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POLYPOLIDY AND SWEETENER INDUCTION IN DIFFERENT STEVIA VARIETIES USING COLCHICINE

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ABSTRACT

The aim of the present investigation was to improve steviol glycosides (SGs) of three stevia varieties; Sponti (SPNT), China1 (CHN) and EGY1 (EGY) by obtaining new genotypes with high ratio of rebaudioside A (rebA)/stevioside (stv) using five different doses of colchicine(0, 0.025, 0.05, 0.1 and 0.2%), during 2018 and 2019 seasons at El-Sabahya Research Station, Alexandria Governorate. Plants with a significant large leaf area/plant and significantly high number of leaves/plant, as well as dry matter content from the three commercial varieties, were selected and evaluated for their steviol glycosides content by using high performance liquid chromatography (HPLC). Results of buds survival rate, showed that SPNT and EGY varieties performed better than CHN. SPNT-22, CHN-31, EGY-22, EGY-23and EGY-31 mutated clones showed a significant superiority for most desirable morphological characters (leaf area/plant, number of leaves/plant as well as dry matter content)compared to other tested genotypes. HPLC showed that the sweetener contents stv and reb A percentages were affected by polyploidy induced by colchicine. Best ratio of reb A/stv was recorded by CHN-31 (3.846) which was 4.2 times higher than CHN (0.919). CHN-31, EGY-22 and EGY-31 clones recorded the highest reb A + stv percentage (77.25, 78.33 and 76.18, respectively). For EGY-31(a new clone), rebA/stv percentage was 2.6 higher compared to its commercial variety, this, consequently, decreases the bitter taste and aftertaste and increases sweet flavor. New genotypes (CHN-31and EGY-31) were obtained and selected as the best clones due to the higher sweeteners percentages recorded, which might help in meeting diabetics needs in the future.

Key words: ***Stevia, Stevia rebaudiana, Colchicine, Polyploidy, Stevioside, Steviol glycosides, HPLC, RebA, Diabetics.***

INTRODUCTION

Stevia rebaudiana (Bertoni) is a perennial herb (2n=22), belongs to family *Asteraceae*, recognized as sugary leaf or sweetie leaf (Lemus-Mondaca *et al* 2012).It is originated from South America northern regions, and distributed along Amambay high lands between Brazil and Paraguay((Madan *et al* 2010).Among 407 species, *S. rebaudiana* Bertoni is a calorie-free, natural sweetener plant, which became an inevitable alternative to sugar especially, with over 346 million diabetic populations across the world (WHO 2011).Stevia leaves produce non-nutritive, non-harmful glycosides with high level of sweeteners, which can be used as a sugar supplement (Mahdi *et al* 2018), but aftertaste of these steviol glycoside (SGs) seem like bitter, metallic, or licorice (Prakash *et al* 2012 and Vouillamoz *et al* 2016). The elimination of the aftertaste in SGs is a key challenge for industrialists (De Roode *et al* 2015). Users and bio-industry need superior quality, higher reb A content, and safe products (Tavarani *et al* 2015), such varieties can be bred specifically for improving quality by

increasing reb A amount, which does not have an aftertaste. Stevia leaves usually contain 0.5 or less of reb A/stv ratio (Yadav *et al* 2011).

S. rebaudiana has a wide range of pharmaceutical values and uses (Mahdi *et al* 2018). Its main medical role is due to the presence of glycosides in its leaves, which includes stevioside, rebaudioside A, C, D, and dulcoside A, with high sweetening potency (nearly 300–400 times sweeter than the daily used sucrose sugar) (Chaturvedula *et al* 2011). Rebaudioside-A is the superior among them due to its taste profile, with no liquorice or metallic after taste and it is slightly sweeter than them all (Brandle and Telmer, 2007 and Blinstrubienė *et al* 2020). U.S. Food & Drug Administration (2018) has approved diterpene glycosides produced by stevia as a dietetic supplement, and accordingly, it was highly recommended for diabetic patients (Lee *et al* 2001 and Luwańska *et al* 2015). In 2015, WHO recommended the reduction of free sugars consumption to 5-10% of total energy intake for both grownups and children.

