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Steviol Glycosides and Biomass Variation of *Stevia rebaudiana* Crop Grown under Moroccan Conditions

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ABSTRACT

Stevia is a relatively unknown plant in Morocco. It contains low calorie sweeteners, which are 400 times sweeter than sucrose. The present study deals with the yield and quality response of cultivated stevia to the edaphoclimatic conditions in twelve different areas in Morocco, by measuring the biomass, Stevioside (STV), Rebaudioside A (RA), and Total steviol glycosides (SGtot). It was shown that stevia can grow in different types of soils even with low nutrient levels. Also stevia is suitable to be grown in areas with high electrical conductivity of soil (ECs) and water (ECw) like in Marrakech site with ECs=9.68 dS/cm, and Berkane site with ECw=2.97 and ECs=4.54. The organoleptic quality is increased with low pH as was recorded in Oulmes site where RA and SGtot were the greatest, with 9.88% and 18.94%, respectively. Stevia crop offers a great potential for introduction as a commercial crop for bio sweeteners production under Moroccan conditions.

Keywords: Bio sweeteners, Organoleptic quality, Rebaudioside A, *Stevia rebaudiana*, Steviol glycoside, Stevioside.

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni), an herbaceous perennial plant belonging to the Asteraceae family is native to Paraguay. It is known as a source of steviol glycosides (SG), which are responsible for the typical sweet taste [1]. The stevia plant was recently introduced to many countries in order to produce a natural sweetener that can cover some of the lack of sugar production like in Egypt [2] and Malaysia [3]. Its compounds are considered as a promising sugar substitute and low caloric sweetener.

Most sweet diterpenes are accumulated in leaves, which may contain 4 to 20% in dry leaf matter [4] and they are up to 400 times sweeter than sucrose [1], while, the other plant organs (stems, flowers, seeds and roots) contain significantly a lower rate of these sweeter compounds. The major diterpene glycosides (SGs) are namely stevioside, rebaudiosides A, B, C, D, E, and F, steviolbioside, and dulcoside A [1].

The main sweet components are stevioside (STV) and rebaudioside A (RA). STV is the predominant sweetener, accounting for 3-8% (w/w) of dried leaves [5] and RA is the second most abundant sweetener (approximately 1-2% of dried leaves). STV tastes about 300 times in a 0.4 g/100 ml solution [1], this glycoside is responsible for stevia's aftertaste [6], and RA tastes about 400 times sweeter than 0.4% sucrose solution [1,7]. RA has the greatest organoleptic quality without bitter aftertaste [8]. The stevioside to rebaudioside A ratio (STV/RA ratio) is an indicator of the quality of the biomass. Thus, if leaves contain equal amounts of RA and STV the aftertaste is greatly diminished. The sweetness quality increases the greater the relative concentration of RA is [9].

There have been numerous studies, experimental and simulated, to quantify the sweet diterpene glycosides and to verify the possible mutagenic and genotoxic effects of stevia extracts. It turned out that it exerts several therapeutic benefits, having antihyperglycemic [10], anti-hypertensive [11], anti-inflammatory [12], antioxidant [13], insulinotropic, and glucagonostatic actions in diabetic rat [14-16]. Nowadays, the most common high intensity sweeteners in the world market are made of synthetic compounds. Some kinds of artificial sweetener, such as saccharin is associated with the potential risk of cancer of bladder when they are used heavily [17]. Iyyaswamy and Rathinasamy [18] found that after chronic exposure to aspartame, detectable methanol continues to circulate in the blood; methanol and its metabolites may be responsible for the generation of oxidative stress in brain regions. This study confirms the presence of toxic metabolite after aspartame administration and it

emphasizes the need to caution the people who are using aspartame routinely. Stevia cultivation and production would further help those who have to restrict carbohydrate intake in their diet; to enjoy the sweet taste with minimal calories. Also stevia plants are a good source of carbohydrates, protein, crude fiber, minerals, essential and non-essential amino acid which are vital for human nutrition and maintain a good health [19,20].

