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Interpopulation variations in morphochemical characteristics of *Stevia rebaudiana* Bertoni

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Abstract: The present study was carried out with an aim to evaluate *Stevia rebaudiana* Bertoni segregants and to characterize them morphochemically to facilitate selection of superior genotypes for hybridization in breeding programs. Two identified strains named A and B and their segregant populations (F_1 and F_2) were evaluated with their morphological and chemical characteristics. Various qualitative and quantitative morphological characters including leaf shape, plant type (loose or compact), plant habit, plant height, and shoot collar diameter were considered. Simultaneously stevioside and rebaudioside-A content was evaluated. A few individuals were identified with rebaudioside-A content ranging from 5.11% to 8.55% and the ratio between stevioside and rebaudioside-A was found to be very low. One plant with stevioside of 16.18% was obtained.

Key words: Extraction, morphology, populations, rebaudioside-A, *Stevia*, stevioside

1. Introduction

Low-calorie sweeteners have gained importance due to an increase in the number of diabetic people in the world. As per a World Health Organization report, the number of diabetic people will be 80 million by 2025. Synthetic low-calorie sweeteners have been in use for a long time but are associated with some health risks like carcinogenicity, weight gain, headache, and depression (Tandel, 2011). Consequently, people prefer natural sweeteners rather than synthetic ones. *Stevia rebaudiana* Bertoni is an important natural sweetener-containing perennial plant of the family Asteraceae (Reis et al., 2017). It is a native of Brazil and Paraguay in South America. In these regions, several other *Stevia* species such as *S. salicifolia* Cav., *S. lucida* Lag., *S. rhombifolia* Kunth, *S. eupatoria* Spreng., and *S. balansae* Hieron. are well known for their ethnopharmacological properties. *S. salicifolia* Cav. and *S. lucida* Lag. possess antirheumatic, antihelminthic, and antiinflammatory properties while *S. rhombifolia*, *S. eupatoria*, and *S. balansae* have emetic, antidiarrheal, and diuretic properties, respectively (Ferrazzano et al., 2016).

Stevioside and rebaudioside-A are two main sweetener compounds present in the leaves of the plant (Ramesh et al., 2006) and are widely used as zero-calorie sweeteners in many food and beverage industries (Midmore and Rank, 2006; Herranz et al., 2010). These are utilized to sweeten various products like tea (Vanek et al., 2001), seafood, candy, and ice cream (Ozdemir et al., 2015). In addition, the leaves have antidiabetic, antiviral, antiobesity, anticancerous, diuretic, and antiinflammatory properties (Yadav et al., 2011).

Stevia rebaudiana grows well under semitropical, subhumid climatic conditions with average annual temperature and rainfall of 25 °C and 1375 mm, respectively (Gupta et al., 2013). The species has been introduced as a commercial crop in several countries, such as the United States, Korea, Tanzania, Brazil, and India (Brandle and Rosa, 1992). According to Ramesh et al. (2006), *Stevia rebaudiana* performs well at a temperature range from 28 to 40 °C under subtropical Indian conditions. Dwivedi (1999) also reported the cultivation of the crop in humid hilly regions of Assam. The crop is found to grow well in regions like Maharashtra, Rajasthan, Kerala, Orissa, and Punjab in India (Singh et al., 2014).

Stevia rebaudiana populations grown from seeds show variations in morphology as well as chemical contents (Tamura et al., 1984; Jadeja et al., 2005). Accordingly, morphochemical characterization needs to be applied to natural sweetener plants like *S. rebaudiana*. Although earlier studies reported the selection of elite genotypes in natural populations and segregant generations of *S. rebaudiana* (Abdelsalam et al., 2016; Singh et al., 2017), only limited information is available with regard to the identification of superior plant types in segregant populations. Though the segregating individuals are not needed for crop cultivation as associated with

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heterogeneity, the segregating generations may signify a source of genetic diversity for stevia improvement programs. Thus, the evaluation of segregating generations with the objective of selecting elite genotypes as parents for the development of valuable cultivars is interesting. Therefore, this work was undertaken to identify such elite genotypes from the segregant populations (F_1 and F_2) of the already identified A and B strains.

2. Materials and methods

2.1. Study site

The study was conducted at the experimental farm and laboratory of the Department of Forest Products, College of Forestry, Dr YS Parmar University of Horticulture and Forestry, Nauni (Solan), Himachal Pradesh (India). The experimental farm is located at 30°50′30″N to 30°52′0″N and 77°8′30″0°to 77°11′30″E at 1300 m above mean sea level.

2.2. Plant material

Plant material for the present study was obtained from two already existing strains of *Stevia rebaudiana* (A and B) identified earlier by Raina et al. (2013) and their F_1 and F_2 populations. Strains A and B were allowed to grow in the proximity of the farm. These strains were involved in fertilizing each other by open as well as controlled pollination. Fertile seed sets of these strains were used for raising the F_1 population. For this, seeds of A × B and their reciprocal crosses were sown in nursery beds and seedlings were transplanted to the experimental field. These F_1 plants were also allowed to freely cross among each other to set fertile seeds. Seeds harvested from these F_1 plants were used for growing the F_2 population. Some interesting variants differing in lamina shape, disease resistance, shoot collar diameter, and biomass were selected from the F_1 and F_2 segregating populations. Subsequently, these 12 F_1 plants (P-1 to P-12) and 23 F_2 plants (S-1 to S-23) along with the A and B strains were evaluated for variation in morphological and chemical characters.