Plant breeders showed high interest in polyploidy induction, and considered it a valuable tool to enhance agronomic traits along with essential secondary products quality and quantity of pharmaceutical plants (Ahmadi *et al* 2013).Polyploidy plants are produced in nature as the result of some cytological mechanisms or induced artificially by using mutagenic agents such as colchicine and trifluralin (Manzoor *et al* 2019). Colchicine ($C_{22}H_{25}NO_6$ – MW: 399.44) is a natural product extracted from the *Colchicum autumnale* L which acts as a mutagenic agent and used to enhance polyploidy (Dhooghe *et al* 2011 and Stanys *et al* 2004). The principle role of colchicine is to inhibit the formation of microtubules to enhance polyploidy cells (Otto 2007 and Petersen *et al*2003), which will make some differences at the morphological, physiological, and cytological level, sometimes at the gene expression level (Vainola 2000) that increases the number of chromosomes, which in return might lead to increased level

of the secondary metabolites contents. Mutation breeding approaches are used nowadays, to improve yield productivity and secondary metabolites in medical plants (Talei *et al* 2020).

The aim of this study was to obtain new stevia genotypes with high sweetener profile (reb A/stv ratio) to expand sweetener supplementation for diabetes patients.

MATERIALS AND METHODS

This study was a collaboration between Sugar Crops Research Institute (SCRI) and the Horticulture Research institute (HRI).Three varieties of stevia, namely, Sponti (SPNT) (MH087465), China1 (CHN) (MH087464) and Egy1 (EGY) (MH087463),were supplied by Sugar Crops Research Institute (SCRI) – Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Egypt. Plantlets were grown in pots (25 cm) in the green house of El-Sabahya Research Station, Alexandria Governorate, Egypt – located at 55.55 North, 26.36 East, -6 m under sea level. Stem nodes of stevia were planted in pots containing peat moss, clay and sand (1:1:1) during 2018 and 2019 growing seasons.

Colchicine was used as a polyploidy mutagenic agent, supplied by Sigma-Aldrich Chemie (GmbH Export Department Eschenstr. 5, 82024 Taufkirchen, Germany).Forty five days old plants were treated with colchicine. Five colchicine doses were tested in this study, viz. 0, 0.025, 0.05, 0.1 and 0.2 % w/v. This was done by soaking cotton pads on the colchicine solution and places them on the axillary buds (Mahdi, 2012).Treatments were performed for 3 successive days by dropping the colchicine solution on the cotton pads every morning. Each treatment was replicated 5 times. After one week, untreated buds were removed and 8 weeks later flowering buds were removed too. After 24 weeks of treatment, each branch from each bud was cut and replanted again as a single plant. Twenty weeks later, healthy plants with morphological differences from

control (zero dose) were selected for their morphological characters evaluation. Morphological measurements were: plant height (cm), leaf length (cm), leaf width (cm), leaf area (cm^2)/plant by disk method (Bremner and Taha, 1960), number of leaves/plant and number of branches/plant. Also dry matter content was evaluated using Fisher Scientific oven (model 750G Isotemp, serial 40400055, USA)at 45$^oC$for 4 days.

Superior clones in leaf area/plant and number of leaves/plant as well as dry matter content percentages were selected again for HPLC analysis along with the commercial varieties just before flowering stage. Preparation of samples was performed as described by Ibrahim (2015) using Fisher Scientific oven, model 750G Isotemp, serial 40400055, USA, at 45^oC.Stevioside extraction preparation was modified from Kolb *et al*. (2001) and Abou Arab *et al* (2010): 1g of each dried sample was grinded and extracted by 100ml HPLC grad methanol (95%) by shaking in water bath (70^oC) for 30 min. Extracts were left on the bench to reach room temperature then filtrated twice through filter paper along with activated charcoal, to get rid of pigmentation, for HPLC preparation (Nishiyama *et al* 1992). Stevioside HPLC was performed at the Central Laboratory of Faculty of Science, Alexandria University. Stevia leaves extract was separated and identified on HPLC (Agilent 880952-708) using a column Zorbax NH_2 70 A (5um, 4.6x250 mm), with a linear gradient of 84 to 55% CH_3CN in H_2O (pH = 5, H_3PO_4), over 15 min at a flow rate of 2.0 ml/min), by 210 nm as explained by Ibrahium (2015). Steviol glycosides were identified and quantified by comparing their retention times to a well-known previously injected standard as mentioned by Massoud *et al* (2002).

