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Full Length Article

Comparison of Growth, Leaf Yield and Steviol Glycosides Concentration of Two Stevia Cultivars (*Stevia rebaudiana*) Grown in a Sandponics System

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Abstract

Sandponics, a low-cost, low-tech, environmentally friendly soilless cultivation technique, is consistent with Egypt's national situation and the global development of soilless culture. Two stevia cultivars, Sugar High-A3 (CV_1) and Morita (CV_2), were evaluated for growth, leaf yield and biochemical properties in the three circulatory sandponics substrates, silica (S_1), silica + sand (S_2) and sand (S_3) inside a greenhouse. The cultivars differed significantly in their plant height, branch number, fresh weight and dry weight of leaves. The cv. Morita showed a clear superiority in the growth and leaf yield traits as compared to the cv. Sugar High-A3. In most cases, there was the same trend for the influence of substrate cultivation on the growth and leaf yield attributes of both cultivars. For all harvests, the content of Morita of stevioside was higher than the content of Sugar High-A3 in all cultivation substrates, the highest content of stevioside was recorded 14.31% achieved when Morita was grown in sand substrate. The content of Sugar High-A3 of rebaudoside was higher than the content of Morita in all cultivation substrates. With Sugar High-A3, the concentration of rebaudoside was higher with sand substrate at all harvests. The cultivation of medicinal plants under controlled conditions, particularly sandponics technology, seemed viable to improve accumulation of high-quality biomass and optimized secondary metabolite production. © 2022 Friends Science Publishers

Keywords: Soilless; Stevia; Stevioside; Rebaudoside; Sand substrates; Leaf yield

Introduction

Soilless culture uses substrate instead of soil, which avoids the factors of continuous cropping obstacles. Rock wool and peat are currently the main soilless culture substrates. The Dutch substrate cultivation accounts for 90% of soilless culture (Jiang *et al*. 2015) and 80% of the vegetables use rock wool as the substrate (Mu *et al*. 2019). After 1–2 years of use, such substrates as rock wool and peat need to be replaced response to alterations in physical and chemical properties (Liu *et al*. 2006). Rock wool is difficult to degrade in the natural environment and a large amount of rock wool waste causes serious environmental damage. Peat is a non-renewable resource. Excessive exploitation causes resource depletion and damages the ecological environment. Therefore, it is an inevitable trend for the development of soilless culture to find sustainable cultivation substrates and develop eco-environmental, low-cost and low-tech (Mu *et al*. 2019).

Sandponics is a soilless cultivation method in which fine sand with low salt, mud content is used as a growing substrate and a small amount of low-dose nutrient solution is used for multiple irrigation. The physical structure and chemical composition of the sand cannot be changed for decades and the sand can still maintain good air permeability and will not cause the problem of hardening (Mu *et al*. 2019).

Sandponics cultivation can produce for up to 25 years without needing to replace the substrate. It is an ecological and environmentally friendly soilless cultivation method that is simple to operate, can be produced by non-professionals, and has low-cost (Masayoshi 2015). This will increase the chances of entering new producers due to increased production and lower costs, thus increasing profitability in the cultivation of medicinal and aromatic plants. As an excellent alternative to traditional agriculture, which has many problems, for example, the continued cultivation of crops for a long time leads to soil degradation and reduces its fertility, continuous cropping over a long period will cause soil deterioration, reduces soil fertility and increases the development of diseases and insect pests, all of which impair plant production and quality (Wang *et al*. 2010). Most farmers in the cultivation areas primarily use pesticides to preserve crop yields, but the results are frequently unsatisfactory, increasing production costs, polluting the environment, and causing the deterioration of farmland ecosystem functions (Yang *et al*. 2017). The sand itself contains very little carbon and nitrogen and the carbon-nitrogen ratio is low, which is not conducive to the reproduction and growth of microorganisms in the matrix, and there are fewer soil-borne pathogens (Mu *et al.* 2019). Continuous crops retain auto toxic substances in the soil through leaching, root exudation and root decomposition. The known auto toxic substances are mainly phenolic acids such as ferulic acid, p-hydroxybenzoic acid, cinnamic acid and vanillin (Bouhaouel *et al*. 2015), these auto toxic substances can be easily removed from the sand by fresh water flushing.