2.3. Chemicals and solvents used

For Soxhlet extraction of stevioside and rebaudioside-A, dichloromethane and methanol of CDH brand (Central Drug House) were used. A Waters HPLC (high-performance liquid chromatography) unit with 515 pumps, Merck Purospher Star NH_2 column (4.6 × 250 mm, 5 µm), Rheodyne injector, and dual λ absorbance detector 2487 were used for the analysis of leaf samples (Kumari et al., 2017). HPLC analysis was performed by using HPLC grade solvents, i.e. acetonitrile and water of Merck brand and 0.22-µm filter paper (catalog no. GVWPO4700, Millipore). Reference compounds (stevioside and rebaudioside-A) used for HPLC analysis were of Chromadex brand, procured from Life Technologies Pvt. Ltd. (India).

2.4. Evaluation of morphological characteristics

The plant height, plant type, and other leaf characteristics were measured as per standard methodology (Weberling, 1981; Kaufman et al., 1989). The plant height (cm) was measured from the base to the tip of the main shoot. Shoot collar diameter was measured with a vernier caliper (2 cm above ground level) by taking two observations at a right angle. Gross appearance of the plants was noted and classified as loose or compact type. Five mature leaves per plant were taken for morphological studies. The lamina shape, tip, base, and margin were observed as per the standard plant taxonomic literature. The leaf length was measured as the length of the lamina from its base to tip and width was recorded at its broadest point.

2.5. Extraction of stevioside and rebaudioside-A

Mature leaves were harvested from the plants prior to the flowering stage (August). The harvested leaves were first dried in shade and then in an oven at 60 °C until a constant weight was obtained (Kumari et al., 2017). The dried leaves were then powdered and 300 mg of powdered material was used for estimation of stevioside and rebaudioside-A. Powdered samples packed in thimbles were extracted with dichloromethane on a water bath using a Soxhlet apparatus for extracting the dichloromethane soluble fraction (Sasidharan et al., 2011). The residual samples in the thimbles (preextracted with dichloromethane) were then extracted with methanol to obtain the sweet compounds of stevioside and rebaudioside-A (Pol et al., 2007; Kumari et al., 2016). Methanol from the methanolic extract was removed by distillation and the sample was dried under vacuum.

2.6. Stevioside and rebaudioside-A analysis through HPLC

The concentrated vacuum-dried extract was completely dissolved in HPLC grade acetonitrile and water mixture (78:22) through ultrasonication and the final volume was made to 15 mL in a volumetric flask. The flow rate of the solvent was 1 mL/min (Woelwer et al., 2010). Detection of the compounds was carried out at 210 nm wavelength (Bovanova et al., 1998; Woelwer et al., 2010). The samples were filtered (0.22-µm filter, catalog no. GVWPO4700, Millipore) and injected (20 µL) for analysis of stevioside and rebaudioside-A content. Standard curves of stevioside and rebaudioside-A were prepared separately.

2.7. Statistical analysis

To quantify the amount of morphochemical variation among the populations, one-way ANOVA was performed in a completely randomized design using IBM SPSS statistics software (Version 20.0, IBM Corp., Armonk, NY, USA). The least significant difference test was used for comparison of data at the $P \leq 0.05$ level. The variable values were expressed as the mean ± standard error of the

mean (SE). Pearson's correlation coefficient was calculated for all the quantitative morphochemical parameters of *Stevia rebaudiana*.

3. Results

3.1. Morphological and chemical studies of parental A and B strains

Morphological characters and chemical contents (stevioside and rebaudioside-A) of the A and B strains are presented in Table 1. Strain A was characterized by compact growth with obtuse leaf tip, whereas B was characterized by acute leaf tip. However, in both strains, obovate leaf shape and attenuate base were found. Leaf length and width and petiole length were also found similar in the two strains; however, shoot collar diameter in strain B was longer (9.87 mm) as compared to A (5.95 mm). Stevioside content was higher in strain B (7.81%) whereas rebaudioside-A content was higher in A (3.05%).

3.2. Interpopulation variations in morphological characters

All the 12 F_1 individuals (P-1 to P-12) studied were compact as far as plant type was concerned. However, the leaf shape varied in the ratio of 33.33:66.67 (obovate:lanceolate), leaf tip varied in the ratio of 16.67:83.33 (acute:obtuse), leaf base varied in the ratio of 33.33:66.67 (cuneate:attenuate), and leaf margin varied in the ratio of 8.33:16.67:75.00 (crenate:lobate crenate:serrated) (Table 2; Figure 1). All the plants of F_2 progeny (S-1 to S-23) exhibited the compact plant type. However, the leaf shape ranged between obovate, lanceolate, and orbicular, which segregated in the ratio of 47.83:47.83:4.35, respectively. As far as leaf tip was concerned, plants with obtuse, acute, and retuse leaf tips segregated in the ratio of 91.30:4.35:4.35, respectively. Plants with attenuate, cuneate, and rounded leaf bases segregated in the ratio of 30.43:56.52:13.04, respectively. These F_2 plants exhibited great variations with regard to leaf margins with crenate, lobate crenate, dentate, incised, and serrated margins segregating in the ratio of 34.78:8.70:8.70:4.35:43.48, respectively.

