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Characterization of Lebanese Germplasm of Snake Melon (*Cucumis melo* subsp. *melo* var. *flexuosus*) Using Morphological Traits and SSR Markers

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Abstract: Snake melon (*Cucumis melo* subsp. *melo* L. var. *flexuosus* (L.) Naudin) is an ancient and traditional crop in the Mediterranean region. Nevertheless, there has been poor interest in assessing snake melon germplasm where its genetic resources have not been surveyed before despite their potential in adaptation to environmental changes. In this study, we assess the genetic diversity of snake melon landraces collected from different Lebanese regions at both morphological and molecular levels. Morphological characterization using a set of 18 descriptors revealed an important phenotypic variability among the landraces studied. Principle component analysis indicated that fruit hair and its consistency, fruit size, and skin color pattern were good criteria for discriminating among landraces. Based on the scatter plot diagram, landraces of snake melon formed five different groups with one being defined as typical var. *flexuosus*. Ten simple sequence repeat (SSR) markers were used for the molecular characterization. Fifty-six different alleles were detected, with an average of 5.6 alleles per locus. Polymorphism information content of SSR markers ranged from 0.06 to 0.84 (average 0.38). Cluster analysis based on molecular markers showed high genetic diversity and divided the landraces into five distinct genetic groups, confirming thereby the morphological variability. Findings of this study indicate a significant diversity for the Lebanese snake melon germplasm that must be further conserved and considered in improvement programs of this ancient crop.

Keywords: Armenian cucumber; fruit morphological descriptors; SSRs markers; germplasm diversity

1. Introduction

Snake melon or Armenian cucumber, also known as snake cucumber (*Cucumis melo* subsp. *melo* L. var. *flexuosus* (L.) Naudin; $2n = 2x = 24$), is a member of the cucurbits family. It is an ancient crop that has been enjoyed and esteemed in Mediterranean antiquity by several civilizations [1]. Snake melon is a rain-fed crop where it flourishes in sunny and warm climates and it is commonly grown in the Levant area, Asia Minor, and North Africa. Snake melon fruits are harvested immature and green and consumed when fresh or pickled [2,3]. It earned its common name “snake melon” from its very long, slender, curved, and twisted fruits [4]. However, it is recognized around the world by different

names where it's called fakous in North Africa and some Arabic countries, alficoz in Spain, tortarello, citrangolo, or cucummaru in Italy, hiti in Turkey, and mekte in Lebanon and Syria [5].

Unlike most melon fruits that are sweet and have a 1:1 fruit length-to-width ratio, snake melon fruits are non-sweet and have long fruits with a length-to-width ratio of 4:1 or higher [4,6]. Cultivars of snake melon differ from one another in the external color of the immature fruits, which can be light green, dark green, or a mixture of both colors [3,7]. They can also be distinguished by the presence or absence of pubescence on the skin and by the longitudinal ribbing of the fruits from being very shallow to very deep. Furthermore, the 4:1 length-to-width ratio is often greatly exceeded, where fruits over 50 cm long and only 5 cm wide may be observed [8].

The diversity of snake melon has been investigated using several morphological characters [9] and molecular markers [10]. It is difficult to distinguish genotypes based only on their external morphology. Molecular markers offer more informative and accurate data because they are numerous, easy to use, and are not related to the agroclimatic factors that have uncontrolled effect on the expression of the morphological traits [11–13]. To date, only few studies are available regarding the characterization and evaluation of snake melon. Varieties have been assessed in Turkey [14,15], Greece/Cyprus [16], Iran [12], Sudan [17], Jordan [13], Tunisia [18], and Palestine [9,10]. Several molecular markers have been effectively used to assess the genetic diversity of melons. However, simple sequence repeats (SSRs) have proven to be useful marker systems in recent diversity studies [15,16,19,20].

