Journal of Agricultural Chemistry and Biotechnology

Journal homepage & Available online at: www.jacb.journals.ekb.eg

Molecular and Phenotypic Diversity Assessment of Some Selected *Stevia rebaudiana* Hybrids Based on Yield Traits and Quality of Sweetness

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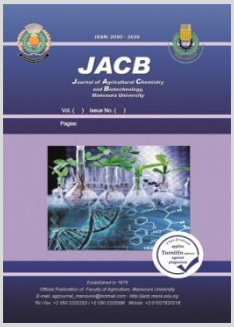

ABSTRACT

To increase the knowledge about *Stevia rebaudiana* variability and to develop a breeding strategy through hybridizations and selection to get cultivars adapted to Egyptian conditions. We performed hybridizations for three Stevia varieties with half-diallel design then studied the molecular and phenotypic diversity. Based on leaf dry weight, we selected the top five hybrids from each cross. From these hybrids, the top two hybrids were selected for genetic diversity assessment based on the RA/ST ratio. Molecular diversity assessment was done using ISSR and SCoT techniques. SCoT exhibited a higher discriminatory potential compared to ISSR technique. Both were effective tools for assessing genetic relationships among studied genotypes. H1-9 hybrid exhibited high values for most traits. While H1-1 exhibited exceptional performance of plant height and RA/ST ratio. The greatest molecular and phenotypic distance between parents was observed between the Spanti and China1. The H1-9 and H1-1 hybrids, resulting from the crossing of these parents, were characterized by distinct positive unique molecular markers. H1-9 was distinguished by three markers: one identified by the SCoT-22 primer and two by ISSR primers. H1-1 was distinguished by four markers: one identified by the SCoT-12 primer and three by ISSR primers. High genetic divergence values were observed between H1-9 and the other genotypes. Indicating the possibility of improving stevia in future breeding programs by crossing the H1-9 with any of the studied genotypes and utilizing these genotypes and their unique markers to achieve satisfactory improvements aligned with sustainable development goals.

Keywords *: Stevia,* phenotypic diversity, genetic diversity

INTRODUCTION

Stevia is a perennial herb which belongs to the Asteraceae family. It is a natural sweetener plant. Stevia leaves are the source of diterpene glycosides, in addition to. stevioside (ST) and rebaudioside-A (RA) (Goettemoeller and Ching 1999). But rebaudioside-A is particularly prized for its superior taste (DuBois, 2000). Stevioside, the primary sweetener in stevia (60-70% of total glycosides), is 110-270 times sweeter than sugar but often has a bitter aftertaste, sometimes described as licorice. Additionally, stevioside can have a lingering or pungent quality that decreases its overall acceptability. In contrast, rebaudioside-A, typically comprising 30-40% of the sweetener, is remarkably sweet (180-400 times sweeter than sugar) without the bitter aftertaste. The ratio of rebaudioside-A to stevioside (RA/ST) determines the overall sweetness quality; higher rebaudioside-A content correlates with improved taste. When these components are present in equal amounts, the aftertaste seems to disappear (Yadav *et al.*, 2011). There are much international food companies using *stevia* in their products among them some famous juice which uses *Stevia* in Japan and other countries for its Diet juice. Stevioside at high temperature in tea and coffee beverages for 1 h showed good stability up to 120ºC. Stevioside is remarkably stable in the pH range of 2–10 in aqueous solution. This observation seems to be essential for its effective application in hot coffee and tea beverages (Abdalbasit 2014). Stevia populations had grown from seeds exhibit variations in morphology as well as chemical contents (Jadeja *et al.*, 2005 and Elsheikh *et al.*, 2019). Plant leaf yield is proportional to branch number, leaf number and (some time) plant height (Buana and Goenadi 1985 and Buana 1989). Total stevioside content is positive correlated with leaf/stem ratio (Tateo *et al.*, 1998). Whereas earlier studies reported the selection of elite genotypes in natural populations and segregating generations of stevia (Abdelsalam *et al.*, 2016; Singh *et al.*, 2017 and Elsheikh *et al.*, 2019). Although by the segregating individuals are not needed for crop cultivation as associated with heterogeneity, the segregating generations may be a source of genetic diversity for improvement stevia breeding programs. Thus, the evaluation of segregating generations with the selecting elite genotypes objective as new variety or parents for the development of valuable cultivars is interesting (Elsheikh *et al.*, 2019).