In F_1 plants (P-1 to P-12), the plant height ranged between 50 and 85 cm, collar diameter between 0.42 and 0.72 cm, leaf length between 5.85 and 8.49 cm, leaf width between 1.93 and 4.02 cm, and petiole length between 0.31 and 0.89 cm (Figure 2). Among F_2 plants, plant height ranged between 45 and 97 cm, collar diameter between 0.40 and 0.89 cm, leaf length between 4.59 and 8.08 cm, leaf width between 1.81 and 5.04 cm, and petiole length between 0.20 and 0.77 cm (Figure 3).

3.3. Interpopulation variations in chemical content

Stevioside content varied from a minimum of 3.18% in P-10 to a maximum of 16.18% in P-11 (Figure 4). Rebaudioside-A content significantly varied from 0.00% (P-1 plant) to 5.12% (P-9 plant). First generation plants showed a minimum stevioside:rebaudioside-A ratio (S:R ratio) of 1.37 in P-9 followed by 1.47 in P-10 plants. However, in F_2 populations, stevioside content ranged from 0.64% in S-7 to 9.71% in S-15 plants. All the results obtained were statistically significant. S-2 and S-1 plants had stevioside contents of 7.41% and 6.67%, respectively (Figure 5). Rebaudioside-A was totally absent in S-2, while maximum rebaudioside-A content of 8.55% was recorded in S-21 (Figure 6). Rebaudioside-A content in S-23, S-20, and S-19 plants was 8.26%, 6.24%, and 6.03% respectively. The S:R ratio ranged from ∞ to 0.27, showing great

Table 1. Morphochemical characteristics of strains A and B.

Parameter		Strain A	Strain B
Plant type		Compact	Loose
Leaf	Shape	Obovate	Obovate
	Tip	Obtuse	Acute
	Base	Attenuate	Attenuate
	Length (cm)	6.54 ± 0.36*	7.46 ± 0.14
	Width (cm)	2.43 ± 0.06	2.74 ± 0.17
	Petiole length (cm)	0.50 ± 0.01	0.52 ± 0.02
Plant height (cm)		70	92
Collar diameter (mm)		5.95 ± 0.09	9.87 ± 0.20
Stevioside %		4.16	7.81
Rebaudioside-A %		3.05	2.84

*Values are given as means ± SE.

Table 2. Qualitative morphological characteristics of F_1 and F_2 populations.

Plant number (F_1)	Plant type	Leaf				Plant number (F_2)	Plant type	Leaf			
		Shape	Tip	Base	Margin			Shape	Tip	Base	Margin
P-1	C	Ov	O	Cn	Cr	S-1	C	L	O	Cn	Cr
P-2	C	L	O	Cn	Sr	S-2	C	Ov	O	Cn	Sr
P-3	C	L	O	Cn	Sr	S-3	C	Ov	O	Cn	Cr
P-4	C	L	O	Cn	Lcr	S-4	C	Ov	O	Cn	Cr
P-5	C	L	O	At	Sr	S-5	C	L	O	At	D
P-6	C	L	O	At	Sr	S-6	C	Ov	O	Ro	Cr
P-7	C	L	O	At	Sr	S-7	C	L	O	Cn	Sr
P-8	C	Ov	A	At	Sr	S-8	C	Ov	O	At	Sr
P-9	C	Ov	O	At	Sr	S-9	C	L	O	At	D
P-10	C	L	A	At	Sr	S-10	C	L	O	Cn	I
P-11	C	L	O	At	Sr	S-11	C	Ov	Rt	Cn	Cr
P-12	C	Ov	O	At	Lcr	S-12	C	Ov	O	Cn	Lcr
-	-	-	-	-	-	S-13	C	Or	O	Ro	Sr
-	-	-	-	-	-	S-14	C	L	O	Cn	Sr
-	-	-	-	-	-	S-15	C	Ov	O	Ro	Cr
-	-	-	-	-	-	S-16	C	Ov	O	Cn	Lcr
-	-	-	-	-	-	S-17	C	L	A	At	Sr
-	-	-	-	-	-	S-18	C	Ov	O	Cn	Sr
-	-	-	-	-	-	S-19	C	Ov	O	Cn	Cr
-	-	-	-	-	-	S-20	C	L	O	At	Sr
-	-	-	-	-	-	S-21	C	L	O	Cn	Sr
-	-	-	-	-	-	S-22	C	L	O	At	Cr
-	-	-	-	-	-	S-23	C	L	O	At	Sr
Frequency (%)	C - 100	Ov - 33.33 L - 66.67	O - 83.33 A - 16.67	At - 66.67 Cn - 33.33	Cr - 8.33 Lcr - 16.67 Sr - 75.00		C - 100	Ov - 47.83 L - 47.83 Or - 4.35	O -91.30 A - 4.35 Rt - 4.35	At -30.43 Cn - 56.52 Ro - 13.04	Cr - 34.78 Lcr - 8.70 D - 8.70 I - 4.35 Sr - 43.48

C- Compact; Ov- obovate; L- lanceolate; O- obtuse; A- acute; At- attenuate; Cn- cuneate; Cr- crenate; Lcr- lobate crenate; Sr- serrated; Or- orbicular; Rt- retuse; Ro- rounded; D- dentate; I- incised.

variation. The minimum ratio of 0.27 was observed in S-21, which was followed by 0.42 (S-19), 0.45 (S-23), 0.68 (S-22), 0.69 (S-20), 0.82 (S-7), and 0.91 (S-3), indicating lower stevioside than rebaudioside-A content.