In Lebanon, snake melon is an ancient vegetable crop commonly grown in different agroclimatic areas across the country. The long history of agriculture in this part of the world and the effect of anthropogenic activities, along with the diverse topographic and bioclimatic conditions present in the country, in addition to the outcrossing nature of snake melon, suggest the existence of a wide genetic diversity of this crop that remains to be explored [1,13,21]. As to the statistics of the Ministry of Agriculture in 2016, snake melon growing in Lebanon covers 1600 ha distributed at different altitudes with an annual production fluctuating from 12 to 40 tons per ha [22]. It relies almost exclusively on traditional local varieties known as landraces that are often distributed within an informal traditional seed system through farmer-to-farmer seed exchange or bought directly from farmers' seed stores [21,23]. Landraces are generally characterized by their specific adaptation to the environmental conditions of the region of domestication. They are named and maintained by farmers to meet their social, economic, cultural, and environmental needs [21,24]. Snake melon *flexuosus* landraces with typical long and slender fruits are globally threatened by severe genetic erosion due to the underestimation of their valuable genetic importance by commercial growers mainly, where they are substituting local cultivars by improved ones introduced into the country [21,23]. Therefore, it is very important to characterize and evaluate the snake melon local germplasm for further conservation and sustainable utilization actions.

The main objective of this study was to establish an inventory of the Lebanese snake melon landraces growing in Lebanon, and to assess their phenotypic and molecular variability. Results are expected to pave the road for founding the first Lebanese snake melon collection in the country. These snake melon genetic resources will be further evaluated for their agronomic traits, their genetic variability, and their adaptation to harsh conditions prior to their utilization in breeding programs.

2. Materials and Methods

2.1. Collection of Landraces

The collection missions were performed during the period extending from January to March 2019. Seeds of snake melon landraces (also called here accessions) were collected from various areas across Lebanon obtained from local farmers and farmers' seed stores (Figure 1). Vernacular names along with the geographic data were recorded for each accession (Table 1). A total of 70 accessions were identified and shared with the national gene bank at the Lebanese Agriculture Research Institute.

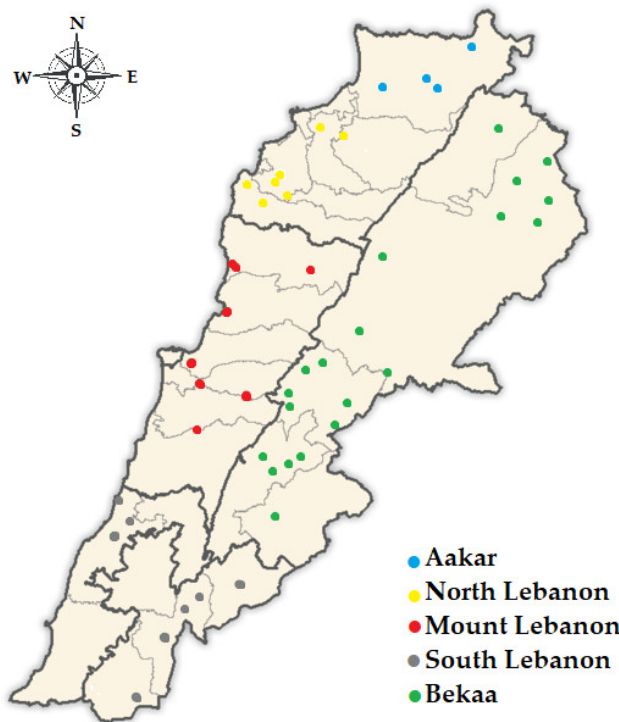


Figure 1. Geographic distribution of collection sites of the snake melon seeds landraces.

Table 1. Details of collected Lebanese snake melon accessions used in this study.