Statistical analysis, for the collected data of the three commercial varieties (SPNT-CHN-EGY) and the new clones was performed using CoStat software (version 6.400) design as One-Way Randomized Complete Blocks with three replicates. Comparisons among tested genotypes were

carried out using Duncan's multiple range tests at 0.05 significance level of probability, as recommended by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

Polyploidy of three stevia varieties was carried out during 2018 and 2019 seasons using five colchicine doses (0, 0.025, 0.05, 0.1 and 0.2%). Results of buds survival rate, after two weeks of treatment, were illustrated in Figure (1). Data showed that SPNT has the best bud survival percentage towards colchicine followed by EGY. Survival rate of SPNT ranged from 100% (control) to 26.67% (0.2% colchicine treatment).

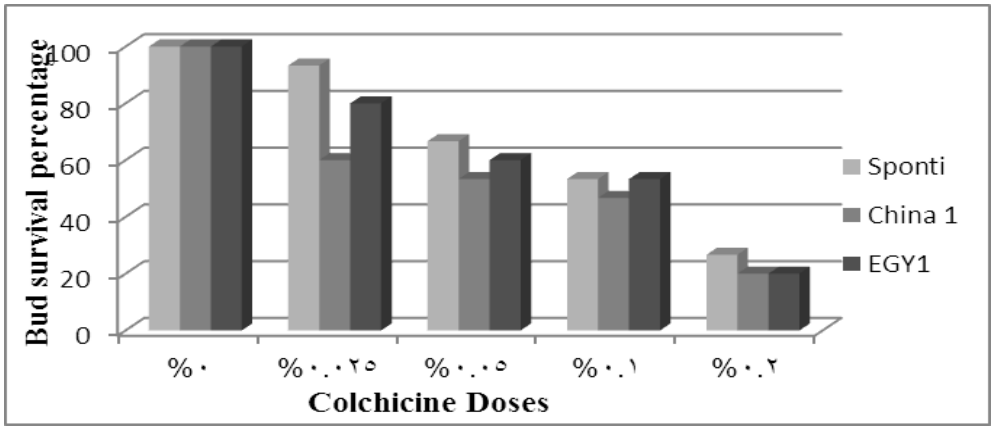

Fig. 1. Bud survival rate of stevia varieties; after 2 weeks of treatment with five different colchicine doses.

Data presented in Table (1) clearly showed that there were significant differences among the tested genotypes regarding the studied traits, such as plant height (cm), leaf length (cm), leaf width (cm), leaf area (cm^2), number of leaves, number of branches and dry matter content (%).

Table 1. Mean performances of the studied vegetative characters for stevia varieties and their mutated clones.