The size of the particle of sand will affect the air permeability, water retention and nutrient absorption. Sand with a larger particle size has good air permeability, but plant roots cannot absorb necessary trace elements in the coarse sand. Sand with a small particle size has good water retention performance, but excessive irrigation will affect air permeability and cause water stagnation (Mu *et al.* 2019). Douglas (1985) believed that as a cultivation substrate, sand with particle sizes less than 0.6 mm should account for 50% and sand with particle sizes greater than 0.6 mm should account for 50%.

Stevia (*Stevia rebaudiana*) is well-known for its sweet-tasting compounds, steviol glycosides (SG), which are abundant in the leaves (Ramesh *et al*. 2006). The approval of steviol glycosides as strong sweeteners, first in Australia, and in the United States, and most lately in Europe, has increased interest in commercial stevia production (Anon 2011). Stevia leaf extract has been used traditionally in many remedial applications as a natural product with zero calories and confirmed non-toxic effects on human health (Megeji *et al.* 2005; Dushyant *et al.* 2014).

Stevia plantations in north Egypt may be harmed by the frequent low temperatures during the winter season because the plant grows in tropical conditions. Planting stevia in greenhouses and hydroponic systems is one solution to this problem. On the other hand, using hydroponic systems to grow plants of human interest has become a viable option. It is important to note that plant material for pharmaceutical use must be free of heavy metals, soil and soil-borne organisms, herbicides and pesticides. Thus, sandponics is a promising new tool for the production of pharmaceutically relevant plants, as well as an optimum growing system for high-quality plant biomass production. In addition, adoption of hydroponics system can improve water-use efficiency (Putra and Yuliando 2015).

High SG concentrations in abundant leaf biomass are an important component of commercial stevia production. The yield component attributes show a wide variation between cultivars. Particularly, significant differences in SG yield components are reported for the cultivars (Huber and Wehner 2021). The content of SVglys varies greatly depending on cultivar (Nakamura and Tamura 1985; Tateo *et al*. 1998).

Sandponics is a promising new tool for the production of pharmaceutically relevant plants, as well as an optimal growing system for the production of high-quality plant biomass. Thus, the objectives of this study were to investigate the effects of three sand substrates on growth, leaf yield and steviol glycosides concentration of two stevia cultivars (cv. Sugar High-A3 and cv. Morita) under controlled conditions.

Materials and Methods

Location

The trial was conducted on a private plantation in El-Obour City's Orabi association (30° 13′, 59"N, 31° 32 31"E), Egypt, during two successive seasons (2020 and 2021), using a closed sandponics system inside a translucent polycarbonate greenhouse and 75% sunlight. The average temperatures of day and night were 28 and 23°C, respectively, with a relative humidity of 65%.

Sandponcis cultivation structure

Three cultivation troughs were built on the ground uses galvanized steel with a height of 60 cm and width of 2 m. The planting troughs were lowered with a 2% slope to the nutrient solution recovery tank. The inner wall of the trough was covered with plastic film (1000 μm) as a waterproof layer. The bottom section of the cultivation trough was V-shaped, and the drainage tubing was placed in the center of the V-shape, which has the roles of ventilation and nutrient solution recycling. The pipe body was separated by 20 cm with holes. The pipe body was covered with gauze to prevent sand from clogging the drainage pipe hole. The first trough was filled with silica, the second trough with sand, and the third trough was filled with a 1:1 blend of the two types. Fresh water was used to rinse the three troughs to remove excess salinity and fine sand.

Plant growth conditions and experimental design

Seeds of the two-stevia cultivars, cv. Sugar High-A3 and cv. Morita (obtained from the Institute of Sugar Crops Research, Giza, Egypt), were planted in a potting medium comprising a mixture of peat moss, vermiculite, and perlite (1:1:1 v/v) in 1st February of the two seasons. One month after germination, at the 6–8 leaf stage, with plant height ranging from 6 to 8 cm, seedlings were transplanted into sand cultivation troughs connected to a 10,000 L tank containing nutrient solution (1 m*M* $Ca(NO_3)_2$; 1 m*M* KNO_3; 1 m*M* $(NH_4)2HPO_4$; 1 m*M* $NH_4H_2PO_4$; 0.02 m*M* Fe-EDTA; 1 m*M* $MgSO_4$; 0.05 m*M* KCl; 0.025 m*M* H_3BO_3; 0.002 m*M* $ZnSO_4$; 0.002 m*M* $MnSO_4$; 0.0005 m*M* MoO_3; 0.0005 m*M* $CuSO_4$) (modified after Epstein 1972).

