CORRELATION BETWEEN GLYCOSIDES IN LEAVES AND AGRONOMIC TRAITS OF *STEVIA REBAUDINA* BERTONI

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Keywords: Agronomic traits, Correlation, Glycosides

Abstract

According to results of SDS-PAGE of *Stevia rebaudina* Bertoni germplasm materials from Canada, Israel, Japan and China, 36 germplasm materials were selected from 78 to study the correlation analysis among the mainly agronomic traits, the leaf yield and the glycosides in the leaves. The results indicated that, the longer vegetative growth time was the plant height and the yield would be higher. The greater the leaves aspect ratio was, the leaves would be thicker, the dry matter content and the total glycosides content in leaves be higher, the Rebaudioside A (RA), Rebaudioside C(RC) content would be higher, but the Stevioside (STV) content be lower. The RA and RC content increased with the increase of total glycoside, and the STV content significantly decreased with the increase of the RA and RC content. The results of this study defined the correlations among the agronomic traits, yield of leaves and glycoside quality, and provided theoretical support for the high-quality *Stevia* variety breeding.

Stevia rebaudina Bertoni (Compositae), the sweetness of the glycosides in the leaves is 200 to 400 times to sucrose, but the caloric value is just one three-hundredth to the sucrose, so the glycosides is called natural saccharin. The glycosides could prevent the hypertension, diabetes, obesity, cardiopathy, caries etc. (Aleksandar *et al*. 2008, Velbor *et al*. 2006), which is widely used in food, beverage, pharmacy, cosmetics etc. The demand of the glycosides is increasing in the world, and the quantity is about 1.0×10^6 kg, but the production could not meet the market in a large extent. The first mainly reason is the lower yield, and the other is the lower content of the glycosides in the leaves. So variety breeding and expanding the cultivated area are the mainly ways to increase the content of glycosides and the production of the leaves. There have been some researches on the *Stevia* cultivation (Lv *et al.* 2009, Ma *et al.* 2010), the glycoside extraction and the glycoside's application in agricultural (Liu *et al.* 2010) on the nutrition and health. In this study, the correlation of the glycosides in the leaves and the mainly agronomic traits of the *Stevia* germplasm had been analysed to find the correlativity between them and provided theoretical support for the high-quality *Stevia* breeding.

According to results of SDS-PAGE of *S. rebaudina* Bertoni germplasm materials from Canada, Israel, Japan and China, 36 germplasm materials (Table 1) were selected from 78 to carry out this study. The materials from Israel were showed with IS, from Canada were showed with CA, from Dongtai City, Jiangsu Province, China were showed with JD, from Mingguang City, Anhui Province, China were showed with AM, from Linquan City, Anhui Province, China were showed with AL, from Qingdao Agricultural University, Qingdao, China were showed with QT in this study.

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The field study was conducted in the Qingdao Agricultural University (north latitude 36°.11', east longitude 120°17') experimental plot in 2009. The seeds of the materials were seeded in the greenhouse on April 13, 2009, and the seedlings with the same growth were transplanted in the field on May 15, 2009. The plot was 1.00 m². The contents of the organic matter, hydrolysable nitrogen, available phosphorus and available potassium were 1.35%, 86.53, 24.34 and 87.53 mg/kg, respectively. As the base fertilizer for every plot 30.00 g urea, 52.00 g KH_2PO_4, 65.00 g KCl were used. There were 15 plants in every plots, and the plants space was 0.25×0.25 m². There was no topping in *Stevia* growing season and other managements were as that in the field.

The vegetative growth (d), the plant height (cm) and the SPAD value (the relative chlorophyll content) of the *S. rebaudina* Bertoni were measured in the field when the blossom rate was about 10%, and then the plants were harvested. The other agronomic traits determined in the Key Laboratory of Shandong Province after harvest, included stem diameter (cm), branches, the leaf length (cm), the leaf width (cm), the leaf aspect ratio, the leaf area (cm²), the leaf thickness (μm), the fresh leaves weight/plant (g), the dry leaves weight/plant(g).

The contents of glycosides, rebaudioside A (RA), stevioside (STV) and rebaudioside C (RC) were determined in the Central Laboratory of Qingdao Agricultural University. The glycoside was measured with the macroporous resin method of Liu Zonglin (Liu *et al.* 2002), the RA, RC and STV were measured with HPLC (High Performance Liquid Chromatography) method (Yu and Shi 2009). The data processing and statistical analysis were done with the SPSS 18.0.

Table 2 shows that the fresh leaves yield of per plant and dry leaves yield had the positive correlation with the vegetative growth phase, the fresh leaves yield had notable negative correlation with the leaves aspect radio, the dry-fresh leaf yield radio per plant had positive correlation with the leaves aspect radio and had significantly positive correlation with the leaf thickness. There was no remarkable correlation among the other agronomic traits and yield traits, respectively.

