Egypt. J. Plant Breed. 23(1):213– 221 (2019)

MORPHOLOGICAL EVALUATION OF SOME HYBRIDS OF STEVIA (*Stevia rebaudiana*) UNDER EGYPTIAN CONDITIONS

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ABSTRACT

The present study was carried out to evaluate some Stevia rebaudiana segregates to facilitate selection of superior genotypes for hybridization in breeding programs. Three varieties, namely Sugar-High-A3, Egy.1 and Shou-2 and their F_1 populations were evaluated for their morphological characteristics at Giza Research Station (latitude of 30.02^o N and longitude of 31.22^o E) in 2017 and 2018. Various quantitative morphological traits including plant height, number of branches/plant, number of leaves/plant, leaf fresh and dry weight/plant, leaf moisture%, stems fresh weight and leaf: stem ratio were recorded. Individuals were identified with leaf fresh weight ranging from 40 to 670 g, while the highest leaf dry weight (215 g) was recorded by strain No. 2 of Egy.1 variety. Shou-2 variety had the highest leaf dry weight (60 g/plant). Stevia Egy.1 variety produced the highest percentage of superior strains (65 %), compared to the other varieties. Correlation indicated that, leaf dray weight was positively and highly significantly correlated with leaf fresh weight and leaf number/plant, recording 0.97 and 0.81, respectively.

Key words**:** ***Morphology, Populations, F_1 strains, Stevia.***

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 lowcalorie 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.

Stevia 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 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. Stevia is cultivated in different places of the world, it is expected that in the Egyptian agriculture environment one Fadden of stevia may produce up to 400 kg of Stevia sugar annually (Allam 2007).

Stevia populations grown from seeds show variations in morphology as well as chemical contents (Tamura *et al* 1984 and Jadeja *et al* 2005). Plant leaf yield is proportional to branch number, leaf number and (not always) plant height (Buana and Goenadi 1985 and Buana 1989). Total stevioside content is positively correlated with leaf/stem ratio (Tateo *et al* 1998).

In nature, seed germination in stevia is poor and unsuccessful commonly due to infertile seed (Goettemoeller and Ching 1999; Kumar 2013) and small endosperm (Yadav *et al* 2011). Stevia is a self-incompatible species that fails to produce any fertile seed upon selfing (Miyagawa *et al*., 1986; Chalapathi *et al* 1997; Ramesh *et al* 2006; Yadav *et al* 2011 and Raina *et al* 2013). There is natural variability in stevia due to self-incompatible flowers and seed germination is very poor due to infertile seed. Artificial cross pollination is required for setting of seeds (Skaria *et al* 2004). Mass selection is a very simple breeding scheme, that allows to create a new population by cross-pollinating two different existing open-pollinating populations (Caligari and Forster 2015). A representative set of individuals from each population will be taken to be crossed. The seed that results from such a set of crosses is grown under field conditions over a number of seasons (Shock 1982). Accordingly, morphochemical characterization needs to be applied to natural sweetener plants like stevia. Although earlier studies reported the selection of elite genotypes in natural populations and segregating generations of stevia (Abdelsalam *et al* 2016 and Singh *et al* 2017), only limited information is available with regard to the identification of superior plant types in segregating populations. Though the segregating individuals are not needed for crop cultivation as associated with 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 elite genotypes from the F_1 populations resulted from crossing among the three varieties Sugar-High-A3, Egy.1 and Shou-2.

MATERIALS AND METHODS

Plant material for the present study included three varieties of *Stevia rebaudiana*, namely Sugar-High-A3 (V.1), Egy.1 (V.2) and Shou-2 (V.3) and their F_1 hybrid populations, which were obtained from Sugar Crops Research Institute (SCRI), Agricultural Research Centre (ARC), Giza, Egypt. The three varieties were grown at Giza Research Station (latitude of 30.02^{o} N and longitude of 31.22^{o} E) in 2017 and 2018. These varieties were involved in fertilizing each other by open as well as artificial cross pollination among the three varieties (Artificial cross pollination is required

for setting of seeds) in the first season. Fertile seeds on female parents of each variety were collected separately and used for raising the F_1 hybrid populations. For this, seeds collections of the three varieties were sown in nursery beds in the greenhouse. Thereafter, seedlings were transplanted to the experimental field as randomized complete block design, in the second season. Some strains having variable distinguished morphological traits were selected from the F_1 segregating populations. Subsequently, 57 F_1 plants (23 plants from V.1, 20 from V.2 and 14 from V.3) along with (V.1), (V.2) and (V.3) were evaluated for variation in morphological characters.