Plant-to-plant and row-to-row spacing were both maintained at 15 cm and 25 cm, respectively and the trial density was 26.66 plants / m^2. A timer was used to irrigate the plants every two days for one hour (until the surface of the sand was immersed in water). To avoid nutrient depletion, the nutrient solution was constantly modified. The experiment was laid out according to randomized complete block design with split plot arrangements. Stevia cultivars were kept in main plot, whereas sand substrates were randomized in sub-plots. Each treatment had three replications and 20 plants were used per replication. The data collected during both seasons was analyzed and presented.

Data collection

In each season, after three and a half months from transplanting the seedlings, three cuts were taken in June then September then December (June 30th, September 15th and December 15th for the first season and June 20th, September 10th and December 15th for the second season, respectively) by cutting the vegetative parts of all plants 10 cm above the soil surface. The number of branches per plant was counted, and the fresh and dry weights of each plant's leaves were recorded (10 plants/replicate).

In the second season, two youngest fully developed leaves from each plant of each treatment were removed for the measurement of leaf steviol glycoside concentration (*i.e.*, amount of SG per unit dry weight of leaf, expressed as %). Stevia leaves were combined and oven-dried for 48 h at 60°C before being ground to a fine powder using a tiny bead-beater and stored in airtight containers. Stevioside and Reb-A concentrations of the samples were analysed with HPLC using a modification of the procedure described by Hearn and Subedi (2009).

Statistical analysis

The data for both the seasons was averaged to determine the effects of three sand substrates on growth and leaf yield of two stevia cultivars (cv. Sugar High-A3 and cv. Morita). Data were analyzed using the statistical package for ANOVA (analysis of variance) through Genstat version 11.1. Difference between means is reported as significant at $P \leq 0.05$. All statistical analyses were performed using SPSS v. 20.0.

Results

Plant height

Averaged over sandponics substrates, there were significant differences among stevia cultivars in plant height at all harvests (Table 1). Sugar High-A3 (CV_1) had the tallest plant at all harvests in both seasons. Plant height for all the harvests had significant differences between sandponics substrates; for the first harvest, sand (S_3) and Silica + Sand (S_2) had greater plant height than Silica (S_1) in both seasons. For the second and third harvests, maximal plant height was observed with Silica (S_1) sandponics substrate, while minimal plant height resulted from sand (S_3) sandponics substrate in both seasons.

For the second and third harvests, the interaction between cultivars and sandponics substrates treatments reveals that the highest plant height value was recorded with Morita (CV_2) planted in silica substrate, while the lowest plant height value was recorded when the Sugar High-A3 (CV_1) planted in sand substrate in both seasons. On the contrary, for the first harvest, the combination of Morita and sand substrate treatment gave the highest positive effect on plant height in both seasons.

Number of branches per plant

There were differences among cultivars in the number of branches per plant, except the first harvest (Table 2). At second and third harvests, cultivar Morita (CV_2) produced the highest number of branches per plant in both seasons compared with Sugar High-A3. Except the first harvest, the effect of sandponic substrates treatments on the number of branches per plant was significant in both seasons. The highest number of branches per plant was obtained when stevia planted in the sand (S_3) and Silica + Sand (S_2) substrates at second and third harvests, respectively. The lowest values in the same regard were noticed by planted both cultivars in Silica (S_1) substrate at first and second harvests. The number of branches per plant demonstrated a significant interaction between sandponic substrates and cultivars. More number of branches of the second cultivar with Silica + Sand (S_2) substrate at third harvest and a decrease in the number of branches of first cultivar with Silica (S_1) substrate at first harvest in both seasons (Table 2).

Leaf fresh weight

The leaf fresh weight was significantly higher in cultivar Morita (CV_2) and significantly lower in cultivar Sugar High-A3 (CV_1). When leaf fresh weight was averaged over cultivars, there were significant differences between sandponics substrates at all harvests. For the first and second harvests, sand (S_3) substrate had significantly higher leaf fresh weight than all other substrates. For the third harvest, Silica + Sand (S_2) substrate had significantly higher leaf fresh weight than all other substrates (Table 3).