The correlation analysis between morphochemical parameters revealed that stevioside percentage had a positive and significant correlation (0.450; $P \leq 0.01$) with petiole length and collar diameter (0.344; $P \leq 0.05$) of the plants. For rebaudioside-A percentages of the plants, we did not find any significant correlation with any of the studied morphological parameters of *Stevia rebaudiana* plants.

4. Discussion

4.1. Morphological characteristics of A and B strains and their F_1 and F_2 populations

Species like *Stevia rebaudiana* represent a wide range of sweetener compounds (stevioside and rebaudioside-A) as well as morphological traits, so it is imperative to identify strains with higher stevioside or rebaudioside-A and to establish the separate identity of such strains for commercial success. Raina et al. (2013) identified two strains, namely strains A and B, that were available during the present study. Observations of clear morphological difference between the two strains (strain A with obtuse

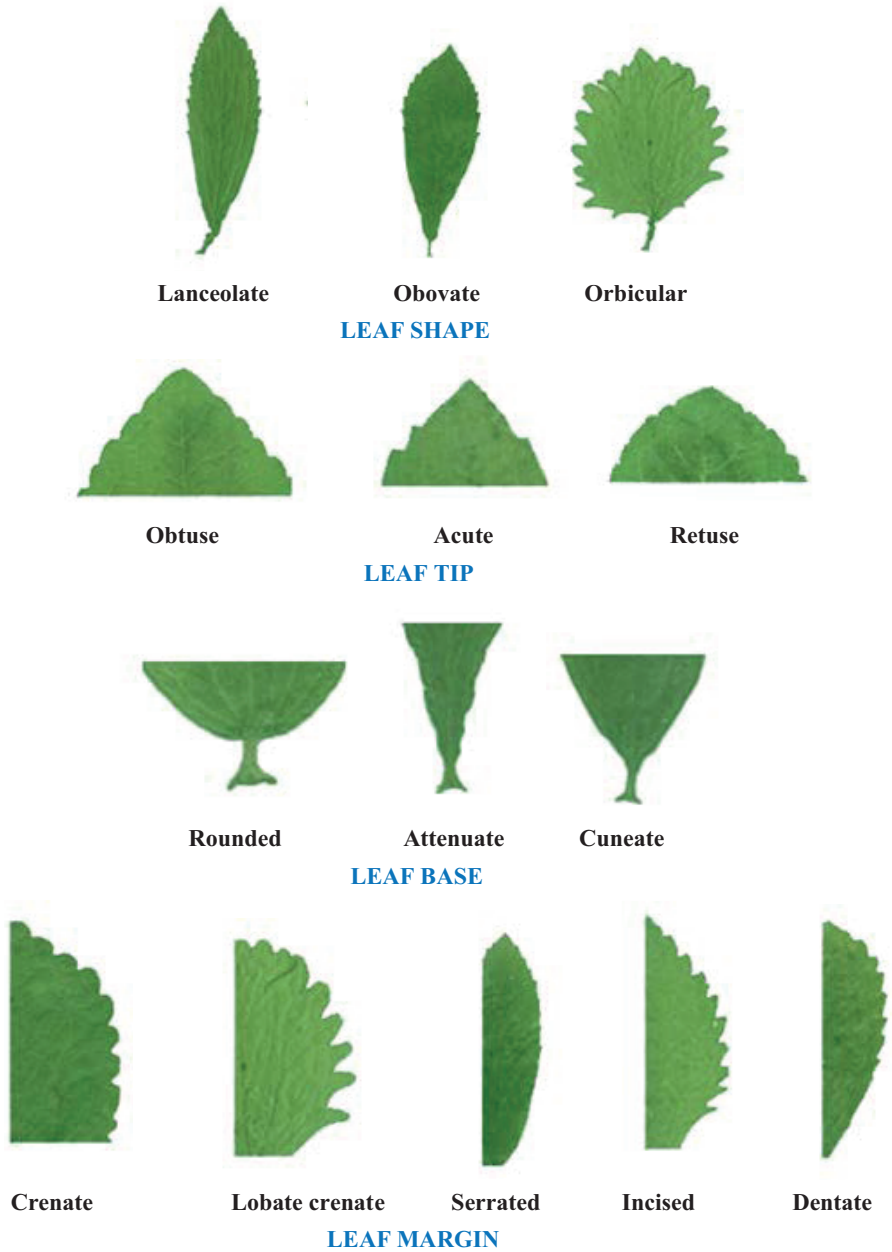

Figure 1. Variation found in leaf characteristics of *Stevia rebaudiana*.