At age of 45 days (before flowering), plants were tagged, and the following morphological characters were recorded on individual plants:

1. Plant height (cm); 2. Number of branches/plant; 3. Number of leaves/plant; 4. Fresh weight of leaves; 5. Dry weight of leaves; 6. Leaf moisture %; 7. Stems fresh weight (g) and Leaf: Stem ratio.

Data were analyzed using t-Test by the analytical package MSTAT-c v 2.1. (1988), the variable values were expressed as the mean ± standard error of the mean (SE). Phenotypic correlation coefficients were estimated according to Cardinal and Burton (2007).

RESULTS AND DISCUSSION

Stevia rebaudiana plants were evaluated for different morphological characters before flowering stage. After statistical analysis for F_1 hybrid populations, Sugar-High-A3 variety and its hybrid strains are presented in Table (1). However, data of 23 strains showed significant differences between strains and most strains were superior to Sugar-High-A3 variety in all studied traits. Strain no 14 had superior values to Sugar-High-A3 variety and other strains in branches height, leaves number/plant, leaves fresh and dray weight and stems fresh weight. While, strain no 1 was superior to other strains in branches number/plant (21 branch) and without significant differences with strains no 14, 21 and 6 in leaves number/plant, as well as, strain no 2 had higher ratio of leaf/stem (1.97) than other strains. Whereas, leaf moisture ranged from 58.67% to 81.25% (strains no 21 and 15, respectively), while was 66.27% for Sugar-High-A3 variety. Similar results were recorded by Singh *et al* 2017 and Kumari *et al* 2018.

Strains mean in Table (1) indicated that, 14 strains had values of leaves dray yield higher than Sugar High A3 variety, that mean we can select them as new strains under Egyptian conditions.

Table 1. Means for studied characters of stevia variety Sugar-High-A3 and its strains.

Genotypes	Plant height (cm)	Branches number /plant.	Leaves number /plant	Leaves fresh weight (g)	Leaves dry weight (g)	Leaf moisture %	Stems fresh weight (g)	Leaf/ stem Ratio
Sugar-High-A3	61	10	334.6	133	45	66.27	136	1.20
1	75	21	1064	230	77	66.52	160	1.44
2	80	10	786	285	75	73.68	145	1.97
3	80	11	855	160	55	65.63	180	0.89
4	80	9	354	115	42	63.48	125	0.92
5	70	9	435	160	45	71.88	155	1.03
6	75	12	1036	170	65	61.76	130	1.31
7	70	6	211	80	25	68.75	50	1.60
8	70	3	469	105	38	63.81	75	1.40
9	70	6	463	150	50	66.67	120	1.25
10	70	7	432	180	65	63.89	149	1.21
11	70	9	788	270	80	70.37	238	1.13
12	80	7	690	190	65	65.79	190	1.00
13	70	3	375	115	28	75.65	90	1.28
14	100	11	1200	350	124	64.57	296	1.18
15	75	10	400	240	45	81.25	130	1.85
16	65	6	318	140	45	67.86	95	1.47
17	85	6	438	199	50	74.87	151	1.32
18	85	6	369	170	52	69.41	110	1.55
19	75	8	283	134	33	75.37	86	1.56
20	70	14	407	180	55	69.44	160	1.13
21	70	9	1180	225	93	58.67	245	0.92
22	70	6	189	73	30	58.90	57	1.28
23	80	8	558	225	80	64.44	200	1.13
Mean	74.8	8.6	568.1	178.3	56.8	67.87	144.7	1.29
t values	45.5	11.3	9.1	13.1	12.1	60.34	11.9	22.82
Standard Deviation	8.06	3.74	305.90	66.57	23.05	5.51	59.63	0.28
Std. Error	1.65	0.76	62.44	13.59	4.71	1.12	12.17	0.06

Data in Table (2) are presented for Egy.1 variety and its hybrid strains. The data of 20 strains cleared, significant differences between strains and most strains were superior to Egy.1 variety in all studied traits. Strain no 2 had the highest values compared to Egy.1 variety and other strains in branches height, branches number/plant, leaves number/plant and leaves fresh and dry weight. While, strain no 4 were superior to other strains in stems fresh weight (350 g) with insignificant difference with strains no 2. On the other hand, strain no 1 had high ratio of leaf/stem (2.67) compared with other strains. While, leaf moisture ranged from 32.50% to 80.0% (strains no 18 and 16, respectively). It recorded 67.48% for Egy.1 variety. Same trends were reported by Singh *et al* (2017) and Kumari *et al* (2018).