At all harvests, there were significant interactions of sandponics substrates by cultivars on leaf fresh weight. At the second harvest, second cultivar (Morita) grown in Silica + Sand (S_2) substrate had the highest leaf yields (int the first season leaf fresh weight recorded 1503.00 and in the second season was 1611.00 g/m^2, respectively); whereas first cultivar (Sugar High-A3) grown in Silica (S_1) substrate at

Table 1: Effect of stevia cultivars and sandponics substrates on plant height, during 2020 and 2021 seasons

Treatments	Plant height (cm)											
	First harvest				Second harvest				Third harvest			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
						Season 2020						
Sugar High-A3	55.00 f	59.44 f	66.44 g	60.29 a	70.00 ef	72.33 f	68.80 e	70.38 a	88.80 f	84.13 e	82.47 e	85.13 a
Morita	69.67 g	74.89 g	85.56 h	76.71 b	88.00 h	86.00 h	77.93 g	83.98 b	107.00 h	92.33 g	91.40 fg	96.91 b
Mean	62.34 c	67.17 d	76 e		79 d	79.17 d	73.37 c		97.9 d	88.23 c	86.94 c	
L.S.D at 0.05	CV = 3.81; S = 4.66; CV × S = 6.59				CV = 1.93; S = 2.36; CV × S = 3.34				CV = 1.76; S = 2.15; CV × S = 3.05			
						Season 2021						
Sugar High-A3	55.67 f	58.80 f	67.40 g	60.62 a	72.78 e	69.00 e	68.22 e	60.62 a	88.44 gh	86.33 g	79.78 f	84.85 a
Morita	69.27 gh	75.07 h	87.13 i	77.16 b	87.11 g	86.67 g	80.22 f	77.16 b	107.11j	94.56 i	93.44 hi	98.37 b
Mean	62.47 c	66.94 d	77.27 e		79.95 d	77.84 cd	74.22 c		97.78 e	90.45 d	86.61 c	
L.S.D at 0.05	CV = 3.47; S = 4.25; CV × S = 6.01				CV = 3.65; S = 4.47; CV × S = 6.31				CV = 3.06; S = 3.75; CV × S = 5.30			

Means with the same letter are not significantly different at 5% level of probability **S_1** = Silica, **S_2** = Silica + Sand and **S_3** = Sand

Table 2: Effect of stevia cultivars and sandponics substrates on number of branches per plant, during 2020 and 2021 seasons

Treatments	Number of branches per plant											
	First harvest				Second harvest				Third harvest			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
						Season 2020						
Sugar High-A3	5.20 e	5.40 e	6.67 g	5.76 a	12.20 f	10.67 f	16.33 g	13.07 a	15.47 f	20.67 g	19.87 g	18.67 a
Morita	5.87 ef	5.93 f	6.00 fg	5.93 a	12.13 f	17.60 g	19.60 h	16.44 b	19.80 g	22.93 h	20.13 g	20.95 b
Mean	5.54 c	5.67 c	6.34 d		12.17 c	14.14 d	17.97 e		17.64 c	21.80 e	20.00 d	
L.S.D at 0.05	CV = N.S.; S = 0.51; CV × S = 0.72				CV = 1.06; S = 1.29; CV × S = 1.82				CV = 0.51; S = 0.62; CV × S = 0.88			
						Season 2021						
Sugar High-A3	5.22 c	5.78 cd	6.56 d	5.85 a	12.33 f	9.11 e	16.67 g	12.70 a	17.78 f	21.89 h	19.67 g	19.78 a
Morita	6.22 cd	6.44 cd	5.78 cd	6.15 a	12.44 f	16.56 g	19.78 h	16.26 b	18.00 f	22.11 h	21.44 h	20.52 b
Mean	5.72 b	6.11 b	6.17 b		12.39 c	12.84 c	18.23 d		17.89 c	22.00 e	20.56 d	
L.S.D at 0.05	CV = N.S.; S = N.S; CV × S = 1.31				CV = 1.16.; S =1.41; CV × S = 2.00				CV = 0.67.; S = 0.82; CV × S = 1.15			