In Morocco, the statistics are alarming, health minister Houssaine Louardi during the celebration of World Diabetes Day 2016, stated that between 2011 and 2015 the number of diabetics in Morocco increased from 1.5 million individuals to more than 2 million, aged 20 and over, that is 25% more in 5 years, besides 80% of diabetes cases are type 2, related to obesity and lifestyle. According to Yves Souteyrand, The world health organization representative in Morocco, 55.1% of the population is overweight and 21.7% is obese, which is alarming. This study is directed to elucidate the factors controlling development of stevia as a new crop in Morocco. Accordingly we investigated the effect of different environmental parameters (soil, water, altitude, temperature, day length) in 12 different sites on stevia yield, STV, RA and SG_{tot}. This will allow us the characterization of water, soil and climatic parameters of potential areas for stevia cultivation in Morocco as a new crop.

MATERIAL AND METHODS

Experimental locations characteristics

In this study, 12 different regions were chosen to cultivate stevia in Morocco, with different edaphoclimatic characteristics (Figure 1). All parcels were carried out at the experimental field of the National Agricultural Research Institute (INRA), Morocco. The seeds, originating from Germany, were sowed during March 2014 in the greenhouse of INRA-Rabat. The soil is a mixture of 50% peat and 50% sand. Six to seven weeks old stevia seedlings (6 leaf stage) were transplanted in the open field in the 12 sites on April 2014 with a spacing of 50 cm x 50 cm. Before transplanting, the fields were fertilized with manure. All the present cultures were conducted in irrigated drip mode. The crop was irrigated two to three times per week except at the areas that are characterized by hot flushes (Agadir and Marrakech) where the irrigation was given as per requirement. Weeding operation was done every month.