Table 2. Means for studied characters of stevia variety Egy.1 and its strains.

Genotypes	Plant height (cm)	Branches number /plant.	Leaves number /plant	Leaves fresh weight (g)	Leaves dry weight (g)	Leaf moisture %	Stems fresh weight (g)	Leaf/ stem Ratio
Egy.1	67	10	341	169	55	67.48	88	1.94
1	90	16	884	400	140	65.00	150	2.67
2	100	22	1238	670	215	67.91	330	2.03
3	90	8	638	250	85	66.00	200	1.25
4	80	13	673	430	155	63.95	350	1.23
5	70	10	671	239	75	68.62	208	1.15
6	70	8	245	110	30	72.73	100	1.10
7	70	9	600	285	90	68.42	170	1.68
8	70	16	743	360	125	65.28	290	1.24
9	60	11	770	225	77	65.78	263	0.86
10	50	12	496	180	60	66.67	111	1.62
11	70	13	481	255	90	64.71	195	1.31
12	60	16	617	288	90	68.75	170	1.69
13	80	15	725	330	110	66.67	230	1.43
14	50	9	450	145	50	65.52	97	1.49
15	50	14	630	220	70	68.18	164	1.34
16	50	7	213	75	15	80.00	55	1.36
17	40	3	107	40	20	50.00	20	2.00
18	45	5	100	40	27	32.50	16	2.50
19	45	4	123	55	25	54.55	25	2.20
20	45	8	128	50	26	48.00	30	1.67
Mean	64.4	10.9	517.8	229.3	77.6	63.65	1.6	1.61
t values	17.3	10.8	8.0	6.8	6.9	29.18	7.0	15.65
Standard Deviation	17.1	4.6	296.4	155.6	50.9	9.99	101.0	0.47
Std. Error	3.7	1.1	64.7	33.9	11.1	2.18	22.0	0.10

Data in Table (2) indicated that, 13 strains had higher values of leaves dry weight than Egy.1 variety; they may be selected as new strains under Egyptian conditions.

Data in Table (3) cleared that, Shou-2 variety and its hybrid strains differed significantly. Fourteen strains had significant differences between and Shou-2 variety, they were superior to most of strains in all studied traits. Strain no 8 had the highest values compared to other strains, but without significant difference with Shou-2 variety in leaves number/plant, as well as, strain no 7 in branches fresh weight (g) trait and strain no 3 in L/S Ratio trait which were superior to Shou-2 variety (137 g and 2.22 respectively). Data (Table 3) showed that, leaf moisture ranged from 44.44 to 63.64% for strains no 12 and 11, respectively, while, was 71.87% for Shou-2 variety. These results are in agreement with results by Singh *et al* (2017) and Kumari *et al* (2018).

Table 3. Means for studied characters of stevia variety Shou-2 and its strains.

Genotypes	Plant height (cm)	Branches number /plant.	Leaves number /plant	Leaves fresh weight (g)	Leaves dry weight (g)	Leaf moisture %	Stems fresh weight (g)	Leaf/ stem ratio
Shou-2	68	11	477	216	60	71.87	112	1.99
1	50	13	271	75	40	46.67	115	0.65
2	50	5	167	50	25	50.00	58	0.86
3	45	6	75	40	15	62.50	18	2.22
4	35	18	309	95	44	53.68	95	1.00
5	43	11	260	55	30	45.45	69	0.80
6	40	15	285	70	28	60.00	53	1.32
7	40	16	353	110	44	60.00	137	0.80
8	50	16	481	140	55	60.71	130	1.08
9	45	13	409	95	42	55.79	105	0.90
10	45	15	289	70	35	50.00	90	0.78
11	33	11	175	55	20	63.64	49	1.12
12	45	3	311	45	25	44.44	65	0.69
13	45	12	430	80	42	47.50	110	0.73
14	35	29	338	65	28	56.92	55	1.18
Mean	44.6	12.9	308.7	84.1	35.5	55.28	84.1	1.1
t values	20.4	8.1	10.5	7.2	10.8	26.98	9.5	9.01
Standard Deviation	8.4	6.2	114.3	45.2	12.7	7.93	34.4	0.5
Std. Error	2.2	1.6	29.5	11.7	3.3	2.05	8.9	0.12

