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Manvendra Singh
Department of Molecular Biology & Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Vinod Saharan
Department of Molecular Biology & Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Deepak Rajpurohit
Department of Molecular Biology & Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Yadunandan Sen
Department of Molecular Biology & Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Ajay Sharma
Department of Molecular Biology & Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Arunabh Joshi
Department of Molecular Biology & Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Correspondence
Manvendra Singh
Department of Molecular Biology & Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur, Rajasthan, India

Criteria for selection of superior *Stevia rebaudiana* plant for propagation establishment

Manvendra Singh, Vinod Saharan, Deepak Rajpurohit, Yadunandan Sen, Ajay Sharma and Arunabh Joshi

Abstract
Stevia rebaudiana (Bertoni) is a calorie free sweet herb belongs to Asteraceae family which have high-potency sweeteners and excellent substitute to sugar. Stevia plants collected from various places of Rajasthan were grown in poly-house to evaluate different morphological characters before flowering stage. Five superior stevia plants (S36, S53, S81, S34, S37) were selected on the basis of morphological characters association and evaluated for stevioside and rebaudioside-A contents by High performance liquid chromatography (HPLC). Among the selected plants 'S36' mother plant exhibited highest leaf length (7.84 cm), leaf width (2.68 cm) and stevioside (3.4%) and rebaudioside (1.0%) content. In this study, leaf length and leaf width is positively correlated with stevioside and rebaudioside-A content. Therefore, this method is use for selection of superior stevia plant having high stevioside and rebaudioside-A content for propagation establishment.

Keywords: Propagation, Rebaudioside-A, Stevioside, Stevia

1. Introduction
Stevia rebaudiana (Bertoni) is a valuable medicinal plant belongs to Asteraceae family. The leaves of stevia produced diterpene glycosides (stevioside and rebaudiosides) which have high-potency sweeteners and excellent substitute to sugar (Megeji *et al*., 2005; Singh and Rao, 2005; Madan *et al*., 2010; Yadav *et al*., 2011) [1-4]. Stevia is a well-known therapeutic agent serve as an efficient medication for diabetes, hypertension, myocardial and antimicrobial infections, dental troubles, and tumors (Shivanna *et al*., 2013; Philippe *et al*., 2014; Gantait *et al*., 2015; Singh *et al*., 2015; Marcinek and Krejpcio, 2016) [5-9]. In present time people are very calorie conscious which increases use of stevia in preparation of non-calorie food stuffs and become a major sweetening agent in food products in South-east Asia (Salunkhe and Bhise, 2010; Savita *et al*., 2010; Marcinek and Krejpcio, 2016) [9-11]. India is supposed to have suitable conditions for the cultivation of stevia. It has been found that Indian stevia plant gives a higher stevioside yield of 10–18 percent in comparison to the reported 8–12 percent from other countries (Yadav *et al*., 2011)[4]. India itself is also stepping forward to compete in the stevia sweetener international market (Savita *et al*., 2010; Yadav *et al*., 2011) [4, 11].
In nature, seed germination in stevia is poor and unsuccessful commonly due to infertile seed (Goettemoeller and Ching, 1999; Kumar, 2013) [12-13] and small endosperm (Yadav *et al*., 2011) [4]. Even some plant selections produce virtually no viable seed due to their self-incompatibility (Ramesh *et al*., 2006; Yadav *et al*., 2011; Raina *et al*., 2013) [14-15, 4]. Plant raised from seed does not allow the production of homogenous plant population resulting in great variability in important features like sweetening levels and compositions (Brandle and Telmer, 2007; Kovylyaeva *et al*., 2007) [16-17]. Mass multiplication of stevia is usually done by conventional methods (Chalapathi *et al*., 1999; Yadav *et al*., 2011) [18, 4]. Some plant varieties/selections produce virtually no viable seed and vegetative propagation is the only way of multiplication. Stem cuttings were the prime means for the propagation of stevia. However, stevia plants have variability in production of stevioside and rebaudiosides.
Literature survey revealed that various morphological characters which direct positively correlated with stevioside and rebaudioside-A content. Information and understanding of the interrelationships among characters are important for selection of superior stevia plants without adversely affecting another important character. Therefore, aim of this study, development of method for selection of superior stevia plants with high content of sweeting compound on the basis of morphological and important biochemical characteristics for commercial cultivation.

2. Materials and Methods

In the present investigation, rooted seedlings were collected from Jaipur, Jodhpur and Udaipur. These seedlings were grown under poly-house condition at High-tech horticulture farm, RCA, MPUAT, Udaipur. Laboratory studies were undertaken at the Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Udaipur.