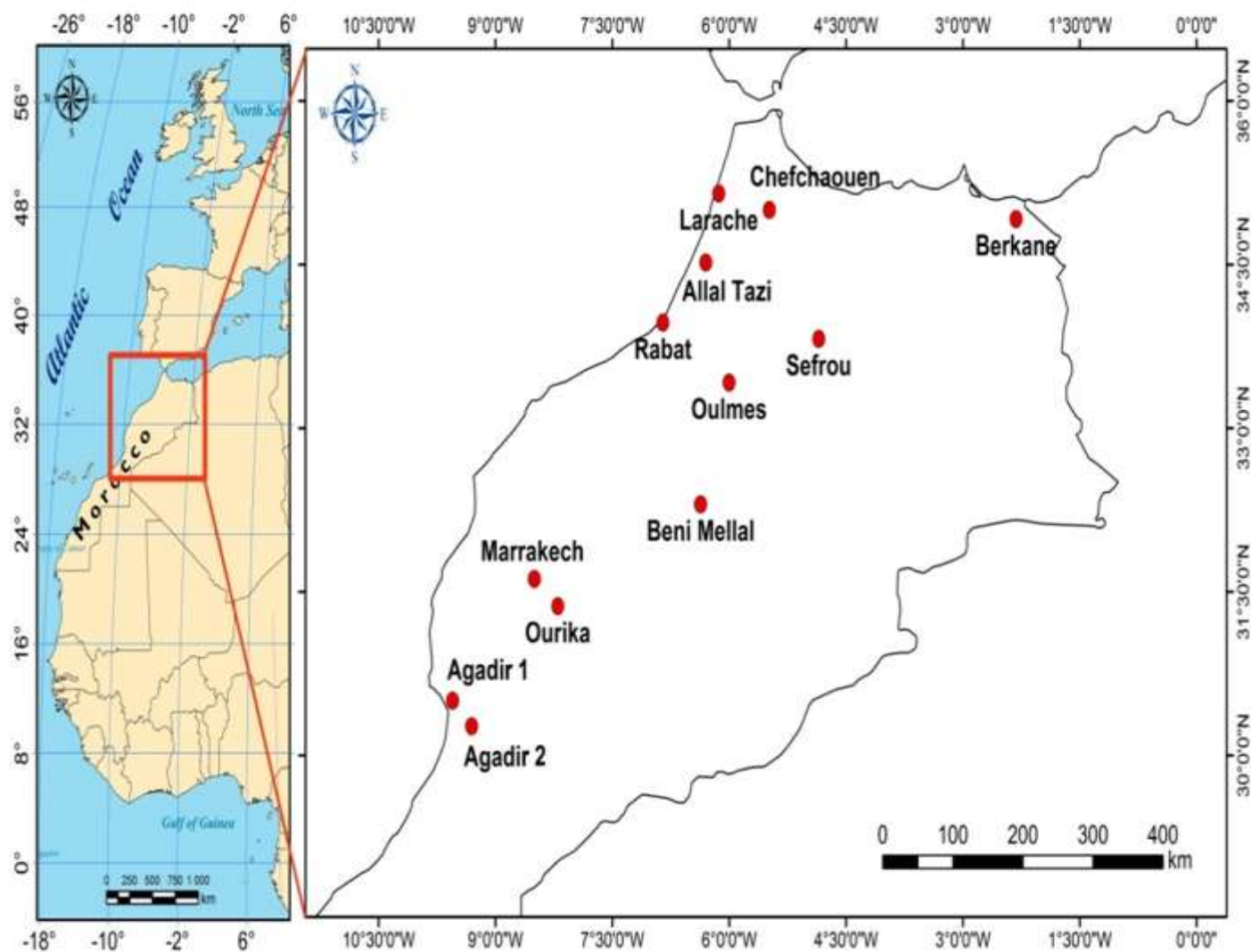


Figure 1: Location map of the twelve study sites

The plants were harvested at the end of August 2014; five individual plants were randomly selected at each site. The height, fresh weight and leaf to stem ratio (L/S ratio) per plant were recorded at the time of harvest (Table 1). The brown and yellow leaves were removed from the plants then the green leaves were dried in heated greenhouses avoiding direct sunlight for 48-72 h.

Table 1: Physical characteristics of the plants harvested

	Height (cm)		Biomass (g/plant)		L/S ratio	
	Mean \pm SE	Min-Max	Mean \pm SE	Min-Max	Mean \pm SE	Min-Max
Agadir 1	92.60 ^{ab} \pm 6.65	73-110	661.54 ^a \pm 40.59	456.70- 865.20	1.14 ^a \pm 0.037	0.84-1.32
Agadir 2	80.20 ^{cd} \pm 4.19	71-93	426.21 ^{bc} \pm 37.05	273.19- 564.37	0.80 ^b \pm 0.032	0.55-1.08
Allal Tazi	78.14 ^{cd} \pm 6.09	61-96	177.89 ^e \pm 17.93	123.93- 233.71	0.93 ^{ab} \pm 0.086	0.70-1.23
Beni Mellal	83.60 ^{bcd} \pm 2.21	77-89	61.73 ^f \pm 11.07	15- 91	0.89 ^{ab} \pm 0.016	0.61-1.12
Berkane	79.60 ^{cd} \pm 4.99	71-100	345.63 ^{cd} \pm 23.14	217.01- 456	0.97 ^{ab} \pm 0.051	0.41-2.17
Chefchaouen	72.14 ^{cd} \pm 2.18	64-82	498.21 ^b \pm 7.73	133.90-916.60	0.63 ^b \pm 0.035	0.55-0.75
Larache	95.91 ^a \pm 4.18	83-107	346.13 ^{cd} \pm 28.38	239.80- 489.62	0.96 ^{ab} \pm 0.039	0.63-1.21
Marrakech	85.31 ^{abc} \pm 5.02	71-110	405.11 ^{bc} \pm 39.84	223.33-615.87	0.82 ^{ab} \pm 0.046	0.61-1.20
Oulmes	83.53 ^{bcd} \pm 3.09	77-110	265.39 ^{de} \pm 25.51	167.92- 436.68	0.76 ^b \pm 0.093	0.49-1.13
Ourika	75.89 ^{cde} \pm 5.50	60-95	192.43 ^e \pm 20.87	138.22- 307.67	0.93 ^{ab} \pm 0.051	0.80-1.00
Rabat	66.60 ^e \pm 1.91	60-71	205.96 ^e \pm 22.43	114.85- 320.88	0.71 ^b \pm 0.060	0.62-0.91
Sefrou	81.80 ^{bcd} \pm 5.21	67-94	304.56 ^{cde} \pm 38.70	169.64- 485.90	0.76 ^b \pm 0.071	0.53-1.13