and strain B with acute leaf tip, with the plant type of the former being compact and the latter being loose) and strain A with lower stevioside and rebaudioside-A content as compared to strain B (Table 1) were registered. As was clearly established by Raina et al. (2013), *S. rebaudiana* is a self-incompatible species that fails to produce any fertile seed upon selfing. Reciprocal crosses between strains A and B set dark-colored seeds (fertile seeds), indicating that the two strains are genetically compatible with each other. The F_1 progeny of the two strains (A and B) revealed complete uniformity as far as plant type was concerned, which was compact, a characteristic of the strain A parent. Regarding leaf tip type, a segregation ratio of 83.33:16.67 (obtuse:acute) was observed among the F_1 progeny. This indicates that the compact habit of the plant is dominant over the loose plant type and the gene responsible for obtuse leaf tip is expressed more in comparison to the acute leaf tip types. Although both strains (A and B) were characterized by obovate leaf shape, the F_1 progeny revealed a segregation ratio of 33.33:66.67

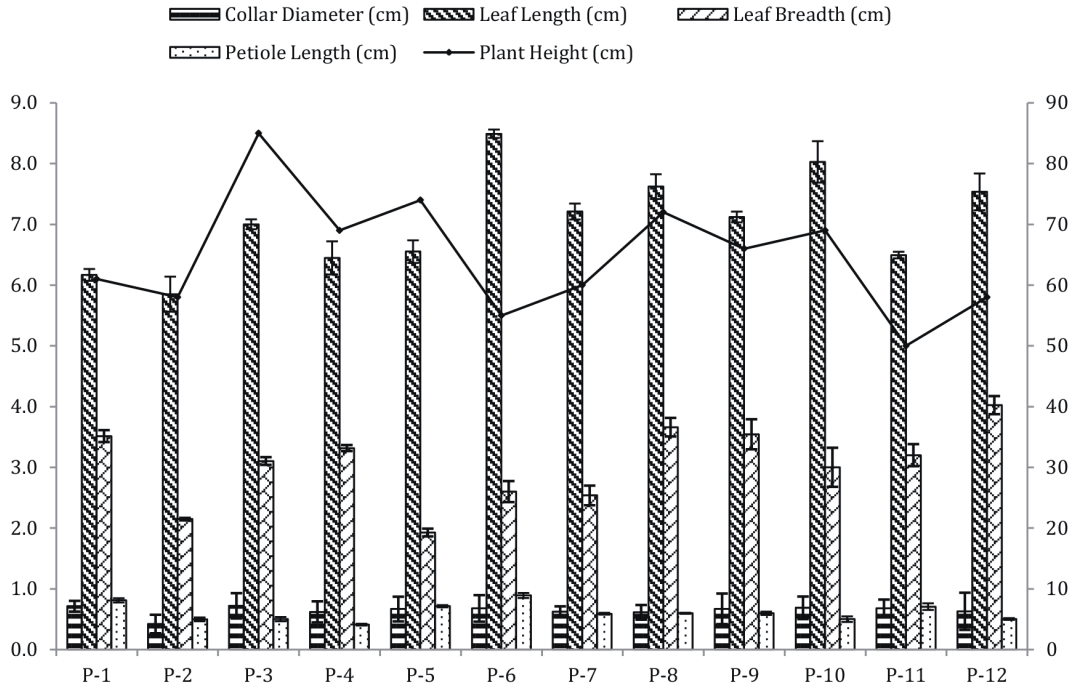

Figure 2. Quantitative morphological characteristics of F_1 population. Error bars denote mean ± SE.

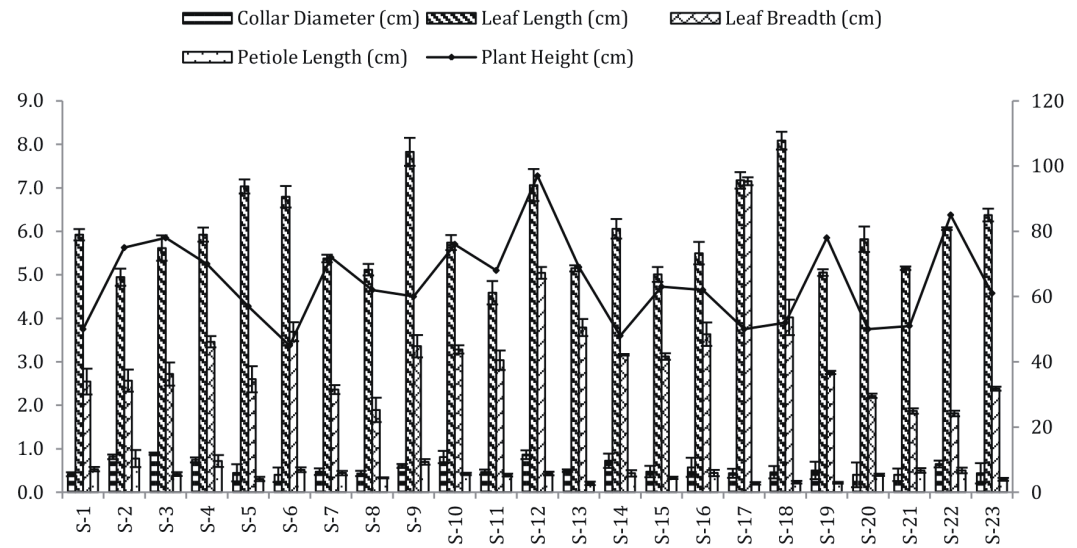

Figure 3. Quantitative morphological characteristics of F_2 population. Error bars denote mean ± SE.