Means with the same letter are not significantly different at 5% level of probability **S_1** = Silica, **S_2** = Silica + Sand and **S_3** = Sand

Table 3: Effect of stevia cultivars and sandponics substrates on leaf fresh weight m^{-2}, during 2020 and 2021 seasons

Treatments	Leaf fresh weight (g/m^{-2})											
	First harvest				Second harvest				Third harvest			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
						Season 2020						
Sugar High-A3	486.82 e	540.00 ef	711.00 f	579.27 a	1053.00 e	1152.00 e	1219.50 e	1141.50 a	999.00 e	918.00 e	949.50 e	955.50 a
Morita	702.00 fg	792.00 g	967.50 h	820.50 b	1303.50 f	1503.00 f	1470.75 f	1425.75 b	1218.50 f	1417.50 g	954.00 e	1196.67 b
Mean	594.41 c	666.00 c	839.25 d		1178.25 c	1327.50 d	1345.13 d		1108.75 d	1167.75 d	951.75 c	
L.S.D at 0.05	CV = 109.19; S = 133.73; CV × S = 189.12				CV = 129.27; S = 158.32; CV × S = 223.9				CV = 76.05; S = 93.14; CV × S = 131.72			
						Season 2021						
Sugar High-A3	535.50 f	603.00 f	891.00 g	676.50 a	1255.50 d	1314.00 d	1359.00 d	1309.50 a	1155.50 e	1026.00 e	1039.50 e	1073.67 a
Morita	783.00 g	970.75 h	1053.00 h	935.58 b	1476.00 e	1611.00 e	1521.00 e	1536.00 b	1278.00 f	1539.00 g	1071.00 e	1296.00 b
Mean	659.25 c	786.88 d	972.00 e		1365.75 c	1462.50 c	1440.00 c		1216.75 d	1282.50 d	1055.25 c	
L.S.D at 0.05	CV = 75.89; S = 92.95; CV × S = 131.45				CV = 112.33; S = N.S.; CV × S = 194.56				CV = 107.67; S = 131.53; CV × S = 186.02			

Means with the same letter are not significantly different at 5% level of probability **S_1**= Silica, **S_2** = Silica + Sand and **S_3** = Sand

the first harvest had the lowest leaf yields in both seasons (int the first season leaf fresh weight recorded 486.82 and in the second season was 535.50 g/m^2, respectively).

Leaf dry weight

Averaged over sandponics substrates, there were significant differences among stevia cultivars in leaf dry weight, sandponics substrates and interaction among them at all harvests (Table 4). Morita (CV_2) showed a higher leaf dry weight as compared to Sugar High-A3 (CV_1) at all harvests in both seasons. For the first harvest, sand (S_3) substrate had greater leaf dry weight than Silica (S_1) substrate in both seasons. For the second and third harvests, maximal leaf dry weight was observed with Silica (S_1) sandponics substrate (in the first season recorded 387.00 and 366.20, while in the second season recorded 423.00 and 401.10 g/m^2, second and third harvests, respectively), while minimal leaf dry weight resulted from sand (S3) sandponics substrate in both seasons (243.88 and 254.70 g/m^2, third harvest in the first and the second seasons, respectively). The leaf dry weight demonstrated a significant interaction between sandponic substrates and cultivars. The most interesting result was an increase in the leaf dry weight of Morita with Silica (S_1) substrate at second and third harvests and a decrease in the leaf dry weight of Sugar High-A3 with Silica + Sand (S_2) substrate at first harvest in both seasons (Table 4).

Stevioside and rebaudoside A content

Table 5 and Fig. 1, 2 and 3 showed that for all harvests, the content of the Morita of Stevioside was higher than the

Table 4: Effect of stevia cultivars and sandponics substrates on leaf dry weight m^{-2}, during 2020 and 2021 seasons