Irrigation water and soil analysis

Irrigation water and soil samples were collected from the 12 parcels to analyze the main physico-chemical properties. Concerning water samples, the electrical conductivity (EC_w) and potential hydrogen (pH_w) were determined by standard methods [21]. As of the soil analyses, 0-20 cm depth samples were collected from each site and the main physico-chemical parameters measured were the salinity (dS/m) [22], organic matter (OM %) [23], available phosphorus P₂O₅ (ppm) [24] and exchangeable potassium K₂O (ppm) [21]. Granulometric analysis was performed according to Robinson [25] to determine the soil texture. The results are resumed in Table 2.

Table 2: Physical and chemical characteristics of the soil and water, and geoclimatical characteristics (altitude, day length and temperature) of the twelve experimental sites

T mean (°C)	T (Min-Max) (°C)	DL (h) Min-max	Texture	pHs	ECs (mS/cm)	pHw	ECw (mS/cm)	OM (%)	N	K ₂ O (ppm)	P ₂ O ₅ (ppm)
24	15.4-36.2	12.52-14.62	Sandy	9.16	0.52	7.92	0.53	1.42	0.06	373.55	12.96
24	15.4-36.2	12.52-14.62	Sandy loam	7.4	1.83	7.24	1.56	1.24	0.05	590.45	6.48
30.3	13.8-30.3	12.58-14.45	fine Silt	7.56	1.2	7.3	1.5	2.98	0.23	617.56	14.31
29.6	16-43.7	12.55-14.28	Silty sand	7.89	0.39	7.79	0.55	1.38	0.06	66.28	16.2
27.1	14.6-40.2	12.58-14.60	fine Silt	7.9	4.54	7.74	2.97	0.83	0.05	542.25	1.89
25.9	12.5-41.5	12.58-14.50	Clay	7.24	0.75	7.14	0.7	1.63	0.16	168.7	5.4
22.9	15.4-39.8	12.60-14.53	Silty sand	7.04	0.57	7.7	0.7	1.28	0.06	204.85	28.08
29	17.5-41.1	12.53-14.22	Sandy-clay	7.56	9.68	8.14	0.69	1.15	0.06	144.6	4.86
22	14-30	12.90-14.40	Loamy	6.86	1.85	5.78	0.12	8.62	0.52	60.25	15.66
25	20-30	12.53-14.22	Clay	8	1.53	6.26	0.9	2.15	0.14	388.61	14.58
26.9	13.8-30.3	12.57-14.42	Silty sand	7.75	0.53	7.69	0.63	1.05	0.42	36.15	14.58
26.9	13.2-42.1	12.57-14.40	Silty clay	7.7	1.38	8.32	0.41	4.94	0.24	530.2	35.1

DL: day length in August (the period of harvest); T: temperature in August (the period of harvest); ECs: Electrical conductivity of soil; ECw: Electrical conductivity of water; pHs: Hydraulic potential of soil; pHw: Hydraulic potential of water; N: available nitrogen in soil; P₂O₅: available phosphorus in soil; K₂O: Available potassium in soil

Steviol glycosides analysis

The dried leaves of 2 plants which had a higher yield were selected and blended to powder form with a high speed blender. Dried ground stevia leaves were analyzed by High Performance Liquid Chromatography (HPLC) in Stevia Natura France analysis laboratory. The SGs were determined according to the JECFA (2010) method and the rate of STV, RA, and SG tot are showed in the Table 3.