Genotype	Code	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area/ plant (cm^2)	Number of leaves/ plant	Number of branches/ plant	Dry matter content (%)
SPNT	SPNT	89.67	5.10	2.39	6.03	458.00	12.67	18.01
SPNT-Col 0.025%-1	SPNT-11	96.33	4.39	1.30	8.52	240.33	11.67	12.52
SPNT-Col 0.025%-2	SPNT-12	98.00	5.00	1.93	8.71	355.33	11.00	19.60
SPNT-Col 0.025%-3	SPNT-13	75.00	4.47	1.70	8.61	237.00	11.67	21.19
SPNT-Col 0.025%-4	SPNT-14	89.00	4.90	1.87	8.33	244.33	11.67	18.75
SPNT-Col 0.05%-1	SPNT-21	84.00	4.10	1.48	8.53	275.33	13.33	23.94
SPNT-Col 0.05%-2	SPNT-22	134.33	6.23	2.73	9.96	711.67	21.33	23.97
SPNT-Col 0.05%-3	SPNT-23	70.00	5.60	2.27	8.62	298.33	15.33	21.25
SPNT-Col 0.05%-4	SPNT-24	86.33	4.60	1.60	8.34	298.33	14.00	20.24
SPNT-Col 0.1%-1	SPNT-31	94.17	4.32	1.54	8.72	296.33	9.00	24.62
SPNT-Col 0.1%-2	SPNT-32	96.00	4.40	1.70	9.34	296.67	10.33	22.56
SPNT-Col 0.1%-3	SPNT-33	73.00	4.87	2.13	9.24	289.33	9.67	19.69
SPNT-Col 0.1%-4	SPNT-34	96.33	4.87	1.87	8.96	309.33	11.00	21.28
SPNT-Col 0.2%-1	SPNT-41	98.83	4.48	1.52	4.75	519.00	14.33	21.77
SPNT-Col 0.2%-2	SPNT-42	93.00	5.03	2.17	4.94	519.33	13.33	14.15
SPNT-Col 0.2%-3	SPNT-43	108.33	5.03	1.94	4.84	542.00	15.00	19.85
SPNT-Col 0.2%-4	SPNT-44	93.33	4.80	1.97	4.56	528.67	14.00	10.13
CHN	CHN	87.75	5.79	1.83	8.05	355.17	9.50	21.11
CHN -Col 0.025%-1	CHN-11	103.00	6.41	2.63	9.60	685.33	20.67	20.78
CHN -Col 0.025%-2	CHN-12	90.33	4.93	1.93	8.18	613.00	18.67	21.34
CHN -Col 0.025%-3	CHN-13	74.67	3.87	1.53	8.08	289.00	14.67	9.51
CHN -Col 0.025%-4	CHN-14	101.33	5.90	2.27	7.80	335.33	19.33	15.11
CHN -Col 0.05%-1	CHN-21	113.67	6.34	2.47	7.12	463.67	11.33	19.54
CHN -Col 0.05%-2	CHN-22	72.00	5.63	2.47	7.31	477.33	12.67	21.30
CHN -Col 0.05%-3	CHN-23	75.00	4.50	2.60	7.21	490.00	12.00	21.46
CHN -Col 0.05%-4	CHN-24	79.33	5.00	1.93	6.93	480.00	13.33	21.17

Table 1. Cont.