Data in Table (3) indicated that, one strain had high value of leaves dray yield close to (without significant difference) Shou2 variety, that mean we can select it as new strain under Egyptian conditions.

From data in tables (1,2 and 3) reported that, Sugar high A3 and Egy.1 more favorable varieties for produced strains with high superior to female parents percentage were 61 and 65 %, respectively, while Shou-2 which had highest morphological traits values compared to other varieties hadn't superior strains from its hybrid seeds that tested.

Phenotypic correlations between most studied traits were highly significant and positive. Leaves dry weight (g) and all other studied traits were highly significant correlated (Table 4). Leaves dry weight was positively and highly correlated with leaves fresh weight (g) and leaves number per plant (r= 0.97 and 0.81, respectively).

Table 4. Phenotypic correlation coefficients among leaves dray weight (g) and other studied traits.

Traits	Plant height (cm)	Branches number /plant	Leaves number /plant	Leaves fresh weight (g)	Leaves dry weight (g)	Stems fresh weight (g)
Plant height (cm)	**1**	**-0.07**	**0.65****	**0.72****	**0.65****	**0.65****
Branches number/plant		**1**	**0.39****	**0.39****	**0.43****	**0.34****
Leaves number/plant			**1**	**0.79****	**0.81****	**0.81****
Leaves fresh weight (g)				**1**	**0.97****	**0.86****
Leaves dry weight (g)					**1**	**0.88****
Stems fresh weight (g)						**1**

****. Correlation is significant at the 0.01Probability.**

***. Correlation is significant at the 0.05 Probability.**

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التقييم الخضري لبعض هجن الإستيفيا تحت الظروف المصرية

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أجريت هذه الدراسة بهدف تقييم السلالات لبعض هجن نبات الإستيفيا Stevia rebaudiana Bertoni ليسهل انتخاب السلالات المتفوقة من الهجن المقيمة في برامج التربية. تم تقييم ثلاثة أصناف Sugar High A3 و Egy.1 و Shou.2 وسلالات الهجن F_1 الناتجة منهم مع قياس الصفات المورفولوجية لها في تجربة بحثية بمزرعة مركز البحوث الزراعية بالجيزة خلال الموسمين ٢٠١٧ و ٢٠١٨. الصفات المورفولوجية المقدرة هي ارتفاع النبات، عدد الفروع على النبات، عدد الأوراق لكل نبات، وزن الأوراق الغض والجاف/النبات، النسبة المئوية للرطوبة في الأوراق، الوزن الأخضر للأفرع على النبات و نسبة الورق: الفروع. وقد تم تقييم السلالات المختبرة حيث وجد أنها ذات وزن طازج للورقة على النبات تراوح ما بين ٤٠ جم إلى ٦٧٠ جم بينما تم وصول الوزن الجاف للورق على النبات إلى ٢١٥ جم للسلالة رقم ٢ من الصنف Egy.1. كما وجد أيضاً، أن الصنف Shou-2 سجل أعلى قيمة في صفات الوزن الجاف للأوراق/نبات (٦٠ جم/نبات) متفوقاً على الصنفين الآخرين. من ناحية أخرى ، كانت نسبة السلالات المتفوقة الناتجة من الصنف Egy.1 (٦٥٪) أعلى نسبة من السلالات المتفوقة الناتجة من الصنفين الآخرين. في نفس الوقت وجد من دراسة علاقات الإرتباط وجود إرتباط طردي قوي بين الوزن الجاف للأوراق وبين الوزن الطازج للأوراق (جم) وكذلك عدد الأوراق على النبات وكانت قيم معاملات الإرتباط ٠.٩٧ و ٠.٨١، على التوالي.

المجلة المصرية لتربية النبات ٢٣ (١) : ٢١٣ - ٢٢١ (٢٠١٩)