Table 3: The accumulation pattern of STV, RA, SGtot and RA/STV under different Moroccan conditions Statistical analysis

	SG tot		STV		RA		RA/STV	
	Mean \pm SE	Min-Max	Mean \pm SE	Min-Max	Mean \pm SE	Min-Max	Mean \pm SE	Min-Max
Agadir1	11.81 ^{ab} \pm 0.71	11.30- 12.31	6.40 ^{ab} \pm 1.35	5.05-7.74	1.88 ^c \pm 1.17	0.71- 3.05	0.27 ^d \pm 0.125	0.14-0.39
Agadir2	9.40 ^b \pm 1.70	9.10-11.10	3.95 ^b \pm 0.35	3.60-4.30	4.25 ^{bc} \pm 1.25	3.00-5.50	1.06 ^{abc} \pm 0.225	0.83-1.28

Allal Tazi	11.72 ^{ab} ± 0.62	11.28-12.15	6.17 ^{ab} ± 1.32	4.85- 7.49	3.63 ^{bc} ± 0.83	2.80-4.45	0.65 ^{bcd} ± 0.28	0.37-0.92
Beni Mellal	14.69 ^{ab} ± 3.89	10.80-18.57	6.11 ^{ab} ± 0.87	5.24-6.97	4.77 ^{bc} ± 1.72	3.05-6.48	0.76 ^{abcd} ± 0.175	0.58-0.93
Berkane	14.42 ^{ab} ± 2.28	12.14-16.69	5.72 ^{ab} ± 0.69	5.03-6.41	6.36 ^{ab} ± 1.18	5.18-7.54	1.11 ^{abc} ± 0.075	1.03-1.18
Chefchaouen	14.20 ^{ab} ± 2.00	12.20-16.20	5.60 ^{ab} ± 1.50	4.10-7.10	6.80 ^{ab} ± 0.60	6.20-7.40	1.20 ^{ab} ± 0.16	1.04-1.35
Larache	14.05 ^{ab} ± 2.10	11.95- 16.14	8.24 ^a ± 2.72	5.52-10.95	3.53 ^{bc} ± 1.09	2.44- 4.61	0.53 ^{bcd} ± 0.31	0.22-0.84
Marrakech	13.37 ^{ab} ± 0.89	12.48- 14.25	6.09 ^{ab} ± 0.48	5.61- 6.57	4.81 ^{bc} ± 1.30	3.51-6.10	0.81 ^{abcd} ± 0.28	0.53-1.09
Oulmes	17.19 ^a ± 2.47	15.44-18.94	6.18 ^{ab} ± 0.54	5.64- 6.72	8.61 ^a ± 1.28	7.33- 9.88	1.39 ^a ± 0.085	1.30-1.47
Ourika	10.95 ^b ± 0.75	10.20-11.70	4.85 ^{ab} ± 0.15	4.70-5.00	4.50 ^{bc} ± 0.70	3.80-5.20	0.94 ^{abcd} ± 0.175	0.76-1.11
Rabat	10.88 ^b ± 0.57	10.31-11.44	4.22 ^{ab} ± 1.55	2.67-5.76	3.35 ^{bc} ± 0.49	2.86-3.84	0.85 ^{abcd} ± 0.18	0.67-1.03
Sefrou	13.44 ^{ab} ± 0.16	13.28-13.60	7.33 ^{ab} ± 0.64	6.69-7.96	3.36 ^{ab} ± 0.89	2.47-4.24	0.47 ^{cd} ± 0.16	0.31-0.63

All values are means of duplicated determinations ± standard error (SE); Means within columns with different letters are significantly different ($P < 0.05$); SGtot: total steviol glycosides; STV: Stevioside; RA: Rebaudioside A; RA/STV: Rebaudioside A to Stevioside ratio;

Experimental data of STV, RA, SGtot, RA/STV ratio, height, L/S ratio and biomass were subjected to the analysis of variance (ANOVA) using the SAS software (SAS 2004). Furthermore, multiple comparisons of means and their ranking were performed using Student-Newman-Keuls test whenever the ANOVA revealed significant differences.