Study of phenotypic and genotypic diversity is starting step to develop a plant breeding programme through selection. Molecular marker techniques have emerged as valuable tools for assessing genetic diversity in recent years. These methods are neutral, practical, and unaffected by age, tissue type, or environmental conditions (Zietkiewicz *et al.*, 1994). Various molecular techniques can be used to assess genetic diversity in plants. Among the most popular and widely used are SCoT (Start Codon Targeted) and ISSR (Inter-Simple Sequence Repeat) molecular markers. SCoT and ISSR are effective molecular markers for assessing plant genetic diversity (Etminan *et al.*, 2016). SCoT is often considered more efficient than other dominant markers like

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DOI: 10.21608/jacb.2024.314948.1091

RAPD and ISSR due to its gene-targeted nature (Gupta *et al.*, 2018). It also exhibits higher polymorphism and better marker resolution (Gorji *et al.*, 2011). Unlike other dominant markers, SCoT can produce both co-dominant and dominant markers (Aswathy *et al.*, 2017). However, combining ISSR and SCoT markers generally yields more effective, reliable, and superior results for genetic diversity studies compared to using either marker alone (Mao *et al.*, 2018).

To enhance our understanding of *Stevia rebaudiana* variability and develop a breeding strategy to produce cultivars adapted to Egyptian conditions, this study aimed to assess the molecular and phenotypic diversity of three varieties of *S. rebaudiana* and their selected hybrids, focusing on various economic traits associated with yield and overall sweetness quality through greenhouse and field experiments at different phenotypic stages.

MATERIALS AND METHODES

Plant materials

Three *stevia* (*Stevia rebaudiana,* Bertoni) varieties imported from different region, namely; Spanti (P1), China1(P2) and SugarHigh (P3) were obtained on basis of their diversity in agronomic and sweetness traits to achieve this study. The experiment was conducted in November 2019 at the field farm (Google map: 30°1`12"N 31°12`24" E) and laboratories of Genetics and Breeding department, Sugar Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt. Hybridizations were performed after the formation of flower buds and before blooming flowers for each cross in an isolation chamber within a greenhouse. All possible cross combinations, excluding reciprocals, were made among the three varieties, resulting in a total of three F1 crosses (half-diallel hybrids). These genotypes (parents and their hybrids) were assessed in 2021 using the following phenotypic traits: plant height, stem number, leaf number, plant weight, stem fresh weight, leaf fresh weight, leaf dry weight, leaf humidity, and leaf/stem fresh weight. Data were collected from several randomly selected plants within each plot of three replications for each parent variety and hybrid.

Extraction and identification of stevioside and rebauside-A by HPLC:

Dried, mature leaf samples from the top five hybrids of each hybridization group, based on leaf dry weight (the economic trait as described by (Afandi *et al.*, 2013), were powdered and passed through a 20-mesh filter. Sample preparation and analysis were performed according to (Supriyadi and Yawono 2016) using the HPLC-Evaporative Light Scattering Detector (HPLC-ELSD) method to achieve optimal separation of Stevioside (ST) and Rebaudioside-A (RA).

Molecular assessment

Fresh stevia leaves were collected separately from selected genotypes. The bulked DNA extraction from each genotype was performed using CTAB/Chloroform-Isoamyl Alcohol DNA Extraction Protocol (Doyle and Doyle 1987; Doyle and Dickson,1987). Bulked DNA from each sample was used as a template for PCR amplification using ^ SCoT and ° ISSR primers (Table 1 & 2) . DNA amplifications were performed in an automated thermal cycle (model Techno 512) programmed for one cycle at 95°C for 5 min followed by 40 cycles of 1 min at 95°C, 1 min at 56°C (for all primers), and 2 min at 72°C, finally one cycle 72°C for 10 min and stored at 20°C. PCR products for each primer were loaded on a 1.2 % agarose gel mixed with ethidium bromide and electrophoresed against a DNA ladder (0.1 to 3.0 kbp or 0.25 to 10 kbp). The run was performed at 100 V for about 30 min in BioRad mini-submarine gel. DNA banding patterns were photographed using a UV light on the Bio-1D Gel documentation system.

DNA profiles and Molecular data analysis were conducted using binary data (0&1 data) of PCR-banding patterns for SCoT and ISSR techniques as outlined by (Ola-Ahmed and Abd EL-Aziz 2021). Subsequently, a Molecular distance matrix (Dice dissimilarity distance matrix) and an agglomerative hierarchical clustering (AHC) dendrogram were constructed using the Unweighted Pair Group Method with Arithmetic Means (UPGMA) method depending on Dice-similarity index, with the aid of XLSTAT.7 software, as outlined by (Abd El-Aziz *et al.*, 2019).