Treatments	Leaf dry weight (g/m^{-2})											
	First harvest				Second harvest				Third harvest			
	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean	S_1	S_2	S_3	Mean
Season 2020												
Sugar High-A3	157.36 e	153.00 e	211.50 e	173.95 a	373.50 ij	319.50 gh	283.50 fg	325.50 a	305.55 hi	275.85 gh	258.30 fg	279.90 a
Morita	175.50 e	207.00 e	252.00 f	211.50 b	400.50 j	346.50 hi	252.00 f	333.00 a	426.85 j	339.75 i	229.45 f	332.02 b
Mean	166.43 c	180.00 c	231.75 d		387.00 e	333.00 d	267.75 c		366.20 e	307.80 d	243.88 c	
L.S.D at 0.05	CV = 35.45; S = 43.42; CV × S = 61.4				CV = N.S.; S = 37.25; CV × S = 52.69				CV = 26.65; S = 32.64; CV × S = 46.16			
Season 2021												
Sugar High-A3	180.00 e	162.00 e	234.00 f	192.00 a	405.00 gh	373.50 fg	328.50 f	369.00 a	304.95 g	310.95 gh	274.95 fg	296.95 a
Morita	229.50 f	229.50 f	270.00 f	243.00 b	441.00 h	387.00 gh	315.75 f	381.25 a	497.25 i	372.15 h	234.45 f	367.95 b
Mean	204.75 c	195.75 c	252.00 d		423.00 e	380.25 d	322.13 c		401.10 e	341.55 d	254.70 c	
L.S.D at 0.05	CV = 23.79; S = 29.14; CV × S = 41.21				CV = N.S.; S = 42.01; CV × S = 59.41				CV = 37.06; S = 45.38; CV × S = 64.18			

Means with the same letter are not significantly different at 5% level of probability **S_1**= Silica, **S_2** = Silica + Sand and **S_3** = Sand

Table 5: Effect of stevia cultivars and sandponics substrates on Stevioside and Rebaudoside A concentration, during 2021 season

Treatments	Sugar High-A3				Morita			
	Stevioside (%)	Rebaudoside A (%)	Total Svglys (% of leaf dry matter)	Reb A/Stev Ratio	Stevioside (%)	Rebaudoside A (%)	Total Svglys (% of leaf dry matter)	Reb A/Stev ratio
First harvest								
Sand	6.72	4.32	11.04	0.64	14.31	2.57	16.88	0.18
Silika	7.12	3.65	10.77	0.51	7.87	2.27	10.14	0.29
Sand+ Silika	9.93	3.73	13.66	0.38	14.27	2.23	16.50	0.16
Second harvest								
Sand	4.40	3.21	7.61	0.73	9.63	1.93	11.56	0.20
Silika	10.78	3.02	13.80	0.28	11.12	2.06	13.18	0.19
Sand+ Silika	9.75	2.46	12.21	0.25	11.57	2.21	13.78	0.19
Third harvest								
Sand	3.09	1.87	4.96	0.61	5.77	1.00	6.77	0.17
Silika	5.74	1.2	6.94	0.21	6.80	1.65	8.45	0.24
Sand+ Silika	4.45	1.30	5.75	0.29	5.27	0.98	6.25	0.19

(SVglys : Steviol glycosides = Stevioside +: RebaudiosideA; Stev : Stevioside ; Reb A: RebaudiosideA)

content of the Sugar High-A3 in all cultivation substrates, whereas the content of the Sugar High-A3 of Rebaudoside was higher than the content of the Morita in all cultivation substrates. The concentration of Rebaudoside was higher with sand substrate at all harvests in the first cultivar (Sugar High-A3) while, in the first harvest, Stevioside had the highest concentration (9.93%) with (sand + silica) substrate. Moreover, Stevioside had the highest concentrations (10.78 and 5.74%) with silica substrate in the second and third harvests, respectively.

It is clear that there was harmony between the two compounds in the second cultivar (Morita) as they recorded the same trends. In the first harvest, the highest content of Stevioside and Rebaudoside was recorded with sand substrate (14.31 and 2.57%, Stevioside and Rebaudoside, respectively), while in the second harvest, the highest content of the two compounds was recorded with sand and silica substrate (11.57 and 2.21%, Stevioside and Rebaudoside, respectively), and in the third harvest, the highest content of the two compounds was with silica substrate (6.80 and 1.65%, Stevioside and Rebaudoside, respectively). For the first cultivar (Sugar High-A3), the ratio between (Reb A/Stev ratio) was higher in the three harvests with sand (0.64, 0.73 and 0.61%, in the first, second and third harvests, respectively), while with second cultivar (Morita), the highest ratio was achieved with silica in the first and third harvests, and with sand in the second harvest.