(obovate:lanceolate), indicating polygenic control of leaf shape in this species (Table 2). A perusal of the literature reveals the existence of lanceolate (Dwivedi, 1999) as well as elliptic (Goettemoeller and Ching, 1999) leaves in *S. rebaudiana*. However, Tan et al. (2008) also reported oblanceolate, ovate, and spatulate leaf shapes in MSR-007, MSR-012, and MSR-028 accessions of *S. rebaudiana* introduced from Canada and Malaysia, which were high-yielding. Both parents (strains A and B) had attenuated leaf bases. However, 33.33% of the F_1 progeny were with cuneate leaf base. This again indicates that the character of leaf base is controlled by heterozygous alleles that segregate in the progeny. Apart from a single individual with orbicular leaf shape, only two leaf shapes, i.e. obovate and lanceolate, were observed in equal proportion in F_2 plants. Although both strains (A and B) had attenuate leaf bases, in F_2 progeny, plants with attenuate bases (30.43%) were far less common than those with cuneate leaf base

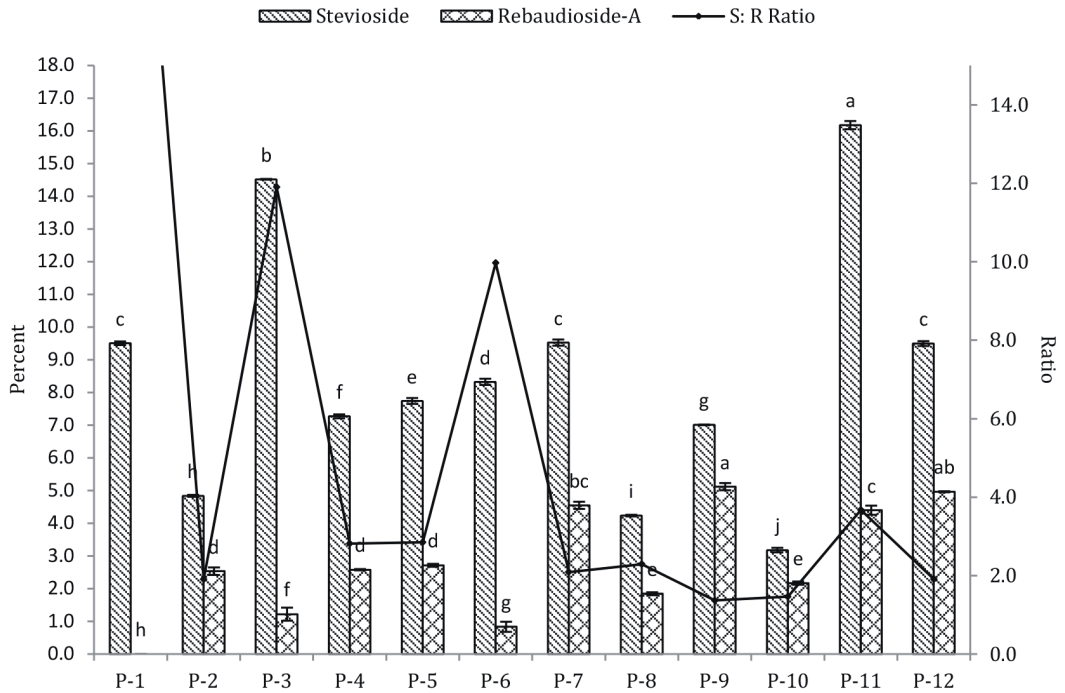

Figure 4. Stevioside and rebaudioside-A contents of F_1 population. Error bars denote mean ± SE. Different letters depict significantly different groups at the $P \leq 0.05$ level.