Table 1. List of SCoT primers name and their sequences

No	Primers	Sequence 5`-3`
1	SCoT 12	ACGACATGGCGACCAACG
2	SCoT-13	ACGACATGGCGACCATCG
3	SCoT-14	ACGACATGGCGACCACGC
4	SCoT- 22	AACCATGGCTACCACCAC
5	SCoT-26	ACCATGGCTACCACCGTC
6	SCoT-28	CCATGGCTACCACCGCCA
7	SCoT-31	CCATGGCTACCACCGCCT
8	SCoT-70	ACCATGGCTACCAGCGCG

Table 2. List of ISSR primers name and their sequences

No	Primers	Sequence 5`-3`
1	UBC-811	$(GA)_8C$
2	UBC-823	$(TC)_8C$
3	UBC-849	$(GA)_8T$
4	844A	$(CT)_8AC$
5	TA-2	$(CT)_{10}G$

Phenotypic assessment

Phenotype data were recorded at chosen plants based on economic traits. These traits included plant height, stem number, leaf number, plant weight, stem fresh weight, leaf fresh weight, leaf dry weight, leaf humidity, and leaf/stem fresh weight ratio as well as percentage of Stevioside and Rebaudioside-A. Based on these data, Phenotypic distance matrix (Euclidean dissimilarity distance matrix) and AHC dendrogram were constructed using the UPGMA method, with the aid of XLSTAT.7 software, as outlined by (Abd El-Aziz *et al.* 2019).

RESULTS AND DISSCUSION

Stevioside and rebaudioside-A content:

To achieve this study's objective, dried mature leaf samples were collected from the top five F1 hybrids resulting from a half-diallel cross of three varieties. The selection was based on leaf dry weight, considered the plant's economic component (Ashwell, 2015). These samples were used to estimate the active ingredients in stevia, (stevioside and rebaudioside-A). Based on the RA/ST ratio (Table 3), the top two hybrids from each of the three crosses were selected for genetic diversity analysis. In cross 1, H1-1 (3.82) ranked highest, followed by H1-9 (2.07). In cross 2, H2-5 (3.77) was the top performer, followed by H2-10 (2.64). For cross 3, H3-6 (1.76) and H3-8 (1.04) were selected. (Fu *et al.*, 2012)

described this procedure as an effective method for preparative separation and comprehensive characterization of steviol glycosides in stevia. Following this approach, these six genotypes and their parents were chosen to assess genetic diversity.

Table 3. Relative content of Stevioside (ST) and Rebaudioside A (RA) and their ratio in leaves of three Stevia varieties and their selected F1 hybrids.

Genotypes	code	ST %		RA %		RA/ST
Spanti	P_1	9.29		6.99		0.75
China 1	P_2	7.98		8.1		1.02
SugarHigh	P_3	4.91		5.3		1.08
	H1-1	1.85	bcd	7.07	a	3.82
	H1-6	5.14	a	0.93	bc	0.18
(P1 x P2)	H1-9	2.65	bc	5.48	abc	2.07
	H1-11	2.33	bcd	0.72	bc	0.31
	H1-14	0.45	cd	0.12	c	0.27
	H2-2	3.29	ab	0.85	bc	0.26
	H2-5	1.86	bcd	7.01	a	3.77
(P1 x P3)	H2-6	1.53	bcd	1.06	bc	0.69
	H2-9	0.12	d	0.03	c	0.25
	H2-10	2.54	bcd	6.71	a	2.64
	H3-6	3.57	ab	6.3	ab	1.76
	H3-8	2.03	bcd	2.12	abc	1.04
(P2 x P3)	H3-9	1.31	bcd	1.09	bc	0.83
	H3-10	1.47	bcd	0.62	c	0.42
	H3-14	2.18	bcd	0.88	bc	0.40

Means in the same column having non-similar letters signify differ significantly at P≤0.05 (Duncan's test).

Molecular assessment

Assessing molecular diversity among available genotypes is a crucial initial step in breeding programs. By identifying genotypes with high diversity and superior performance, breeders can make informed selections for parental lines. PCR-based molecular markers, such as AFLP, RAPD, SSR, ISSR, and SCoT (Hashem *et al.*, 2021), provide efficient tools to elucidate genetic relationships and diversity prior to breeding in crops like stevia.

In this study Fresh stevia leaves were collected separately from the two highest RA/ST value hybrids selected from each cross (based on HPLC results). Where a total of six hybrids, in addition to the three parent varieties (9 samples), were included in molecular diversity study.

Eight SCoT and five ISSR primers were used to molecular assess the selected genotypes of *S. Rabaudiana.* Banding patterns and DNA Profiles of these primers are presented in Figures 1, 2, 3, and 4. These figures reveal that ISSR and SCoT primers except SCoT-14 and SCoT-31 generated 26 unique amplicons (16.5% of 158 total amplicons), of which five were negative and 21 were positive, and thus considered useful as unique markers. This suggests that these techniques are suitable for molecular identification and could be applied to assess the genetic diversity of Stevia genotypes. These findings align with those reported by (Ataweel *et al.*, 2021) in stevia.