Code	VN ¹	Area	Province ²	Source	Code	VN ¹	Area	Province ²	Source
ML1	Akhdar	Aley	ML	Store	SL36	Akhdar	Bint Jbeil	SL	Store
ML2	Akhdar	Mecherfeh	ML	Farmer	SL37	Abyad	Bint Jbeil	SL	Store
ML3	Abyad	Aley	ML	Store	SL38	Akhdar	Saida	SL	Store
ML4	Abyad	Aley	ML	Store	ML39	Abyad	Dekweneh	ML	Store
B5	Abyad	Kherbet Kanafar	B	Farmer	SL40	Abyad	Tebnine	SL	Store
B6	Abyad	Terbol	B	Farmer	B41	Abyad	Kaa	B	Farmer
B7	Abyad	Deir el Ahmar	B	Farmer	ML42	Akhdar	Mrouj	ML	Store
SL8	Azrak	Tebnine	SL	Store	ML43	Akhdar	Mrouj	ML	Store
B9	Abyad	Hermel	B	Farmer	NL44	Akhdar	Aabrine	NL	Farmer
B10	Abyad	Hermel	B	Farmer	NL45	Mdalaa	Kfifan	NL	Farmer
B11	Abyad	Jeb Jenin	B	Store	NL46	Akhdar	Bechmezzine	NL	Farmer
ML12	Abyad	Jounieh	ML	Store	NL47	Akhdar	Bechmezzine	NL	Farmer
B13	Abyad	Temnine el Foka	B	Farmer	NL48	Akhdar	Bechmezzine	NL	Farmer
B14	Abyad	Hermel	B	Farmer	NL49	Akhdar	Bteram	NL	Farmer
SL15	Akhdar	Houmin	SL	Farmer	NL50	Akhdar	Amyoun	NL	Farmer
SL16	Abyad	Zefta	SL	Farmer	NL51	Akhdar	Kfar Habou	NL	Store
SL17	Abyad	Khiyam	SL	Store	NL52	Akhdar	Aalma	NL	Store
B18	Abyad	Baalbak	B	Farmer	ML53	Akhdar	Baakleen	ML	Farmer
ML19	Chikawi	Jbeil	ML	Farmer	ML54	Akhdar	Laqlouq	ML	Farmer
ML20	Akhdar	Jbeil	ML	Store	A55	Abyad	Halba	A	Store
NL21	Akhdar	Bechmezzine	NL	Farmer	A56	Abyad	Halba	A	Store
SL22	Abyad	Hasbaya	SL	Store	A57	Hayye	Halba	A	Store
SL23	Abyad	Hasbaya	SL	Farmer	A58	Akhdar	Borj	A	Farmer
SL24	Akhdar	Qlayaa	SL	Store	ML59	Mshakal	Amchit	ML	Farmer
B25	Abyad	Rachaya	B	Store	A60	Akhdar	Beino	A	Farmer
B26	Tawile	Saad Nayel	B	Farmer	B61	Abyad	Hrbata	B	Store
B27	Abyad	Rachaya	B	Farmer	B62	Abyad	Deir el Ahmar	B	Store
B28	Azrak	Rachaya	B	Store	B63	Abyad	Nabi Othman	B	Store
B29	Abyad	Rachaya	B	Store	B64	Abyad	Kaa	B	Store
B30	Abyad	Niha	B	Store	B65	Abyad	Jabouleh	B	Farmer
B31	Abyad	Jeb Jenin	B	Store	B66	Abyad	Labweh	B	Store
B32	Mshakal	Ghaze	B	Store	B67	Abyad	Sariin	B	Farmer
B33	Abyad	Hosh el Harim	B	Store	A68	Abyad	Qoubayat	A	Store
B34	Abyad	Sariin	B	Farmer	A69	Abyad	Qoubayat	A	Store
B35	Abyad	Aanjar	B	Store	B70	Abyad	Kab Elias	B	Store

¹ VN: Vernacular name; ² A: Akkar; B: Bekaa; ML: Mount Lebanon; NL: North Lebanon; SL: South Lebanon.

Collected accessions were submitted to both morphological and molecular assessments that have been carried on at the experimental field and laboratories of Warsaw University of Life Science, thus within the in-kind collaboration and documented exchange of seed plant genetic resources between Lebanon and Poland.