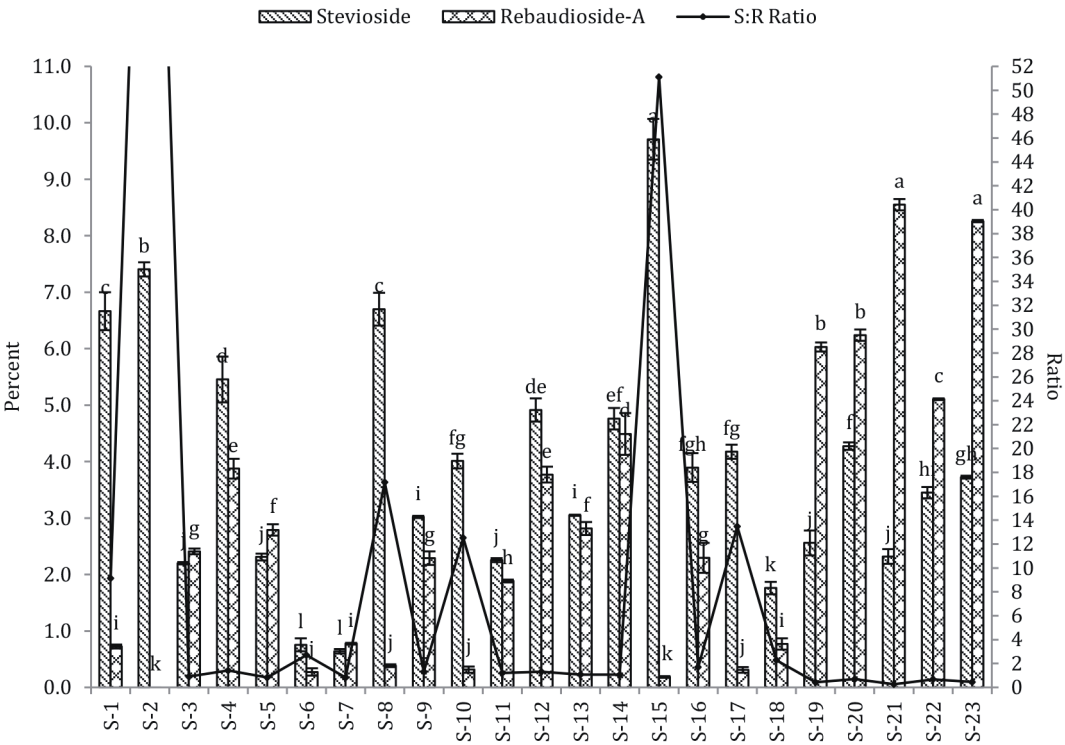

Figure 5. Stevioside and rebaudioside-A contents of F_2 population. Error bars denote mean ± SE. Different letters depict significantly different groups at the $P \leq 0.05$ level.

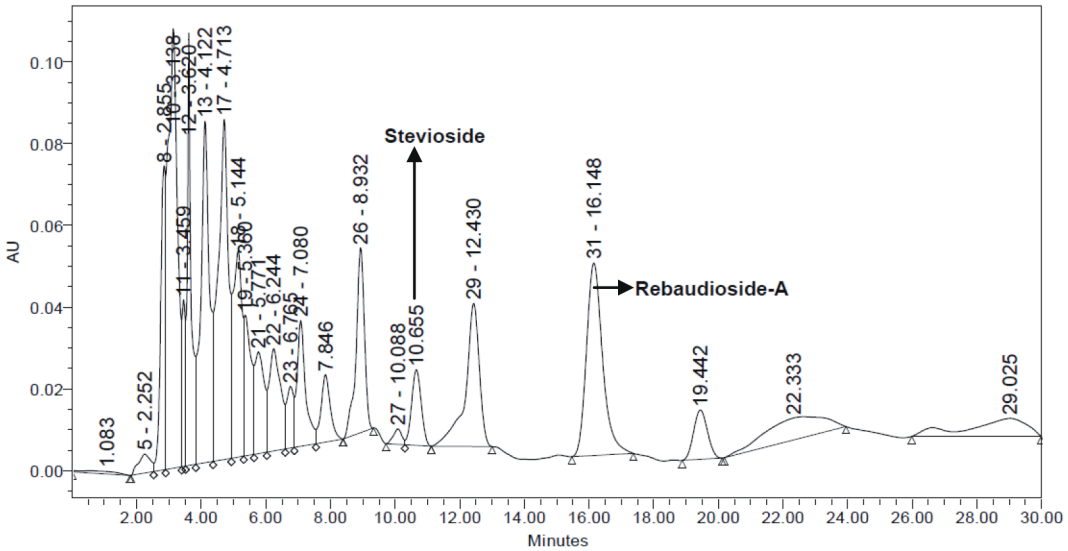

Figure 6. HPLC chromatogram of leaf samples of S-21 (F_2 population) individual.

plants (56.52%), indicating polygenic control of this character. With regard to leaf margin, a similar trend was observed in F_2 individuals as compared to F_1 individuals with crenate, dentate, incised, serrated, and lobate crenate margins.

The plant height of strain A and B was 70 and 92 cm, respectively. F_1 plant height varied from 50 to 85 cm, which indicates that in most of the progeny members the height is reduced in comparison to the parents. A similar trend was noticed with respect to collar diameter (0.42–0.72 cm), leaf length (5.85–8.49 cm), leaf width (1.93–4.02 cm), and petiole length (0.31–0.89 cm) (Figure 2). In the F_2 population, the variations in terms of plant height (45–97 cm), collar diameter (0.40–0.89 cm), and leaf length (4.59–8.08 cm) are significant (Figure 3) and generally indicate a low linkage of genes due to which no strong correlation could be drawn, at least within the morphological parameters studied. These results are similar to those reported by Tateo et al. (1998). Furthermore, Tateo et al. (1998) reported leaf variants differing on the basis of L/W (leaf length/leaf width) ratio ranging from 1.2 to 4.1. Similarly, Radusiene et al. (2004) also reported three leaf variants (narrow, intermediate, and broad) differing on the basis of L/W ranging from 2.05 to 3.89 in the case of *Hypericum perforatum*.