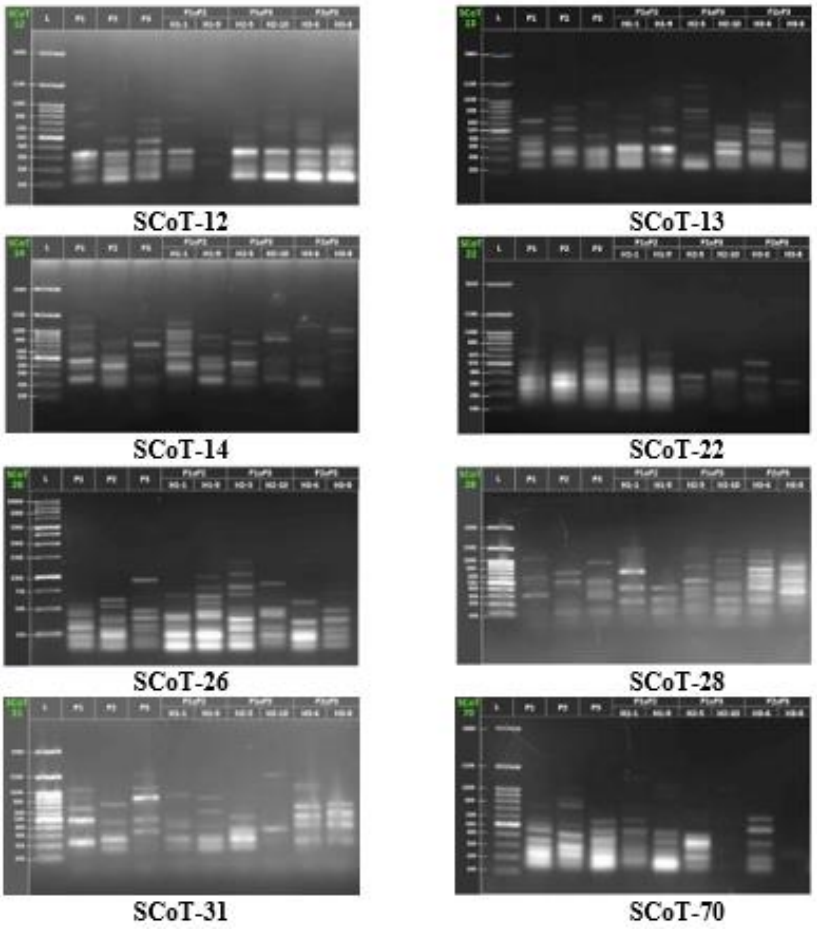


Fig. 1. Banding patterns of SCoT-PCR products for nine genotypes of *stevia* produced with eight primers. M, 10 and 3 k bp ladder and lanes 2 to 10 represent the nine genotypes.

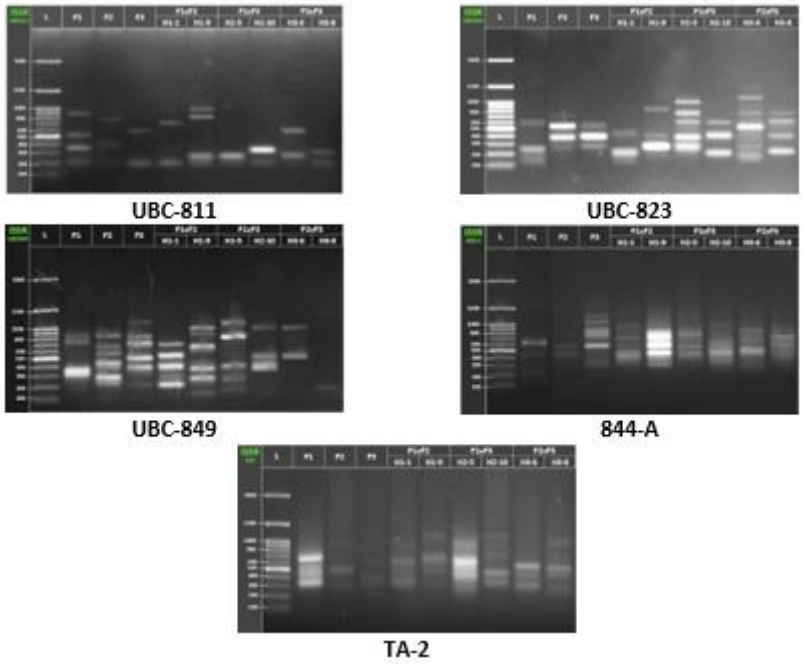


Fig. 2. Banding patterns of ISSR-PCR products for nine genotypes of *stevia* produced with five primers. M, 3 k bp ladder and lanes 2 to 10 represent the nine genotypes.