2.2. Morphological Characterization

For the morphological evaluation 10 seeds from each accession were sown in a compost media. At the 2-leaf stage, seedlings were transplanted to the field. Characterization of the snake melon accessions was done at the production stage based on a list of descriptors previously developed by IPGRI, Stepansky et al., and Youssif et al. [17,25,26]. For each accession, a set of 15 immature fresh fruits were randomly taken five days after fruit set. In total, 18 traits were studied including 12 qualitative and 6 quantitative ones (Table 2): pedicel separation and length, predominant and secondary skin color along with the color pattern covering the skin, presence or absence of fruit hair and their consistency, skin glossiness, fruit shape, texture and weight, ribs appearance and number, flesh color, sweetness, fruit length from stem end to blossom, width at the broadest point, and seed weight per fruit. Also, flower sex expression was recorded for each accession.

Table 2. Morphological traits recorded in the Lebanese snake melon accessions.

No	Trait Code	Trait and Descriptive Value	Reference
1	ST	Sex type: 1 = monoecious; 2 = andromonoecious	[26]
2	PSEP	Pedicel separation from fruit: 1 = easy; 2 = hard	[25]
3	PLEN	Pedicel length: 1 ≤ 5.7 cm; 2 = 5.71–9 cm; 3 ≥ 9.01 cm	[25]
4	PC	Primary fruit skin color: 1 = white; 2 = green	[25]
5	SC	Secondary skin fruit color: 1 = white; 2 = green; 3 = dark green	[25]
6	PAT	Secondary skin color pattern: 1 = absent; 2 = striped	[25]
7	FSH	Fruit shape: 1 = elongate; 2 = semi curved; 3 = curved	[17]
8	FGLO	Fruit glossiness: 1 = dull; 2 = glossy	[25]
9	FTEX	Fruit texture: 1 = smooth; 2 = wrinkled; 3 = ribbed	[26]
10	FH	Fruit hair: 1 = absent; 2 = present	[25]
11	FHC	Fruit hair consistency: 1 = absent; 2 = few; 3 = many	[25]
12	FRIB	Fruit ribs appearance: 1 = absent; 2 = weak; 3 = strong	[17]
13	FRNO	Fruit number of ribs: 1 ≤ 13; 2 = 13–23; 3 ≥ 23	[17]
14	FC	Fruit flesh color: 1 = white; 2 = green	[25]
15	FSWT	Fruit sweetness (Brix): 1 ≤ 4; 2 = 4.01–7.5; 3 ≥ 7.51	[25]
16	FSZ	Fruit size: 1 ≤ 20 g; 2 = 20.01–40 g; 3 ≥ 40.01 g	[25]
17	FLEN	Fruit length/width ratio: 1 ≤ 6; 2 = 6.01–10.5; 3 ≥ 10.51	[25]
18	SW	Seed weight: 1 ≤ 0.3 g; 2 = 0.31–0.45 g; 3 ≥ 0.46 g	[26]

2.3. DNA Isolation

Seedlings of snake cucumber accessions were grown in greenhouse under controlled conditions. At the 2–3 leaf stage, young leaf samples were collected from four seedlings from each accession and directly frozen in liquid nitrogen. Genomic DNA was extracted from the leaf samples by using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The quality and quantity of the isolated DNA was checked spectrophotometrically using Nanodrop 2000 (ThermoFisher™, Waltham, MA, USA). All DNA samples were diluted using distilled water to equal concentration and stored at −20 °C.

2.4. SSR Analysis

Ten simple sequence repeat (SSR) primers previously used in similar analyses and/or developed for melon were selected for this study [27–29]. The polymorphic chain reactions (PCR) comprised of 5 ng of genomic DNA, 5.59 µL RNase free water, 1.5 µL DreamTaq™ 10X Green Buffer (Thermo Fisher Scientific, Waltham, MA, USA), 1.5 µL dNTPs (2 mM), 0.6 µL Forward Primer (5 µM), 0.6 µL Reverse Primer (5 µM), 0.15 µL MgCl₂ (50 mM), and 0.06 µL DreamTaq™ DNA Polymerase (5 U/µL)