Discussion

In this study, there were significant differences among cultivars for plant height, number of branches per plant, leaf yield and glycoside concentration, which can be used to identify superior varieties. The considerable differences in performance between stevia cultivars in this investigation were consistent with previously documented phenotypic variability for plant height and number of branch (Abdullateef and Osman 2011; Othman *et al.* 2015), as well as varinces in yield and steviol glycosides content between accessions and cultivars (Barbet-Massin *et al.* 2016; Parris *et al.* 2016; Hastoy *et al.* 2019). Cultivars with higher yield and reb A concentration have been developed all over the world (Tan *et al.* 2008; Yadav *et al.* 2011; Parris *et al.* 2016). One possible reason for the increased yields is the use of optimized clonal cultivars, which provide a uniform genetic background for the expression of yield traits (Parris *et al*. 2016). Among the steviol glycosides, stevioside is the most frequent (Moraes *et al.* 2013; Vasilakoglou *et al.* 2016). Parris *et al.* (2016) found that when superior cultivars are utilized, reb A concentrations are higher.

In this study, growth, leaf yield and SG content were severely affected by sandponics substrates in both cultivars of Stevia rebaudiana, *viz*., Sugar High-A3 and Morita. Leaf biomass production of stevia, a key factor in yield variability, can vary depending on environmental conditions