Pearson correlation analysis was performed in order to quantify the relationship that may exist between the environmental parameters within twelve study sites and the quality parameters of stevia (STV, RA, SGtot, RA/ST ratio, height, L/S ratio, and biomass).

RESULTS AND DISCUSSION

Characteristics of experimental sites

The results of physico-chemical analysis of water and soil used for the cultivation of stevia in the 12 areas are summarized in Table 1. Soil pH (pHs) is in general alkaline in all sites except for Oulmes, where pH value indicated an acid soil. Soil and water salinity was low for all the sites except Berkane and Marrakech, where salinity was high ($EC_w=2.97$ dS/m at Berkane and $EC_s = 9.68$ dS/m at Marrakech). Organic matter (OM) was also low for all sites except in Oulmes site which resulted from the fact that manure was supplied before soil sampling for analysis. Moroccan soils are in general rich in potassium, which is confirmed by the analysis of all soil samples, except Rabat, Oulmes, and Beni Mellal sites, but nitrogen and available phosphorus were low, meaning that these elements should be applied before planting.

In this study, data showed that there is no big difference in means of day length (DL) between the twelve areas. DL during the period of our study ranged from 11.45 h on March to 14.62 h on August (Table 1). In general, the mean temperature ranged from 13.5 on March to 27.5°C on August.

Biomass

Biomass expressed as g/plant including stems and leaves, L/S ratio, and height are presented in Table 2. Stevia height can reach about 1 m in Moroccan conditions. Heights measured at the twelve sites were ranging from 60 to 110 cm, the highest values were observed in Agadir site 1, Marrakech, Oulmes and Larache (107-110 cm), on the contrary the lowest values were observed in Rabat, Ourika and Allal Tazi (60-61 cm) (Table 2). On the other hand, L/S ratio varied from 0.41 to 2.17, the highest values were recorded in Oulmes and Agadir 1 (Table 2). Data analysis using ANOVA showed that there is significant difference in height between the 12 different locations; the highest means were reported in Larache (95.91 cm) followed by Agadir 1 (92.60 cm) and Marrakech (85.31 cm), respectively as shown in Table 2. Also the study conducted by Angelini and Tavarini [25] showed that the plant height was significantly affected by the cultivation site.

Plants harvested in the twelve locations produced mean biomass varying from 61.73 to 661.54 g/plant, with the largest biomass recorded in Agadir 1, and the lowest values were recorded in Beni Mellal (Table 2).

During the period of our experiment, DL ranged from 11.45 h in March to 14.62 h in August and T° ranged from 5 to 38°C. During the period of transplantation (late April), days were getting longer (>11 h) and warmer (>19°C) which prolonged the vegetative growth stage in stevia and sustained SGs accumulation [26-28]. We observed that seed germination was higher than 50% by the end of March when temperature was higher than 17°C. Also, transplantation is only possible in late April when temperature is higher than 19°C. The bud stage is observed in late August where in general DL begins to decrease, <14 h (Table 1), which promotes flowering. Stevia is an obligate short day plant [29] with a critical DL of about 13 h. SGs synthesis is reduced before flowering, since DL influences yield of aerial biomass [30]. In our investigation, flowering time was about 120 days by date of transplantation in Moroccan conditions. Precise investigations on day length and time required for, described that flowering occurred within 46 days at 11 h day length, while it was extended to 96 days when photoperiod was extended for 12.5 h. [31].