Genotype	Code	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area/ plant (cm^2)	Number of leaves/ plant	Number of branches/ plant	Dry matter content (%)
SPNT	SPNT	89.67	5.10	2.39	6.03	458.00	12.67	18.01
CHN -Col 0.1%-1	CHN-31	121.67	7.80	2.89	10.06	825.67	15.00	22.71
CHN -Col 0.1%-2	CHN-32	79.00	5.23	2.83	7.67	570.67	15.33	18.57
CHN -Col 0.1%-3	CHN-33	78.67	4.27	1.60	7.57	573.33	15.67	19.25
CHN -Col 0.1%-4	CHN-34	70.00	4.40	1.90	7.29	593.33	12.00	23.53
CHN -Col 0.2%-1	CHN-41	84.33	5.61	2.62	8.86	682.67	14.67	19.73
CHN -Col 0.2%-2	CHN-42	55.67	5.73	2.57	9.05	689.67	13.00	17.59
CHN -Col 0.2%-3	CHN-43	77.33	5.60	2.33	8.95	342.33	15.33	20.38
CHN -Col 0.2%- 4	CHN-44	87.67	4.90	1.63	8.67	695.67	13.67	20.51
EGY	EGY	89.33	5.66	1.97	7.99	220.17	5.50	21.98
EGY -Col 0.025%-1	EGY-11	60.00	5.66	2.01	4.49	248.67	5.00	23.42
EGY -Col 0.025%-2	EGY-12	123.00	5.43	1.98	4.68	217.00	3.67	22.55
EGY -Col 0.025%-3	EGY-13	107.33	5.58	2.88	10.06	325.00	4.00	19.26
EGY -Col 0.025%-4	EGY-14	81.33	5.48	2.15	8.07	249.67	4.67	18.77
EGY -Col 0.05%-1	EGY-21	61.83	4.96	1.67	9.01	349.00	9.67	22.51
EGY -Col 0.05%-2	EGY-22	91.33	4.45	1.80	9.20	458.00	13.00	24.41
EGY -Col 0.05%-3	EGY-23	112.00	6.03	2.40	9.10	765.00	16.00	24.63
EGY -Col 0.05%-4	EGY-24	69.00	4.18	1.45	4.58	224.00	4.67	10.62
EGY -Col 0.1%-1	EGY-31	76.33	4.58	1.60	9.41	448.67	5.00	26.40
EGY -Col 0.1%-2	EGY-32	91.33	4.98	1.90	7.48	429.00	5.00	22.35
EGY -Col 0.1%-3	EGY-33	63.00	4.53	1.93	9.50	435.00	5.67	20.42
EGY -Col 0.1%-4	EGY-34	71.00	4.50	1.90	9.22	455.00	5.67	21.89
EGY -Col 0.2%-1	EGY-41	43.00	3.21	1.45	4.30	177.67	3.33	9.73
EGY -Col 0.2%-2	EGY-42	94.33	5.33	2.23	8.26	211.00	5.33	17.58
EGY -Col 0.2%-3	EGY-43	95.33	5.83	1.98	9.67	231.00	5.00	22.50
EGY -Col 0.2%-4	EGY-44	88.00	4.90	1.68	7.88	355.33	6.00	19.34
$LSD_{(p\leq 0.05)}$		11.9572	0.9227	0.3803	0.8103	53.9621	5.7274	2.3159

Concerning plant height (cm), there were significant differences among the tested genotypes. The clone SPNT-22 possessed the highest

value without significant differences with the clone EGY-12. As for leaf length trait, the recorded data showed that CHN-31 clone significantly gave the highest value (3.21 cm). Leaf width (cm) trait possessed significant differences among tested genotypes where clone CHN-31 significantly gave the highest value without significant differences with each of the following clones; SPNT-22, CHN-11, CHN-23 and CHN-42.

Wide variation of plant leaf length and width will lead eventually to variation in leaf shape which in return will lead to variation at the photosynthesis level and development progress. Leaf width (cm) trait possessed significant differences among tested genotypes where clone CHN-31 significantly gave the highest value without significant differences with each of the clones SPNT-22, CHN-11, CHN-23 and CHN-42.

Leaf area/plant (cm^2) trait showed that there were highly significant differences among the tested genotypes, where the recorded values ranged from 10.06 to 4.03 cm^2. In this respect, each of the two clones CHN-31 and EGY-13 gave the highest mean value for leaf area trait without significant differences with each of the clones SPNT-22, SPNT 32, CHN-11, EGY-31, EGY33 and EGY-43.

Number of leaves/plant trait was significantly affected ($p \leq 0.05$) by the genetic differences among the tested genotypes. In this regard, the data clearly showed that the clone CHN-significantly gave the highest mean value for number of leaves/plant.

Number of branches/plant showed a wide range of mean values. The scored values showed that the clone SPNT-22 gave the highest mean value without significant differences with each of the clones CHN-11, CHN-12, CHN-33 and EGY-23.

Dry matter content is a very important factor that determines the percentage of sweetener supplements within the clones under study. Data in Table (1) illustrated that EGY-31 clone gave the highest mean value for dry

matter content without significant differences with each of SPNT-31, EGY-22 and EGY-23 clones. Based on these obtained results, it is evident that the four previously mentioned clones are highly recommended for a high supply of sweetener substitution.

Generally, the obtained data of Table (1) showed that the clones CHN-31, SPNT-22, EGY-22, EGY-23 and EGY-31 gave high mean performances recommended for the most studied characters. Hereby, the authors declare that, colchicine was used as a random mutagen and cannot be expected to give the same action whenever it is used at the same dose. However, stevia can be replicated easily by vegetative reproduction.