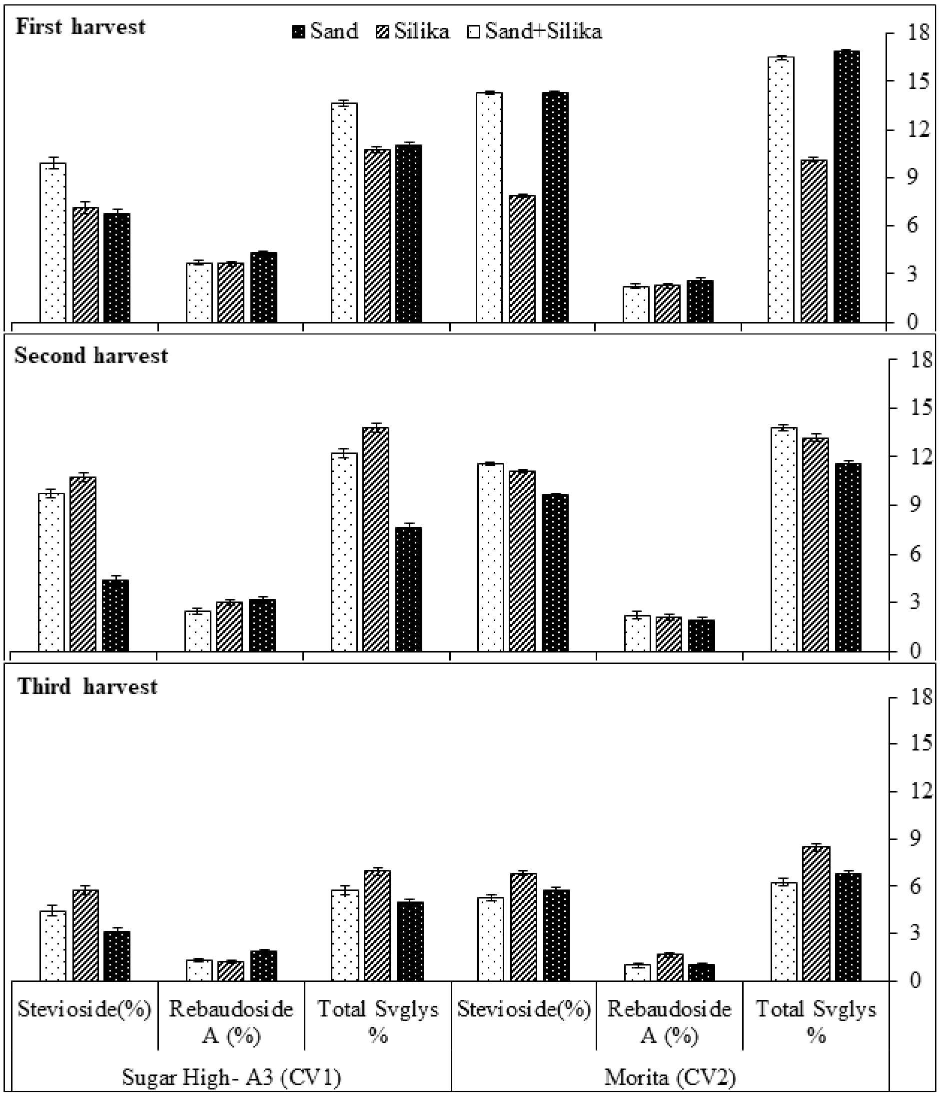

Fig. 1: Effect of stevia cultivars and sandponics substrates on Stevioside, Rebaudoside A and Svglys concentrations at three harvests. Each value is the mean ± S.E

such as cropping system, climate, genetic diversity, production years, and interactions with environmental factors. The interaction of genotypes with environmental factors influences total leaf SG content (Montoro *et al*. 2013; Barbet-Massin *et al*. 2015). Response to nutrients and availability of water is one of the many environmental factors that can influence stevia efficiency (Lavini *et al*. 2008; Angelini *et al*. 2018).

Hydroponic cultivation system was used by Gontier *et al*. (2002), Manukyan (2005) and Xego *et al.* (2017) to grow aromatic and therapeutic plants. According to the findings of these studies, hydroponic cultivation may be a viable option for growing aromatic and therapeutic plants. Hydroponic farming techniques may offer an ideal growth conditions for producing high-quality biomass while controlling secondary metabolism via nutrient solution management (Bolonhezi *et al.* 2010).

According to Tramp *et al.* (2009), water hold capacity allows plants to grow in media. Plant growth is generally stunted when nutrients are deficient, whether due to insufficient quantity or pH-conditioned non-availability in the growing medium, or to insufficient water for uptake. Plant growth and development are generally limited by the availability of water. The variation in particle sizes between the substrates can explain differences in water holding capacity capability. Kukal *et al.* (2012) who investigated the water retention characteristics of growing media discovered that differences in water holding capability among the media could be attributed to differences in total porosity and pore size distribution. When natural soils have been used as a substrate, saturated conditions persist for extended periods of time after irrigation has stopped, whereas this is not the case with a coarse substrate. These saturated conditions may restrict the root system's supply of oxygen. As a result, in thin layer cropping systems, coarse substrates are required (Heinen and de Willigen 1995).

Stevia is cultivated for SVgly extraction, the main source of SVglys is the leaf (Shahverdi *et al*. 2020). The sand substrate treatment increased the relative amount of RA, which is up to 400 times sweeter than sucrose and roughly twice as sweet as ST. These findings imply that cultivating stevia in a sand substrate has the potential to increase RA yield. According to Karimi *et al*. (2019), SVglys biosynthesis is comprised of a complex metabolic pathway, and it is unclear which stage of this pathway is affected by water holding capcity.

In general, the first and second harvests have higher content of the two compounds than the third harvest. This could be due to the difference in day length and plant flowering during this period. These results are consistent with previous findings, which indicated that S. rebaudiana is also highly sensitive to photoperiod variations. A short photoperiod of 12 h of light results in early flowering (Metivier and Viana 1979). A long-day photoperiod of 16 h of light, on the other hand, increases the SG content in leaves by up to 30%, as it contributes to the extension of vegetative growth and increases biomass yield (Ceunen and Geuns 2013).

Conclusions

The cultivation technique of sandponics is consistent with Egypt's national situation and the global development of soilless culture. It is an ecological and environmentally friendly soilless cultivation method that is easy to use, can be produced by non-professionals, and is inexpensive. As a result, sandponics is a promising new tool for the production of pharmaceutically relevant plants, as well as an optimal growing system for the production of high-quality plant biomass. Differences in water holding capacity capability can be explained by differences in particle sizes between substrates. There were significant differences in plant height, number of branches per plant, leaf yield, and glycoside concentration among cultivars in this study, which can be used to identify superior varieties. The cv. Morita showed a clear superiority in the growth and leaf yield traits as compared to the cv. Sugar High-A3.

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Author Contributions

MAA and MYMB planned the experiments, SMB and WHA interpreted the results, MAA, MYMB and SMB made the write up and WHA statistically analyzed the data and made illustrations.

Conflicts of Interest

All authors declare no conflicts of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable in this paper.

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