Fifty two plants were selected for recording observation. The selected plants of 45 days (before flowering) were tagged and data on individual plant were recorded for the different characters. The procedure adopted for recording observations on different morphological characters are followed: Plant height (cm), Number of branches per plant, Numbers of leaves per plant, Leaf length, Leaf width, Fresh weight of leaf, Dry weight of leaf, Leaf: Stem ratio. Selected superior stevia plant's leaves (S36, S53, S81, S34, and S37) were collected on the basis of morphological characters before flowering. Stevia leaves were dried in hot air oven. The samples were ground in a pestle mortar completely. The identification and quantitation of stevioside and rebaudioside-A in samples was done by retention time, UV spectra and by comparing the peak area of sample with that of the standard by high performance liquid chromatography (HPLC) from CSIR-Institute of Himalayan Bio-resource Technology, Palampur.

3. Results and discussions

Stevia rebaudiana plants collected from various places were grown in polyhouse to evaluate different associted morphological characters before flowering stage. After statistical analysis, five most promising mother plants was selected based on significant morphological characters (leaf length and width, Table 1) which are positively correlated to high stevioside and rebaudioside content. These five superior mother plants (S36, S53, S81, S34, S37) were also evaluated for stevioside and rebaudioside contents by HPLC. Among the selected plants 'S36' mother plant exhibited highest leaf length (7.84 cm), leaf width (2.68 cm) and stevioside (3.4%) and rebaudioside (1.0%) content (Table 2).

Table 1: Mean values for associated characters of Stevia

S.N.	Plant code	Treatment	Leaf Length (cm)		Leaf Width (cm)		Fresh Weight (gm)		Dry Weight (gm)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	S1		4.9200	0.5450	1.3600	0.0548	0.0680	0.0130	0.0160	0.0037
2	S2		4.2600	0.7232	1.5000	0.1414	0.0700	0.0158	0.0150	0.0019
3	S3		3.4800	0.2490	1.2400	0.0548	0.0420	0.0045	0.0086	0.0005
4	S4		3.9600	0.2074	1.0800	0.1095	0.0520	0.0084	0.0094	0.0022
5	S5		4.7800	0.2950	1.4200	0.1789	0.0680	0.0130	0.0158	0.0015
6	S7		3.8000	0.1000	1.8800	0.2490	0.0780	0.0045	0.0210	0.0022
7	S9		4.2000	0.2739	1.4600	0.2702	0.0800	0.0100	0.0208	0.0013
8	S10		5.4400	0.2510	2.2000	0.1414	0.1100	0.0200	0.0260	0.0057
9	S11		3.6000	0.5874	1.6200	0.3347	0.0440	0.0134	0.0100	0.0019
10	S12		5.0800	0.4087	1.9000	0.1581	0.1080	0.0164	0.0242	0.0030
11	S13		4.2200	0.5357	1.6400	0.2074	0.0660	0.0134	0.0152	0.0015
12	S14		4.4200	0.5167	1.4800	0.1789	0.0760	0.0089	0.0138	0.0018
13	S15		4.1800	0.4970	1.3600	0.1817	0.0720	0.0084	0.0162	0.0025
14	S16		4.8600	0.1817	2.1800	0.1095	0.1040	0.0055	0.0234	0.0018
15	S18		5.3400	0.6768	1.5400	0.1140	0.0920	0.0179	0.0214	0.0021
16	S19		5.7400	0.4278	1.3800	0.1483	0.0980	0.0179	0.0160	0.0014
17	S20		4.8800	1.0085	1.9800	0.2950	0.0960	0.0358	0.0222	0.0059
18	S21		4.3200	0.3701	1.7800	0.2168	0.0740	0.0167	0.0142	0.0016
19	S22		4.8000	0.2345	1.6600	0.2074	0.1020	0.0164	0.0258	0.0038
20	S24		5.0200	0.2775	1.3200	0.0837	0.0720	0.0045	0.0188	0.0033
21	S25		4.7600	0.2966	1.8000	0.1000	0.0920	0.0130	0.0190	0.0014
22	S27		5.4000	0.6442	1.8400	0.4037	0.1040	0.0313	0.0352	0.0055
23	S28		4.8400	0.1140	1.3000	0.1000	0.0820	0.0045	0.0156	0.0011
24	S29		5.3000	0.7246	1.9800	0.1095	0.1140	0.0207	0.0256	0.0035
25	S31		5.6200	0.2588	2.0200	0.1789	0.1300	0.0212	0.0256	0.0032
26	S32		4.7000	0.0707	1.6400	0.2074	0.1100	0.0255	0.0260	0.0026
27	S34		6.0400	0.3578	2.0000	0.1732	0.1580	0.0164	0.0282	0.0016
28	S36		7.8400	0.1517	2.6800	0.3271	0.2040	0.0114	0.0368	0.0061
29	S37		5.9000	0.3162	2.1800	0.1095	0.1560	0.0230	0.0324	0.0019
30	S38		4.9400	0.2702	1.5600	0.1140	0.0680	0.0217	0.0200	0.0007
31	S40		5.0800	1.0803	1.9400	0.3209	0.1000	0.0308	0.0274	0.0055
32	S41		5.7000	0.8216	2.0200	0.2168	0.1000	0.0122	0.0198	0.0016
33	S42		5.6200	0.3962	1.6200	0.1304	0.1160	0.0195	0.0238	0.0023
34	S43		5.5600	0.4278	1.6600	0.1817	0.0980	0.0239	0.0268	0.0039
35	S44		5.0200	0.3114	1.6200	0.1304	0.0920	0.0164	0.0188	0.0026
36	S45		5.6400	0.3715	1.7600	0.2608	0.1000	0.0255	0.0338	0.0041
37	S46		5.7400	0.3507	1.5600	0.1673	0.0960	0.0089	0.0254	0.0017
38	S48		5.0400	0.2608	1.8800	0.1483	0.0960	0.0089	0.0318	0.0022
39	S49		4.4600	0.3050	1.8400	0.1140	0.0820	0.0084	0.0180	0.0022
40	S50		5.3400	0.3209	2.0600	0.1140	0.1160	0.0167	0.0208	0.0043
41	S52		3.8400	0.2302	1.6600	0.1140	0.0680	0.0130	0.0154	0.0027
42	S53		6.1800	0.2588	1.9800	0.1924	0.1260	0.0365	0.0282	0.0018
43	S57		5.2200	0.4324	2.0400	0.1949	0.1140	0.0230	0.0252	0.0033
44	S58		5.3600	0.1517	1.8800	0.1095	0.0940	0.0055	0.0226	0.0056
45	S64		3.9000	0.2915	1.7800	0.1304	0.0760	0.0089	0.0150	0.0010