The correlation between agronomic and glycosides of *Stevia rebaudina* Bertoni is presented in Table 3. Clearly, the glycosides content and RA content had dramatically positive correlation with the leaf SPAD value, the leaf thickness and the leaf aspect radio, respectively. The STV content had significantly positive correlation with the vegetative growth time, had significantly negative correlation with the leaf SPAD value and the leaf thickness, and had remarkable negative correlation with the leaf aspect radio. The RC content had notable positive correlation with the leaf SPAD value, leaf thickness and the leaf aspect radio, respectively.

The correlations among agronomic traits of *S. rebaudina* Bertoni are presented in Table 4. The plant height had significantly positive correlation with the vegetative growth time. The SPAD value of the leaf had negative correlation with vegetative growth time and plant height, respectively but they were not remarkable. The leaf aspect radio had notably negative correlation with the plant height, had dramatically positive correlation with the SPAD value, and the significantly negative correlation with the leaf width. The leaf thickness had significantly positive correlation with the leaf SPAD value, and the outstanding positive correlation with the leaf aspect radio. The leaf area had the significantly positive correlation with the vegetative growth time, the plant height, leaf length, leaf width, dramatically negative correlation with the branches number, and negative correlation with leaf thickness, respectively.

The correlation between yield traits and quality of *S. rebaudina* Bertoni leaf presented in Table 5 indicated that, the leaf glycosides content had notable negative correlation with the leaf fresh yield and dry yield per plant, but had not remarkable positive correlation with the leaf dry-fresh radio. The leaf RA content had notably negative correlation with the leaf fresh yield and dry

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Table 1. Materials used in agronomic characters analysis.

No.	Sample name	Provenance	Order	Name	Provenance	Order	Name	Provenance
1	IS-1	Israel	13	QT-7	Qingdao Agricultural University	25	JD-6	Dongtai City, Jiangsu Province
2	IS-2	Israel	14	QT-8	Qingdao Agricultural University	26	JD-7	Dongtai City, Jiangsu Province
3	IS-3	Israel	15	QT-9	Qingdao Agricultural University	27	AM-1	Mingguang City, Anhui Province
4	CA-1	Canada	16	QT-11	Qingdao Agricultural University	28	AM-2	Mingguang City, Anhui Province
5	CA-2	Canada	17	QT-12	Qingdao Agricultural University	29	AM-3	Mingguang City, Anhui Province
6	QT-1	Canada	18	QT-13	Qingdao Agricultural University	30	AM-4	Mingguang City, Anhui Province
7	QT-2	Qingdao Agricultural University	19	QT-14	Qingdao Agricultural University	31	AL-1	Linquan City, Anhui Province
8	QT-3	Qingdao Agricultural University	20	JD-1	Dongtai City, Jiangsu Province	32	AL-2	Linquan City, Anhui Province
9	QT-4	Qingdao Agricultural University	21	JD-2	Dongtai City, Jiangsu Province	33	AL-3	Linquan City, Anhui Province
10	QT-5	Qingdao Agricultural University	22	JD-3	Dongtai City, Jiangsu Province	34	AL-4	Linquan City, Anhui Province
11	QT-6	Qingdao Agricultural University	23	JD-4	Dongtai City, Jiangsu Province	35	JP-1	Japan
12	QT-7	Qingdao Agricultural University	24	JD-5	Dongtai City, Jiangsu Province	36	JP-2	Japan

Table 2. The correlation coefficients between agronomic and yield traits of *S. rebaudina*.

	Squaring stage (d)	Plant height (cm)	SPAD value	Stem diameter (cm)	Branches	Length (cm)	Width (cm)	Aspect ratio	Area (cm²)	Thickness (μm)
FY/P (g)	0.22*	0.12	−0.14	0.15	−0.08	−0.11	0.12	−0.39**	0.21	−0.15
DY/P (g)	0.21*	0.15	−0.11	0.18	−0.05	0.18	−0.07	−0.12	−0.08	0.07
D/F	0.04	0.07	0.12	0.14	−0.02	0.13	0.03	0.24*	−0.04	0.31**

*$p < 0.05$ represents significant, **$p < 0.01$ represents highly significant.

Table 3. The correlation coefficients between agronomic traits and quality of *S. rebaudina*.