(Thermo Fisher Scientific, USA). The amplification was performed in a Master Gradient thermal cycler (Eppendorf, Hamburg, Germany). The cycling conditions were as follows: an initial denaturation at 94 °C for 2 min; 35 cycles of 2 min at 94 °C of denaturation, 1 min of primer annealing at 55 °C, and 2 min of extension at 72 °C; the program ended with a 10-min final extension step at 72 °C. PCR products were stored at 4 °C prior to analysis. After amplification, 6 µL of loading buffer (95% formamide, 10 mM EDTA (pH 8.0), 0.025% xylene cyanol, and 0.025% bromophenol blue) was added to each reaction tube. The samples were heat denatured for 5 min at 95 °C and quickly cooled on ice. To detect polymorphism, 1 µL of the amplified product was loaded on a 6% denaturation polyacrylamide gel that had been preheated for 30 min and visualized by silver staining. DNA fragment size was estimated using a 50–350 DNA ladder [30,31].

2.5. Statistical Analysis

For morphological characterization and with the aim to compare together qualitative and quantitative data, a numerical transformation was applied to all traits. For quantitative traits, arithmetic mean, standard deviation (*s.d.*) and coefficient of variation were calculated. The descriptive statistics were later used to classify the accessions into three classes according to Zar [32]; small (<Mean – *s.d.*), medium (>Mean – *s.d.*) and large (>Mean + *s.d.*). The Shannon–Weaver diversity index (H') for qualitative and quantitative traits was estimated as follows [33]:

$$H' = \sum_{i=1}^n (p_i \ln p_i)$$

where, p_i is the proportion of individuals in the i^{th} class of the n class trait and n is the number of phenotypic classes for a given trait.

Principal component analysis (PCA) was performed to generate a cluster diagram. Eigen value and contribution percentage of the first three principal component axes were calculated using the correlation matrix among 15 descriptors for 66 genotypes. All computations were performed using R software version 3.6.1 [9].

Bands revealed by SSR markers were scored as absent (0) or present (1), and then data were used to generate a pair-wise similarity matrix using Jaccard's coefficient [34]. The unweighted pair-group method with arithmetic mean (UPGMA) was employed to create the clustering dendrogram [35]. Polymorphism information content (PIC) values were calculated according to Smith et al. [36], using the algorithm for all primer combinations as follows:

$$PIC = 1 - \sum_{i=1}^n f_i^2$$

where $i = 1$ is the frequency of the i^{th} allele.

3. Results

3.1. General Status of Collected Accessions

A total of 70 snake melon accessions were collected during this survey. Figure 1 and Table 1 show the distribution of the sites visited during the collection missions. Eight accessions were found in Akkar, 10 in North Lebanon, 13 in Mount Lebanon, 11 in Southern Lebanon, and 28 in the Bekaa region. Sources of plant material were obtained from farmers and commercial agricultural stores. The accessions were collected from rural areas, where farmers apply the seed saving system and plant small or vast surfaces of snake melon for their specific uses. When accessions were collected from local farmers' seed stores, efforts were made to obtain seeds from local conservation systems rather than improved materials from breeding programs.

For the 70 collected accessions, only eight vernacular names were found referring mainly to the color of the skin or to the shape. About 38 of the 70 studied accessions were named 'Abyad' (meaning white), 24 were named 'Akhdar' (meaning green), two were named 'Azrak' (meaning blue), and two were named 'Mshakal' (meaning mixed colors). In association to the fruit shape, four accessions were found namely 'Mdalaa' (meaning ribbed), 'Hayye' (meaning snake), 'Tawile' (meaning long), and 'Chikawi' (meaning thorny), represented by one accession each. It is worthy to note that there was no apparent link between the given names for accessions and their geographic distribution. However, the most diversity of vernacular names was observed in the Bekaa area (five names), followed by Mount Lebanon (four names).

3.2. Morphological Characterization

Over the 18 observed traits (Table 2), three were monomorphic and 15 were polymorphic. Studied landraces were found to be monocious and monomorphic in fruit skin glossiness and texture. They had various types of fruits but mostly with ribbed and dull skin (Figure 2).