Various studies have recorded a successful artificial polyploidy induction in different plant species (Otto, 2007 and Dhooghe *et al* 2011). Boonbongkarn *et al* (2013) has used a wide-range of colchicine concentrations for the polyploidy induction in different plant species.

Results came in agreement with Alam *et al* (2011), who reported that, adding colchicine on sprouting buds of potato led to the improvement of yield potential, plant height, number of leaves and fresh weight. Data also agreed with Moussa and Gomaa (2017), who indicated that the change in the genetic pool is relative to the use of colchicine as a mutagen.

Data shown in Table (2) revealed positive direct proportional significant relationship among the studied morphological traits such as: plant height (cm) with each of, leaf length (cm) and width(cm), number of branches/plant, leaf length (cm) with each of leaf width (cm), leaf area (cm^2), number of leaves/plant and dry matter content (%), leaf width (cm) with each of the number of leaves/plant and the number of branches/plant. Leaf area (cm^2) with dry matter content (%), number of leaves/plant with number of branches/plant. These findings are in accordance with Elsheikh *et al* (2019).

Table 2. Correlation coefficient values (r) for pairs of studied characters of three commercial Stevia varieties and their mutated clones.

Traits	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Number of leaves/ plant	Number of branches/ plant
Leaf length (cm)	**0.524****					
Leaf width (cm)	**0.3121***	**0.7561****				
Leaf area (cm^3)	**0.1381^{ns}**	**0.2789***	**0.2395^{ns}**			
Number of leaves/ plant	**0.2747^{ns}**	**0.4161****	**0.4932****	**0.1611^{ns}**		
Number of branches/plant	**0.3034***	**0.2593^{ns}**	**0.3019***	**0.1652^{ns}**	**0.6160****	
Dry matter content (%)	**0.2291^{ns}**	**0.2956***	**0.1495^{ns}**	**0.4354****	**0.2269^{ns}**	**0.0115^{ns}**

*** and ** indicates significant and highly significant at 5% and 1% levels of probability, while ns indicates a non-significant relationship, respectively.**

Only mutated plants with significant differences from the commercial genotypes at leaf area trait and number of leaves were selected for major steviol glycoside (SGs) determination (Steviolbioside, Stevioside, Rebaudioside C and Rebaudioside A) according to the standards previously described by Massoud *et al* (2002). As it was stated by Kaplan and Turgut (2019), breeding programs for stevia should aim at improving the diterpene glycoside content and reb A/stv ratio with a higher leaf yield. Nowadays, breeder efforts with stevia have been mainly focusing on improving leaf yield and amount of reb A in the leaves (Yadav *et al* 2011).

SGs percentages of the three commercial stevia varieties (control) under study were demonstrated below (Figure 2). Commercial varieties showed the same level of steviolbioside percentage, while SPNT was 12% more than CHN and EGY varieties in their stevioside content, which came in agreement with Hussein (2019).

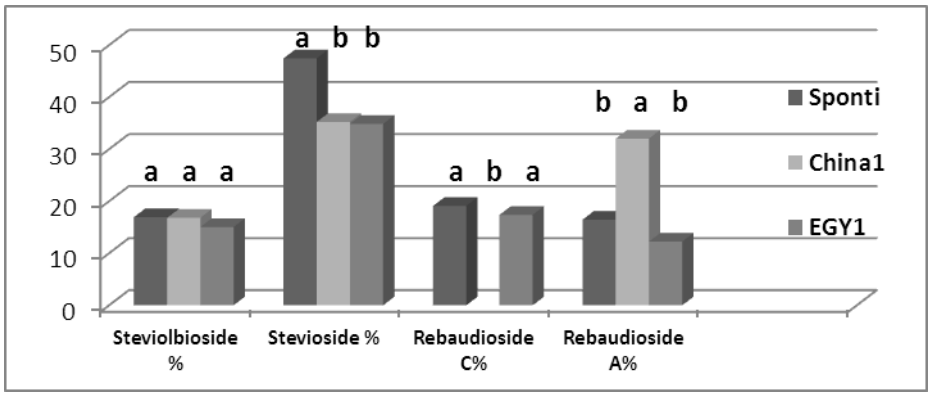

Fig. 2. SGs percentages of *Stevia rebaudiana* varieties (SPNT – CHN - EGY).