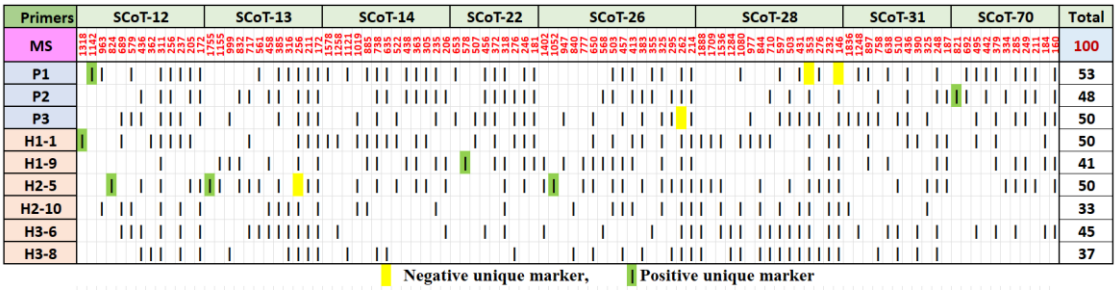


Fig. 3. DNA-profile representation of SCoT fingerprint of nine stevia genotypes based on 100 amplicons 11 of them were marker loci.

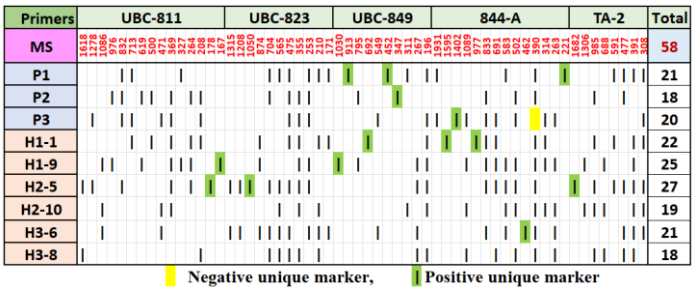


Fig. 4. DNA-profile representation of ISSR fingerprint of nine *stevia* genotypes based on 58 amplicons 15 of them were marker loci.

Molecular data from banding patterns of SCoT and ISSR techniques were recorded in Tables 4 and 5. These tables revealed a total of 158 amplicons, of which 156 were polymorphic. The SCoT-26 and ISSR UBC-811 primers targeted amplification highest number of amplicons (16 and 15, respectively), while SCoT-22 and ISSR TA-2 targeted the lowest (10 and 8, respectively). Amplicon sizes ranged from 160 to 1888 bp for SCoT and 167 to 1931 bp for ISSR.

Polymorphism percentages for SCoT primers ranged from 91.7% to 100% and were consistently 100% for all successful ISSR primers. Resolving power (Rp) values for SCoT and ISSR primers varied between 5.99 and 10.43 (average 7.99) and 3.77 and 8.44 (average 6.13), respectively. The polymorphic information content (PIC) ranged from 0.35 to 0.44 for SCoT primers (with a diversity index, DI, of 0.40) and from 0.29 to 0.40 for ISSR primers (with DI = 0.36). These results suggest that the SCoT technique exhibits a higher discriminatory potential compared to the ISSR technique used in this study, according to (Prevost & Wilkinson 1999). This conclusion is further supported by (Hashem *et al.*, 2021), who validated the SCoT marker as a reliable tool for assessing genetic diversity in stevia.

Table 4. Molecular data estimated from banding patterns of SCoT technique.

Primer	Amplicons						Polymorphism %	Polymorphic index content (PIC)	Resolving power Rp
	Molecular size range	Monomorphic	Polymorphic			Total			
			Without unique	Unique+	Unique -				
SCoT 12	172-1318	1	9	3	0	13	92.3	0.35	7.10
SCoT-13	172-1755	1	9	1	1	12	91.7	0.36	6.67
SCoT-14	206-1578	0	13	0	0	13	100.0	0.44	9.32
SCoT- 22	181-653	0	9	1	0	10	100.0	0.42	5.99
SCoT-26	214-1402	1	13	1	1	16	93.8	0.38	9.32
SCoT-28	146-1888	0	13	0	2	15	100.0	0.42	10.43
SCoT-31	187-1836	0	11	0	0	11	100.0	0.44	7.77
SCoT-70	160-821	0	10	1	0	11	100.0	0.42	7.33
Overall	146-1888	3	87	7	4	101	97.23	0.40	7.99