Steviol glycosides production in the twelve locations

The analysis of the total amount of SGs per leaf (SGtot) revealed interesting results in the different locations with the greatest amount of SGtot and RA found in Oulmes with 18.94 and 8.61 % respectively. The accumulation pattern of STV, RA, and SGtot under different Moroccan conditions is presented in Table 3.

There is a significant variation in STV, RA, and SGtot pattern in the twelve sites, where the highest amount of RA in leaves was observed in Oulmes (8.61%) and the lowers was observed in Agadir 1 (1.88 %).

The leaf content of RA produced in 2014 varied between 1.88 and 8.61% which is higher than the content reported by Moraes *et al.* [32] in Mississippi, 1.98 and 6.44% or in Italy 8.36% (w/w) [19]. The STV content 6.67% was higher than the content reported by Megeji *et al.* [33] in India with 6.09%, when in Mississippi STV produced in 2010 and 2011 was, 10.58% and 11.05% respectively (Moraes *et al.*) [32] and in Italy, 5.72% [19].

In our study SGtot in leaves ranged from 9.30 to 17.19% while in Nakamura et al. [34] study, it ranged from 6.7 to 18.6% (w/w). It can be concluded that stevia can be adapted to all type of soils and poor in nutrients, however for clay soils it is important to drain well, the roots of stevia developing in surface; to prevent the plants suffocate if water is retained by the soil.

The RA/STV ratio in leaves used as an indicator of the organoleptic quality of leaves, was greatest than 1 in Oulmes (1.39), Chefchaouen (1.20), Berkane (1.11) and Agadir 2 (1.06), leaves contains greater amounts of RA than STV especially in Oulmes, with RA having greater economic value than STV as an alternative sweetener. Thus, the sweetness quality is greater in these sites with high concentration of RA.

Relationships between quality parameters, stevia biomass, soil and water characteristics

It was shown that STV ($p < 0.05$) and RA ($p < 0.01$) content were significantly correlated with SGtot, as we can see in Table 4 RA is highly correlated with the total steviol glycosides content.

Table 4: correlation between quality parameters, stevia biomass, and edaphoclimatique characteristics

	STV	RA	S Gt ot	Alt itu de	p Hs	ECs	P ₂ O ₅	OM	K ₂ O	N%	pH w	EC w	DL max Augus t	DL min Augus t	DL mean Augus t	T° max Augus t	T° min Augus t	T° Mea Augus t
ST V		$r^2=0.167$ $p=0.603$	0.64 0.02 0.05	0.165 0.608	0.021 0.948	-0.315 0.092	0.507 0.092	0.36 0.251	0.01 0.975	-0.045 0.890	0.039 0.903	-0.215 0.503	0.475 0.119	0.481 0.114	0.475 0.119	0.406 0.191	0.445 0.147	-0.075 0.817
RA	0.167 0.603		0.795 0.002	0.53 0.077	0.694 0.12	0.169 0.599	-0.231 0.470	0.462 0.131	-0.338 0.283	0.367 0.24	-0.585 0.046	0.079 0.807	0.13 0.687	0.26 0.414	0.443 0.149	0.283 0.373	-0.180 0.576	0.063 0.845
SG tot	0.639 0.025	0.795 0.002		0.483 0.112	0.360 0.251	-0.122 0.706	0.197 0.539	0.598 0.040	-0.346 0.270	0.352 0.264	-0.270 0.039	0.147 0.649	0.377 0.228	0.366 0.242	0.479 0.115	0.494 0.102	-0.406 0.191	0.030 0.926
RA /ST V	0.274 0.388	0.885 0.0001		0.44 0.218 0.063	0.741 0.006	0.333 0.291	0.447 0.145	0.213 0.505	-0.363 0.246	0.336 0.285	-0.558 0.059	0.174 0.589	-0.043 0.895	0.106 0.744	0.357 0.254	0.076 0.814	-0.022 0.946	0.040 0.901
Hei ght	0.531 0.076	-0.055 0.501		0.234 0.046 0.073	0.229 0.474	0.098 0.763	0.372 0.237	0.053 0.869	0.037 0.909	-0.438 0.155	0.028 0.931	-0.164 0.610	0.481 0.275	0.184 0.692	0.392 0.385	0.139 0.766	-0.264 0.567	-0.103 0.825
Bio ma ss	0.795 0.02	0.215 0.501		0.187 0.035 0.054	0.293 0.356	0.149 0.643	0.226 0.480	0.188 0.558	0.037 0.909	-0.334 0.289	0.285 0.369	0.019 0.954	-0.191 0.651	-0.147 0.729	-0.242 0.565	0.188 0.656	0.437 0.279	0.485 0.223