CHN variety showed no content of rebaudioside C in contrast with SPNT and EGY which recorded 19.1 and 17.4%, respectively. On the contrary, CHN variety showed double rebaudioside A% than SPNT and EGY, which is considered the most important component due to the lack of its aftertaste as compared to the rest of components according to Kemp and Lindley (2009) and Carakostas *et al* (2008).

Mean performance of HPLC major SGs components in stevia commercial varieties and their selected clones is presented in Table (3).Data showed that, steviolbioside has significantly decreased by polyploidy among most tested clones when compared to their commercial varieties, stevioside and reb A contents were significantly affected as well. CHN-31, EGY-22 and EGY-31 clones significantly recorded the highest reb A + stv percentage (77.25, 78.33 and 76.18%, respectively). Best ratio of reb A/stv was recorded by CHN-31 (3.846) which is 4.2 times higher than CHN variety (0.919).

Table 3. Mean performance of HPLC major stevioside components in *Stevia rebaudiana* commercial varieties and their clones.

Genotype	Steviolbioside	Stevioside	Rebaudioside (C)	Rebaudioside (A)	Reb A+ Stv.	Reb A/Stv.
SPNT	**16.965**	**47.456**	**18.802**	**16.777**	**64.233**	**0.354**
CHN	**16.841**	**35.240**	**0.000**	**32.374**	**67.614**	**0.919**
EGY	**15.068**	**34.870**	**17.391**	**12.241**	**47.111**	**0.351**
SPNT-22	**21.651**	**51.720**	**8.909**	**11.326**	**63.046**	**0.219**
CHN-31	**1.949**	**15.941**	**0.000**	**61.310**	**77.251**	**3.846**
EGY-22	**6.729**	**46.019**	**14.941**	**32.311**	**78.33**	**0.702**
EGY-23	**6.460**	**35.720**	**18.013**	**26.616**	**62.336**	**0.745**
EGY-31	**9.896**	**39.638**	**12.728**	**36.545**	**76.183**	**0.922**
$LSD_{(p\leq 0.05)}$	**2.2859**	**2.5569**	**1.9265**	**1.9295**	**4.0588**	**0.1285**

Similarly, for EGY-31 new clone, reb A/stv percentage was 2.6 higher compared to its commercial variety. The new clone CHN-31 significantly gave the highest mean performance for reb A/stv compared to other tested genotypes, this consequently decreased the bitter taste and aftertaste and increased sweetness flavor as mentioned by Yadav *et al* (2011) who reported that reb A is directly proportional to the sweetener intensity, so that, if reb A/Stv is equal to1, then aftertaste will be reduced. They also suggested that reb A/stv ratio is the conventional measure of sweetener quality.

These results are in agreement with those reported by Shuichi *et al* (2001) and Oliveira *et al* (2004), who stated that polyploidy plants had an increased amount of rebaudioside A and higher glycosides content. On the contrary, these results disagreed with Ibrahium (2015) who reported that sweeteners decreased in his polyploidy plants.

It was mentioned by Prakash *et al (*2008) that rebaudioside A ($C_{44}H_{70}O_{23}.3H_2O$), is 350-450 times sweeter than sucrose with sweeter and more pleasant taste than pure stevioside. Also, it was stated by Tanaka (1997) that, stevioside was 110 - 270 times sweeter than sucrose while rebaudioside A was 150 - 320 and rebaudioside C was 40 – 60 times sweeter. Rebaudioside A gave the minimal bitter taste, has no long-lasting aftertaste and was considered the most favorable sensory attribute.

Generally, the main steviol glycosides are stevioside, rebaudioside A, rebaudioside C and dulcoside A (Goyal *et al* 2010 and Wöelwer-Rieck 2012), Rebaudioside A and stevioside are the ones in greater quantities among them. Stevioside is 250 – 300 times sweeter than sucrose but with a bitter aftertaste, while rebaudioside A is 350 - 450 times sweeter than sucrose without aftertaste (Goyal *et al* 2010).