Figure 2. Morphological variation observed in some Lebanese snake melon accessions.

Descriptive statistics including mean, minimum, maximum, and coefficient of variation (CV) were recorded for each quantitative trait measured (Table 3). Among all quantitative traits recorded, pedicel length, fruit number of ribs, sweetness, size, length/width ratio, and seed weight, showed relatively low CV values (<45%) ranging from 21.19% to 32.31%. All quantitative traits showed medium to high H' diversity index ranging from 0.51 for fruit number of ribs and 0.99 for pedicel length.

Table 3. Measured characteristics, range of variability, means, coefficient of variation, frequency distribution, and diversity index of the Lebanese snake melon accessions.

Trait	Min Value	s.d.	Mean	Max Value	CV%	Frequency of Distribution			H'
						Small	Medium	Large	
Pedicel length	3.73	1.65	7.31	11.2	22.58	0.19	0.56	0.25	0.99
Fruit number of ribs	14	4.88	18.24	25	26.80	0.10	0.84	0.05	0.51
Fruit sweetness	2.5	1.67	5.781	10.5	29.05	0.13	0.71	0.15	0.80
Fruit size	10.83	9.97	30.89	55.5	32.31	0.15	0.63	0.21	0.91
Fruit length/width ratio	3.13	2.27	8.31	18.52	27.34	0.15	0.71	0.13	0.80
Seed weight	0.15	0.07	0.366	0.61	21.19	0.16	0.66	0.16	0.88

min value: minimum value; s.d.: standard deviation; max value: maximum value; CV: coefficient of variation; H' : diversity index.

The frequency distribution of polymorphic phenotypic traits for the nine qualitative traits is shown in Figure 3. Two phenotypic classes were observed according to pedicel separation from fruit, primary fruit skin color, secondary fruit skin color pattern, fruit hair, and fruit flesh color. Easy pedicel separation, white primary color, the absence of fruit skin color pattern and pubescence, and green flesh color were more predominant among all accessions.