r^2 =Pearson Correlation Coefficients; p= probability; **Correlation is significant at the 0.01 level; *Correlation is significant at the 0.05 level; DL: day length in August (the period of harvest); T: temperature in August (the period of harvest); ECs: Electrical conductivity of soil; ECw: Electrical conductivity of water; pHs: Hydraulic potential of soil; pHw: Hydraulic potential of water; N: available nitrogen in soil; P₂O₅: available phosphorus in soil; K₂O: Available potassium in soil; SGtot: total steviol glycosides; STV: Stevioside; RA: Rebaudioside A., RA/STV: Rebaudioside A to Stevioside ratio

Generally, sites having lower pHw showed dry leaves with higher patterns of RA ($p < 0.05$), this inverse correlation was also found with pHs ($p < 0.05$), the highest amount of RA (8.61% w/w), was recorded in Oulmes with pHw= 5.78 and pHs =6.86.

The highest RA and SGtot levels were observed in regions with pHw and pHs tending towards acidity like in Oulmes with pHw= 8.61. However, RA was the lowest (1.88% Table 2) in Agadir 1 with alkaline pHw and pHs (Table 1). Thus stevia preferred acid pHw and pHs, which promote the accumulation of significant levels of RA and therefore a better tasting quality of the leaves. It is known that more the soil is acid; more aluminum is found in soluble forms and therefore assimilable by the plant; which could cause stress for stevia and therefore producing more RA.

The SGtot, RA and, STV did not change much at different salinities of water and soil in the sites, also the effect of soil texture and nutrients (K₂O, P₂O₅, N%, MO) was not significant (Table 4). At some sites, Marrakech and Berkane, we noticed that despite the high salinity of the soils, SGtot, RA and STV levels were interesting, so the stevia crop can withstand salt stress. Reis observed in his study that plant growth and yield were reduced when the ECw was higher than 2 mS/cm [35]. Two harvests can be obtained if only low salinity irrigation water is used.

CONCLUSION

Morocco is one of the countries whose population, is known by its high consumption of sugar, she also suffers from obesity and diabetes that present an economic burden for the country, and this is making the stevia culture an important and beneficial alternative to cane sugar. In the present study, we tested the feasibility of growing stevia in twelve sites with different geological and edaphoclimatic characteristics in order to better understand the behavior of the crop, the results show that geological and edaphoclimatic factors, such as altitude, day length, temperature, soil and irrigation water in the field affect the quantity and quality of the yield. Stevia was very well adapted in the twelve locations, in fact the production of SGs (STV, RA and SGtot) was very interesting as well as the production of leaves, the main source of extraction of SGs. We found that stevia crop can grow in different types of soils even with low nutrient levels, hence stevia is able to adapt to poor quality soils. It was also recorded that stevia is suitable to be grown in areas with high EC conditions (Marrakech and Berkane). Furthermore the organoleptic quality is greatly increased with low pH (Oulmes), also stevia requires at least DL of 12 h and temperature of 19°C, the production is ideal in areas with long and warm days, so flowering is delayed which allowed more time for glycoside and aerial biomass accumulation. The concentration of secondary metabolites and biomass yield under the influence of these factors can be modulated by cultural practices. In conclusion; *S. rebaudiana* showed interesting potential for introduction as a commercial agricultural crop for steviol glycosides production in Morocco.

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