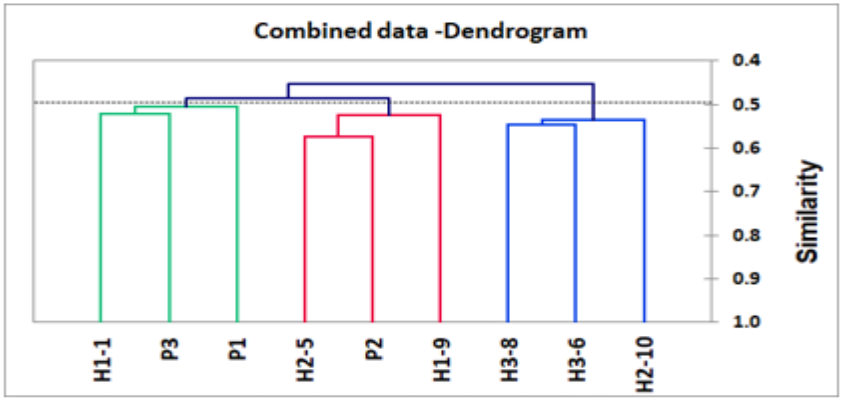
Table 5. Molecular data estimated from banding patterns of ISSR technique.

Primer	Amplicons						Polymorphism %	Polymorphic index content (PIC)	Resolving power Rp
	Molecular size range	Monomorphic	Polymorphic			Total			
			Without unique	Unique +	Unique -				
UBC-811	167-1618	0	13	2	0	15	100.0	0.38	8.44
UBC-823	171-132	0	10	1	0	11	100.0	0.40	6.66
UBC-849	196-1030	0	5	5	0	10	100.0	0.29	3.77
844A	221-1931	0	8	5	1	14	100.0	0.35	7.55
TA-2	308-1682	0	7	1	0	8	100.0	0.40	4.22
Overall	167-1931	0	43	14	1	58	100.0	0.36	6.13

To obtain the most comprehensive molecular assessment, combined SCoT and ISSR data were utilized to estimate molecular distance (MD) values among nine Stevia genotypes (Table 6). MD values among all studied genotypes ranged from 0.427 to 0.678, with the range between the three parents falling within 0.471 (between P1 and P3) to 0.515 (between P1 and P2). The highest MD was observed between H2-10 and P2 (0.678), while the lowest MD was found between H2-5 and P2 (0.427). More clearly, an AHC dendrogram based on combined ISSR and SCoT data (Figure 5) divided the nine Stevia genotypes into three groups at a dissimilarity coefficient of 0.544. The first group comprised genotypes H1-1, P3, and P1. The second group included genotypes H2-5, P2, and H1-9. Finally, the third group consisted of genotypes H3-8, H3-6, and H2-10. These results indicate that SCoT and ISSR markers are effective tools for assessing genetic relationships among different Stevia genotypes. Furthermore, the findings suggest a satisfactory level of genetic diversity among the studied genotypes, which offers potential for achieving hybrid vigor through hybridization of the most divergent genotypes (Abd El-Hadi *et al.*, 2017). Where developing new specific markers is crucial for breeders to evaluate Stevia genotypes in breeding programs according to (Heikal *et al.*, 2008) who used 7 ISSR primers to determine the phylogenetic relationships among six *stevia* accessions.

Table 6. Molecular distance (MD) matrix for nine genotypes of *stevia* based on combined binary data of SCoT and ISSR techniques.

	P1	P2	P3	H1-1	H1-9	H2-5	H2-10	H3-6
P2	0.515							
P3	0.471	0.486						
H1-1	0.507	0.493	0.479					
H1-9	0.557	0.470	0.529	0.522				
H2-5	0.536	0.427	0.510	0.490	0.483			
H2-10	0.540	0.678	0.541	0.548	0.508	0.566		
H3-6	0.486	0.576	0.544	0.551	0.576	0.483	0.475	
H3-8	0.597	0.554	0.504	0.512	0.620	0.485	0.458	0.455



Legend: TL represents truncated line at a coefficient of Similarity=0.544

Fig 5. Dendrogram derived by UPGMA method using Dice-similarity coefficient for combined binary data of SCoT and ISSR techniques for nine lines of stevia.

Phenotypic assessment

According to Table 7, significant variation was observed among the three cultivars and six hybrids in terms of 9 economic traits. Among these genotypes, H1-9 exhibited the highest values for all traits, except for plant height. H1-1 displayed the highest plant height and as previously reported in Table 3, the highest RA/ST ratio.

Table 7. Mean performance range and desirable values for all studied traits in nine genotypes.