According to Thiyagarajan and Venkatachalam (2012) stevia glycosides have no effect on blood sugar level and exhibit a strong antibacterial and antifungal properties.

Several techniques have been used to determine the level of glycosides in stevia, although an HPLC method is recommended by the FAO/WHO-JECFA (González *et al* 2015).

CONCLUSION

Polyploidy was induced in stevia using colchicine. In this content, morphological measurements like plant height, leaf length, leaf width, leaf area, number of leaves and branches showed positive and highly significant differences from the commercial varieties. HPLC stevioside content in the leaves showed higher reb A/stv ratio in some clones when compared to its commercial varieties. New genotypes (CHN-31 and EGY-31) were selected as the best clones due to the higher percentages of sweeteners obtained, which might help in meeting diabetics needs in the future.

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تحفيز التضاعف الكروموسومي والمُحليات في أصناف مختلفة من الإستيفيا بإستخدام الكولشسين

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تم إجراء هذا البحث بصوب محطات مركز البحوث الزراعية بالصبحية بالإسكندرية خلال موسمي ٢٠١٨و ٢٠١٩ ، لتحفيز التضاعف الكروموسوي في نبات الإستيفيا بإستخدام الكولشسين (مطفر كيميائي) وذلك عن طريق معاملة براعم ثلاث أصناف تجارية تحت الدراسة (SPNT, CHN, EGY) بتركيزات مختلفة من مادة الكولسيشين (٠ و ٠,٠٢٥ و ٠,٠٥ و ٠,٠١ و ٠,٠٢ %) . ومن ثم انتخاب افضل السلالات مورفولوجيا؛ كإرتفاع النبات (سم) وطول وعرض الورقة (سم) ومساحة الورقة (سم٢)/نبات وعدد الاوراق/نبات وعدد الفروع/نبات وأيضا الوزن الجاف (%) لتقدير بعض المُحليات الموجودة بالإستيفا عن طريق كروموتوجرافيا السائل عالية الجودة (HPLC). وقد أظهرت النتائج أن براعم الصنفين SPNT و EGY كانت أكثر معدل استجابة وتحمل للمعاملة بالكولشيسين. وقد أظهرت السلالات المطفرة SPNT-22 وCHN-31 وEGY-22 و EGY-23 وEGY-31 فروقا معنوية

فى معظم الصفات المورفولوجية وخاصة مساحة الورقة/نبات، عدد الاوراق/نبات وكذلك محتوى المادة الجافة عند مقارنتهم بجميع التراكيب الوراثية محل الدراسة. وقد بين تحليل المُحليات تأثر جميع التراكيب الوراثية المستحدثة من المعاملة بالكولشيسين فى محتواها من مُحلى الاستيفيوسيد والـ reb A عند مقارنتها بالصنف التجاري. بينما سجلت الأصناف EGY-31 و EGY-22 و CHN-31 أعلى نسبة فى مجموع المُحليات stv +reb A. وقد سجلت أفضل نسبة لـ reb A/stv فى السلالة CHN-31 (3.84) والتي تعادل ٤,٢ أضعاف الصنف التجاريCHN(0.919)، تلاها السلالة EGY-31 التي تضاعفت بها النسبة إلي ٢,٦ ضعف الصنف التجاري مما يقلل الطعم المر ويزيد من المذاق الحلو المستصاغ. وأخيرا، تم الحصول علي سلالتين جديدتين وهما CHN-31، EGY-31 يحتويان علي نسب أعلي من rebA/stv عن طريق إستحداث التضاعف الكروموسومي في نبات الإستيفيا كنتيجة لتحفيز النمو الخضري. وهذا ينعكس إيجابا على كمية المحليات البديلة للسكر لذلك وجب التنويه لتعظيم إنتاج هذه المحليات على نطاق أوسع نظرا لأهميتها الإقتصادية والطبية لمرضى السكر وكذلك إستخدامها في بعض الحِميات الغذائية الخاصة.

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