4.2. Chemical content of A and B strains and their F_1 and F_2 populations

The stevioside content in the F_2 plants exhibited a range of 0.64% to 9.71%, which was considerably low as compared to parents A and B and F_1 progeny (3.18% to 16.18%) (Figure 4). However, rebaudioside-A content exhibited an improvement in F_2 progeny (up to 8.55%) in comparison to F_1 progeny (up to 5.12%) (Figure 5). Generally, the ratio between stevioside and rebaudioside-A content in *Stevia rebaudiana* is in the range of 2:1. As is well known, both stevioside and rebaudioside-A are sweet in taste, with the former being about 300 times sweeter than sucrose (Kohda et al., 1976; Debnath, 2008; Giuffre et al., 2013). However, stevioside has a slightly bitter aftertaste with rebaudioside-A having none. Rebaudioside-A has also been reported to have the most desirable flavor profile (Dubois, 2000). This indicates that apart from strains having higher stevioside plus rebaudioside-A content, strains with higher rebaudioside-A are more desirable and strains having stevioside:rebaudioside-A ratios close to or less than one are much desired to avoid or lessen the bitter aftertaste profile of general strains of *S. rebaudiana*. Some of the individuals with S:R ratios near one, like plants S-13 and S-14, were indeed isolated. Hence, F_2 segregation in the present studies improved the S:R ratio close to one by either decrease of stevioside content, bringing it close to rebaudioside-A content, or by improving the rebaudioside-A content, bringing it closer to the stevioside content. However, the major interesting observation in the F_2 progeny was isolation of individuals with higher rebaudioside-A content than stevioside content. Individuals with higher rebaudioside-A, like S-21 (2.32% stevioside and 8.55% rebaudioside-A), S-23 (3.73% stevioside and 8.26% rebaudioside-A), S-20 (4.28% stevioside and 6.24% rebaudioside-A), S-19 (2.56% stevioside and 6.03% rebaudioside-A), and S-22 (3.45% stevioside and 5.11% rebaudioside-A), were isolated with S:R ratios far below one. These strains, which are much valued in international trade, and strains with higher

Table 3. Correlations between different morphochemical parameters.

	H	CD	LL	LW	PL	St	Rb
H	1.000						
CD	0.642**	1.000					
LL	–0.066	0.239	1.000				
LW	–0.068	0.074	0.318	1.000			
PL	0.008	0.449**	0.274	–0.237	1.000		
St	0.041	0.344*	0.163	–0.010	0.450**	1.000	
Rb	–0.026	–0.090	–0.059	–0.267	–0.119	–0.071	1.000

H- Plant height; CD- collar diameter; LL- leaf length; LW- leaf width; PL- petiole length; St- stevioside %; Rb- rebaudioside-A %. *: Significant difference at $P \leq 0.05$; **: significant difference at $P \leq 0.01$.

rebaudioside-A than stevioside content are reported in the literature (Morita, 1987; Britos, 2012; Milani et al., 2017). Morita (1987), in a patent application, reported plants exhibiting rebaudioside-A to stevioside ratios as high as 9.1:1, while total steviol glycosides were 10.1%. Similarly, Britos (2012) patented a cultivar named AKH L1 characterized by a stevioside content of 1.3% and rebaudioside-A content of 11.5% with total stevioside and rebaudioside-A content of 12.8%. Later, Milani et al. (2017) developed an elite variety named UEM-13 with stevioside and rebaudioside-A content of 4.00% and 9.01%, respectively. Furthermore, the chemical content variations in the present study are in agreement with the previous work by Abdelsalam et al. (2016), who reported significant variations in the stevioside and rebaudioside-A contents of 19 genotypes selected from natural populations of *S. rebaudiana*. Stevioside content varied between 34.4% (genotype 11) to 99.5% (genotype 12) while minimum (0.08%) and maximum (28.2%) rebaudioside-A was found in genotype number 12 and 6, respectively. From the results obtained in the present investigation, it could be concluded that variation in morphological characteristics and chemical contents among the *Stevia rebaudiana* segregants (F_1 and F_2 populations of A and B strains) are evident. Obovate, lanceolate, and orbicular leaf shapes were obtained in the F_1 and F_2 population plants as compared to obovate leaf shapes of parent plants (A and B). Regarding the plant types, all the plants studied were of compact growth. Other leaf characters like leaf margin showed greater variation in the F_2 than the F_1 population with retuse leaf tip. Wide variability was observed among the studied populations related to stevioside and rebaudioside-A percentages. Stevioside percentage was positively and significantly correlated with petiole length and collar diameter of the plants, while there was no effect of plant morphology on rebaudioside-A percentage of the leaves (Table 3). Tateo et al. (1998) found a positive correlation between plant habit and the stevioside content of the plants. Francisco et al. (2018) also found a significant correlation between height and stevioside and rebaudioside-A content of the plant.

Consequently, a few superior individuals in terms of higher rebaudioside-A percentage than stevioside were screened. The study revealed that the quantification of morphochemical characters will possibly favor the selection of potential parents to be involved in future breeding programs. Later, such superior plant types could be commercially utilized to isolate steviol glycosides, chiefly stevioside and rebaudioside-A, from the leaves. Stevioside and rebaudioside-A could be exploited in many food industries for adding sweetness to various products. On an overall basis, much characterization work needs to be done in *Stevia rebaudiana* populations to screen more interesting chemotypes.

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