Traits / Genotypes	Plant height (cm)	St. No.	Leaves No.	Plant W. (g)	St. F.W. (g)	Leaf F.W. (g)	Leaf DW. (g)	leaf Humidity (%)	L/St FW. (g)
P1 (SPANT)	50.00^{bc}	15.00^{b}	240.0^{b}	355.0^{b}	69.4^{b}	180.0^{b}	55.0^{abc}	69.44^{b}	1.03^{abc}
P2 (CHINA1)	45.00^{bc}	15.00^{b}	235.0^{b}	450.0^{b}	71.4^{b}	140.0^{b}	40.0^{c}	71.43^{b}	0.45^{c}
P3 (SUGHHI)	60.00^{ab}	11.00^{b}	245.0^{b}	385.0^{b}	65.0^{b}	145.0^{b}	50.0^{bc}	65.52^{b}	0.60^{bc}
				P1xP2					
H1-1	70.0^{a}	15.00^{b}	370.0^{ab}	540.0^{ab}	69.0^{b}	210.0^{b}	65.0^{ab}	69.05^{b}	0.64^{bc}
H1-9	40.0^{c}	22.00^{a}	495.0^{a}	660.0^{a}	80.8^{a}	390.0^{a}	75.0^{a}	80.77^{a}	1.44^{a}
				P1xP3					
H2-5	50.00^{bc}	18.00^{ab}	295.0^{b}	390.0^{b}	68.0^{b}	205.0^{b}	65.0^{ab}	68.00^{b}	1.11^{ab}
H2-10	60.00^{ab}	11.40^{b}	280.0^{b}	375.0^{b}	66.7^{b}	180.0^{b}	60.0^{abc}	66.67^{b}	0.92^{abc}
				P2xP3					
H3-6	60.00^{ab}	13.00^{b}	305.0^{b}	430.0^{b}	67.6^{b}	185.0^{b}	60.0^{abc}	67.57^{b}	0.76^{bc}
H3-8	60.00^{ab}	13.00^{b}	260.0^{b}	380.0^{b}	66.7^{b}	197.0^{b}	55.0^{abc}	69.27^{b}	0.77^{bc}

Means in the same column having non-similar letters signify differ significantly at P≤0.05 (Duncan's test).
(PH): Plant height, (St.N): Stem number, (L.N): leaf number, (P.W) plant weight, (St.F.W): Stem fresh weight, (L.F.W): Leaf fresh weight, (L.D.W): Leaf dry weight, (L.H): Leaf humidity and (L/St FW): Leaf/stem fresh weight ratio.

Referring to Figures 3 and 4 it is noted that the hybrid H1-9 was distinguished by three unique positive molecular markers: one identified by the SCoT-22 primer with a molecular size (Ms) of 578 bp, and two by ISSR primers (UBC-811 with Ms of 167 bp and UBC-849 with Ms of 1030 bp). While hybrid H1-1 was characterized by four unique positive markers: one identified by the SCoT-12 primer with Ms of 1318 bp, and three by ISSR primers (UBC-849 with Ms of 692 bp and 844-A with Ms of 977 and 1595 bp).

These results align with the findings of (Abdelsalam *et al.*, 2016), who studied the genetic improvement of 19 stevia genotypes through selection at the farm of Faculty of Agriculture Saba Basha, Alexandria University, and Sabahia Agricultural Research Station over two harvest seasons. They also investigated the molecular genetic fingerprints of these genotypes using RAPD markers to elucidate genetic diversity. Their research indicated that most RAPD markers displayed varying levels of genetic polymorphism, corresponding to significant phenotypic variation among studied genotypes. This suggests that divergence genotypes can be served as valuable resources for future breeding programs.

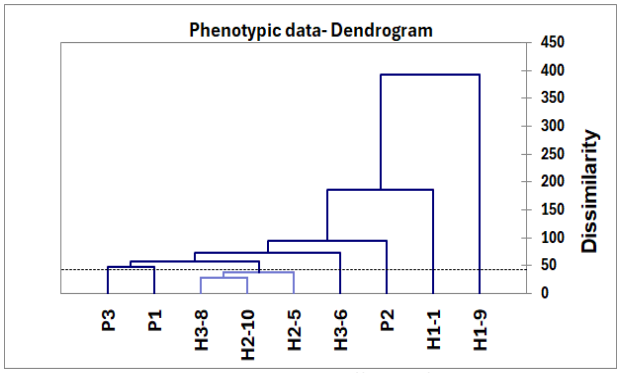
To verify the genetic variation between genotypes in our study, phenotypic distances were calculated based on mean performance for estimated economic traits (Table 8). The results of phenotypic distances (PD) revealed a range of 27.7 to 447. Among the three parents, the range fell within 48.5 (between P1 and P3) to 104.5 (between P1 and P2).

Table 8. Phenotypic distance (PD) matrix for nine studied genotypes of stevia based on phenotypic data.

PD	P1	P2	P3	H1-1	H1-9	H2-5	H2-10	H3-6
P2	104.5							
P3	48.5	69.2						
H1-1	229.3	180.3	210.4					
H1-9	450.6	419.2	447.0	252.5				
H2-5	71.0	110.2	80.8	169.0	384.3			
H2-10	46.9	99.8	51.6	190.7	415.6	35.3		
H3-6	100.2	89.5	85.7	130.7	363.4	47.5	60.7	
H3-8	38.8	96.6	54.9	195.2	414.8	40.5	27.7	68.7

From these results and the molecular distance results in Table 6, it's clear that the highest molecular and phenotypic distance between the tested parents was between P1 (Spanti) and P2 (China1), suggesting the potential for high hybrid vigor through hybridization between them. This was evident in the mean performance of the H1-9 and H1-1 hybrids, which resulted from crossing these parents and were characterized by clear positive unique molecular markers. Additionally, the highest PD values were observed between the genotypes P1 and H1-9, while the lowest PD values were found between H2-10 and H3-8.