46	S66		5.1200	0.6058	1.3800	0.3899	0.0860	0.0055	0.0188	0.0023
47	S67		5.1200	0.3114	2.1000	0.1871	0.1140	0.0134	0.0198	0.0016
48	S69		5.8800	0.5541	2.0800	0.1095	0.0980	0.0084	0.0198	0.0029
49	S71		4.2400	0.5941	1.7400	0.2074	0.0780	0.0217	0.0208	0.0026
50	S77		4.6200	0.2588	1.9000	0.0707	0.0940	0.0114	0.0496	0.0674
51	S79		4.8400	0.2302	1.3800	0.1483	0.0920	0.0130	0.0174	0.0011
52	S81		6.0400	0.6914	2.1000	0.3082	0.1360	0.0261	0.0380	0.0057
		GM	5.0038	0.8764	1.7492	0.3522	0.0954	0.0323	0.0222	0.0117
		Se	0.2039		0.0872		0.0078		0.0044	
		CD5%	0.5685		0.2430		0.0218		0.0123	
		CD1%	0.7498		0.3205		0.0288		0.0162	
		CV	9.11		11.14		18.35		44.23	

*, ** Significant at 5% and 1% respectively

Table 2: Stevioside and Rebaudioside-A content in selected Stevia plant

S.N.	Plant code	Compound analysed	
		Stevioside (% dry weight of leaves)	Rebaudioside-A (% dry weight of leaves)
1	S36	3.4±0.02	1.0±0.04
2	S53	3.0±0.07	0.82±0.05
3	S81	3.0±0.05	0.76±0.09
4	S34	2.2±0.08	0.83±0.07
5	S37	2.1±0.04	0.82±0.02

Interrelationships among plant characters are important to aid selection and set limits of each economic character that a breeder can choose without adversely affecting another important character. Several authors have studied the dependence of yield on various growth parameters as well as stevioside content (Brandle and Rosa, 1992; Chalapathi *et al.*, 1998) [19-20]. Plant height and leaf number at the second and fourth week after planting were positively correlated with biomass production in a greenhouse experiment conducted by Buana and Goenadi (1985) [21]. In another study, Buana (1989) [22] reported that plant height had no significant correlation with production, leaf number, or branch number in the first 4 week. Stevioside content was not correlated with yield or leaf: stem ratio (Brandle and Rosa, 1992) [19]. Further, dry leaf yield was correlated with leaf size and thickness and content of rebaudioside-A was highly correlated with leaf thickness (Shyu, 1994) [23]. Stevioside content is influenced by both leaf length and width; however, the leaf surface has more influence on stevioside content than the number of roots (Yadav *et al*., 2011) [4]. Furthermore, stevioside concentrations were not correlated with yield or leaf:stem ratio indicating that concurrent improvement of agronomic and chemical characteristics is possible (Brandle and Rosa, 1992) [19]. Plant leaf yield is proportional to branch number, leaf number and (not always) plant height (Buana and Goenadi, 1985; Buana, 1989) [21-22]. High rebaudioside-A content is also linked to leaf length and width (Weng *et al.*, 1996) [24].

4. Conclusion

In present investigation, leaf length and leaf width was positively correlated with stevioside content. Therefore, this method is use for selection of the superior stevia plant on the basis of morphological and biochemical characteristics for further improvement programme as well as commercial cultivation.

5. Acknowledgement

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