached to an aglycone steviol ring, while Reb A has one additional glucose molecule [11]. These water-soluble glycosides are 200-300 times sweeter than sucrose [10] and despite strong sweet flavor, are not metabolized by the body (calorie-free) [12]. Hence, stevia is a suitable alternative to artificial sweeteners like saccharin, neotame, aspartame, and *acesulfame K* which is used as the food additive, a flavor enhancer, and a sweetener in beverages and food products in many countries [13, 14]. According to a report of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA), SVglys are safe for human consumption and can be used as a proper sweetener for diabetics and weight watchers [15]. Besides SVglys, many other compounds, such as ascorbic acid, beta-carotene, riboflavin, labdanes, triterpenes, coumarins, flavonoids, sterols, indole-3-acetonitrile, organic acids, cinnamic acids, inorganic salts and some essentials oils have been also found in the leaves of stevia [16, 17].

The leaves are the economic part and a major source of high-potency sweetener in the stevia plant, thus the increase of leaf biomass along with the higher SVglys content is a critical issue for its production [14]. In addition to genetic factors, the growth and the accumulation patterns of secondary metabolites (SVglys) in stevia plants are considerably affected by the nutrient availability [10, 18]. Mineral nutrients based on the relative quantity needed for plants could be divided into two groups, macroelements and microelements [19]. Although microelements are used by plants in small quantities, these elements are needed to improve the physiological and biochemical activities and to ensure the optimum quality. Microelements, especially the elements like manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and molybdenum (Mo) are essential for the normal performance of all higher plants. Also, microelements are important components for enzymatic systems and catalyze the biological reactions [15, 20]. Moreover, it has been reported that the formation of secondary metabolites might be induced by applying the elicitors such as SA in medicinal plant species as well as in the species categorized as functional foods [21]. SA (or 2-hydroxybenzoic acid), is known as an endogenous phytohormone with phenolic nature that has a remarkable role in the growth and development of plants [22, 23]. It has been proven that SA is involved in the regulation of a wide range of diverse physiological and biochemical processes in plant cells like the uptake and transmission of ions, photosynthesis, nitrogen metabolism and proline metabolism [24].

The biosynthesis of SVglys in stevia plants is also controlled by the environmental factors and agronomic management [18]. The reduction of soil moisture content is considered as one of the most major environmental factors, affecting many aspects of morphology, physiology, and biochemistry in plants [25-27]. *S. rebaudiana* originated from a semi-humid and subtropical region with an annual rainfall from 1500 to 1800 mm [28], so soil moisture is an important factor for its growth [26, 29]. The effect of drought stress on the accumulation of secondary metabolites has been frequently studied for many plants [30, 31], while this issue is not well-documented in stevia plant.

The growing demand for stevia sweeteners to use in food and pharmaceutical industries has led to its commercial production across the world. This plant has been recently introduced as a new commercial crop in Iran which is being cultivated in some regions of this country. The cultivation of stevia in Iran for the commercialization of its extract as a natural sweetener requires the comprehensive study of the effects of environmental and nutritional factors on the content of SVglys. According to our knowledge, although the effect