Also, based on phenotypic distances, the dendrogram in Fig. 6 shows that the studied genotypes can be divided into seven groups at a dissimilarity coefficient of 43.19. Each group contains a single genotype, except for group 5, which includes three genotypes: H2-5, H2-10, and H3-8. This indicates significant genetic divergence among most of the studied genotypes. The highest genetic divergence values were observed between the H1-9 hybrid and the other genotypes, particularly with the three parents and the H2-10 and H3-8 hybrids. These results suggest the potential for obtaining high hybrid vigor by crossing the H1-9 hybrid with any of these genotypes.



TL represents truncated line at a dice coefficient of Dissimilarity= 43.19.

Figure 6. UPGMA clustering dendrogram based on Dice-dissimilarity index of phenotypic data for nine stevia genotypes.

These results support findings by (Chung *et al.*, 2011), (Cosson *et al.*, 2019), (Dyduch-Siemińska *et al.*, 2020) and (Azrul-Murad *et al.*, 2022). This bodes well for the possibility of improving stevia in future breeding programs, utilizing

these genotypes and their distinctive molecular markers to achieve satisfactory improvements aligned with sustainable development goals.

CONCLUSION

Our study suggests that hybridization and selection using molecular and phenotypic assessment can be effective tools for improving *Stevia rebaudiana* in Egypt. The identified hybrids and their unique molecular markers offer promising sources for future breeding programs aimed at developing sustainable and high-yielding and sweetness quality cultivars.

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تقييم التنوع الجزيئي والمظهرى لبعض هجن الإستيفيا المنتخبة على أساس صفات المحصول وجودة التحلية

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الملخص

للإرتقاء بالمعرفة حول التنوع الوراثى لنبات الإستيفيا وتطوير استراتيجية تربية فعالة من خلال التهجين والإنتخاب للحصول على أصناف متكيفة مع الظروف المصرية ، أجرينا تهجينات لثلاثة أصناف من نبات الإستيفيا بتصميم نصف دائرى، ثم قمنا بدراسة التنوع الجزيئي والظاهري للهجن المنتخبة . حيث تم الإنتخاب على مرحلتين الأولى بناءً على الوزن الجاف للأوراق، اخترنا فيها أفضل خمسة تراكيب من كل تهجين من هذه الهجن، في المرحلة الثانية تم اختيار أفضل هجينين من حيث نسبة ريبوديوسيد أ إلى إستيفوسيد لتقييم التنوع الجيني. حيث تم إجراء تقييم التنوع الجزيئي باستخدام تقنيات ISSR و SCoT ، وفيه أظهرت تقنية SCoT فاعلية تمييزية أعلى مقارنة بتقنية ISSR. وكان كلاهما بمثابة أدوات فعالة لتقييم العلاقات الوراثية بين التراكيب الوراثية محل الدراسة . ومن خلال التقييم المظهرى تبين أن الهجين H1-9 أظهر قيمًا عالية لمعظم الصفات. بينما أظهر H1-1 أداءً استثنائيًا لارتفاع النبات ونسبة ريبوديوسيد أ إلى إستيفوسيد . وبحساب المسافات الجزيئية والمظهرية تبين أن أكبر مسافة جزيئية وظاهرية بين التراكيب الأبوية كانت بين الأب الأول Spanti والثانى China1. كما تميزت الهجن المنتخبة H1-9 و H1-1 والناتجة عن التهجين بينهما بعلامات جزيئية متفرده موجبة. حيث تميز H1-9 بثلاث علامات واحدة تم تحديدها بواسطة البادئ SCoT-22 واثنتان بواسطة بادئات ISSR . بينما تميز H1-1 بأربع علامات: واحدة تم تحديدها بواسطة البادئ SCoT-12 وثلاثة بواسطة بادئات ISSR. وكان التباعد الوراثي في أعلى قيمه بين H1-9 والتراكيب الوراثية الأخرى. الأمر الذى يشير إلى إمكانية تحسين محصول الإستيفيا في برامج التربية المستقبلية عن طريق تهجين H1-9 مع أي من التراكيب الوراثية محل الدراسة والإستفادة من هذه التراكيب الوراثية وعلاماتها الجزيئية المتفرده لتحقيق تحسينات مرضية تتماشى مع أهداف التنمية المستدامة.