	Squaring stage (d)	Plant height (cm)	SPAD value	Stem diameter (cm)	Branches	Length (cm)	Width (cm)	Aspect ratio	Area (cm²)	Thickness (μm)
Steviaside content	-0.05	-0.09	0.41**	-0.17	0.10	0.14	-0.12	0.31**	-0.07	0.33**
RA "	-0.02	-0.06	0.39**	-0.11	0.15	0.17	-0.18	0.36**	-0.14	0.37**
STV "	0.21*	0.14	0.33**	0.14	-0.09	-0.19	0.11	-0.28*	0.19	-0.32**
RC "	-0.03	-0.01	0.21*	-0.18	0.11	0.08	-0.27	0.19*	-0.15	0.24*

*$p < 0.05$ represents significant , **$p < 0.01$ represents highly significant.

Table 4. The correlation coefficients among Agronomics of *S. rebaudina*.

	Squaring stage (d)	Plant height (cm)	SPAD value	Branches	Length (cm)	Width (cm)	Aspect ratio	Thickness (μm)	Area (cm²)
Squaring stage (d)	1.00								
Plant height (cm)	0.47**	1.00							
SPAD value	-0.04	-0.11	1.00						
Branchs	0.17	-0.03	0.14	1.00					
Length (cm)	-0.05	-0.06	0.02	0.08	1.00				
Width (cm)	0.07	0.11	-0.04	-0.08	-0.01	1.00			
Aspect ratio	-0.19	-0.21*	0.34**	0.14	0.12	-0.38**	1.00		
Thickness (μm)	-0.06	-0.14	0.42**	0.17	0.06	-0.14	0.29*	1.00	
Area (cm²)	0.31**	0.47**	-0.07	-0.44*	0.63**	0.49**	-0.13	-0.21*	1.00

*$p < 0.05$ represents significant, **$p < 0.01$ represents highly significant.

yield of per plant, but significantly positive correlation with the leaf dry-fresh radio. There was notable positive correlation between the leaf STV content and the leaf fresh weight of per plant, and the correlation was marked negative between leaf RC content and leaf dry-fresh radio. The leaf RC content had negative correlation with the leaf fresh weight and dry fresh weight of per plant, and had notable positive correlation with the leaf dry-fresh radio.

Table 5. The correlation coefficients between yield traits and quality of *S. rebaudina*.

	FY/P (g)	DY/P (g)	D/F
Steviaside content	–0.26*	–0.34*	0.19
RA content	–0.25*	–0.21*	0.36**
STV content	0.21*	0.18	–0.25*
RC content	–0.19	–0.15	0.21*

*p < 0.05 represents significant, **p < 0.01 represents highly significant

The leaf RA content had notable positive correlation with the leaf glycosides content, and the leaf RC content had positive correlation with leaf glycosides content and leaf RA content respectively, but they were not notable. The positive correlation between leaf STV content and leaf glycosides content was not outstanding, and the leaf STV content had the significantly negative correlation with the leaf RA content and leaf RC content respectively(Table 6).

Table 6. The correlation coefficients among qualities of *S. rebaudina*.

	Stevia side content	RA content	STV content	RC content
Stevia side content	1.00			
RA content	0.21*	1.00		
STV content	0.07	–0.71**	1.00	
RC content	0.15	0.18	–0.39**	1.00

*p < 0.05 represents significant, **p < 0.01 represents highly significant.

The results obtained in the correlation analysis indicated that, the longer the vegetative growth was, the smaller the leaf aspect radio was, the fresh leaves yield of the *S. rebaudina* be was higher. The bigger the leaf aspect radio was, the thicker the leaf, the dry matter content of the leaves be higher. The bigger the leaf SPAD value was, the bigger the aspect radio was, the thickness the leaf was, the glycoside content, the RA content and RC content in the leaf be higher, and the STV content be lower. The longer the plant vegetative time was, the plant height be higher, the branch number be fewer, the leaf thickness be smaller, the leaf SPAD value be smaller, the leaf be wilder, the leaf aspect radio be smaller, the leaf area be bigger. The leaf yield of per plant was bigger, the glycoside content, the RA content and the RC content would be lower, STV content would was higher in the appropriate range. And the higher the leaf dry-fresh radio was, the glycoside content, the RA content and the RC content qouls were higher, and the STV content was lower. The leaf RA content would be higher when the leaf glycosides content was higher, and the leaf STV content would be lower when the leaf RA content and leaf RC content were higher.

The production of *S. rebaudina* Bertoni leaf is about 20,000,000 kg at present, and the glycoside is about 2,000,000 kg every year, but the demand is about 10,000,000 kg. Leaf production and glycoside production are the major indicators for the variety and quality of *S. rebaudina*, and different consumer group has different demand for the radio of RA and RC.

In this study, the correlations among agronomic traits, yield traits and glycoside quality index had been analyzed, and it was defined clearly. Thus this study had provided a theoretical guidance for the high quality variety breeding for different markets in the world.

Acknowledgements

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(Manuscript received on 1 May, 2017; revised on 23 June, 2017)