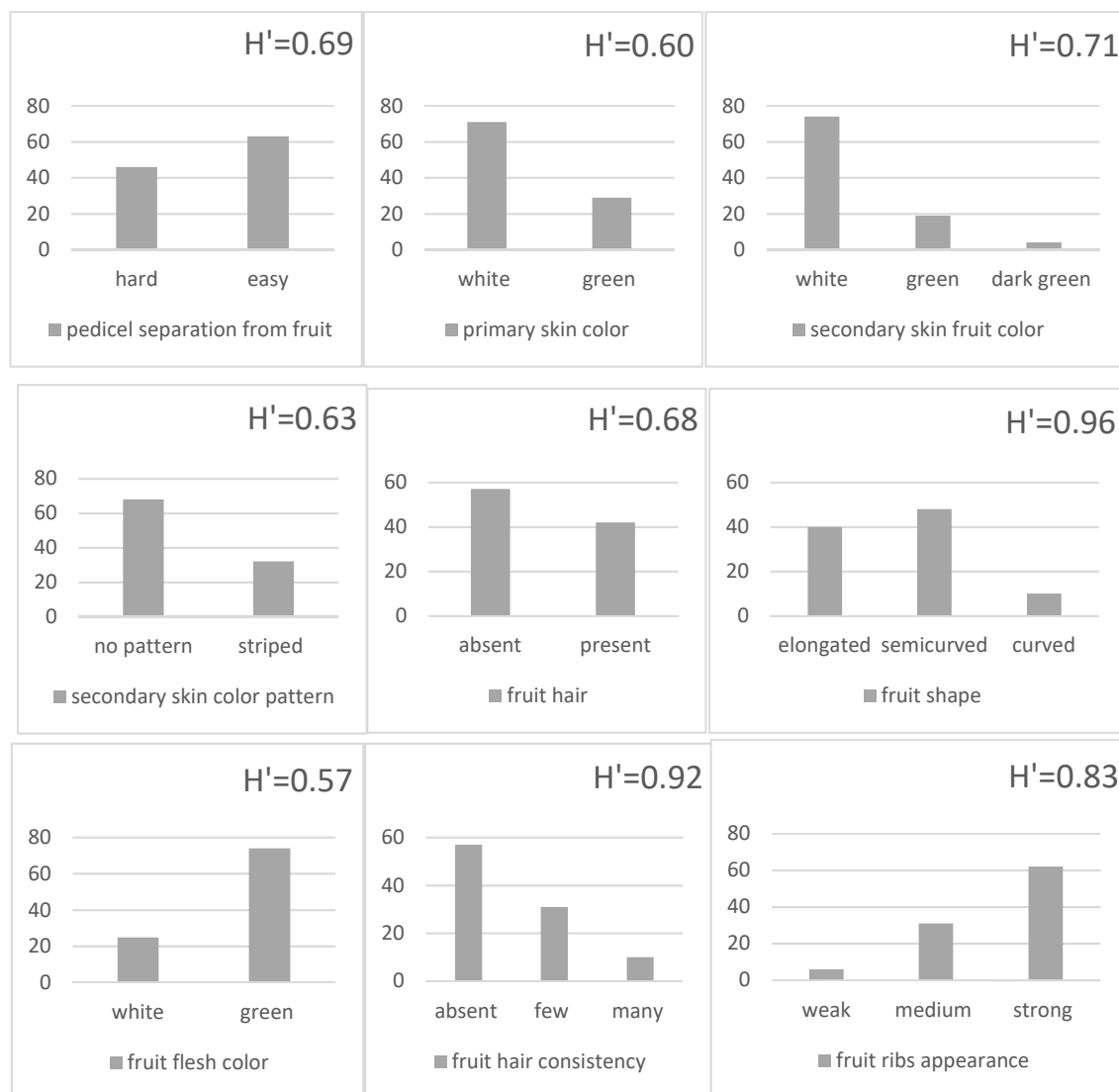


Figure 3. Diversity indices and frequencies for polymorphic qualitative traits recorded for the Lebanese snake melon accessions.

On the other hand, three phenotypic classes were generated by secondary skin fruit color, fruit shape, hair consistency, and fruit ribs appearance. The white secondary color, semi-curved fruit shape, absence in hair consistency, and strong appearance of fruit ribs were predominantly observed in the studied accessions.

The H' diversity index among classes of the studied traits was calculated (Figure 3). Overall, the qualitative traits showed intermediate to high diversity indices. The highest H' was observed with fruit shape (0.96) followed by hair consistency (0.92), while the lowest diversity among trait classes was obtained with fruit flesh color (0.57).

3.3. Principal Component Analysis

Principal component analysis (PCA) involving 15 polymorphic morphological traits (nine qualitative and six quantitative descriptors) was performed to identify the most discriminating characteristics (Table 4). The first three components present 51.51% of the total variation. The first component is characterized by a percentage of variation of 28.39% and is mainly represented by fruit hair and its consistency, skin primary and secondary colors and their respective pattern, and fruit ribs appearance. The second component explains 12.42% of the total variation and includes principally fruit size, flesh color and length to width ratio. The third component was characterized by a percentage of 10.69% and is dominated by fruit shape and sweetness.

Table 4. Contribution percentages and eigenvectors in the three first principal component analysis (PCA) components analysis of the measured traits for the Lebanese snake melon accessions.

	Component 1	Component 2	Component 3
Explained proportion of variance (%)	28.39	12.42	10.69
Cumulative proportion of variance (%)	28.39	40.81	51.51
Traits	Eigenvectors		
Pedicle separation from fruit	0.2910	0.1171	−0.3832
Pedicle length	0.3567	0.0542	0.3998
Primary fruit skin color	0.6631 *	0.0246	0.0432
Secondary fruit skin color	0.5993 *	0.1416	0.1692
Secondary skin color pattern	0.7588 *	0.0541	0.0347
Fruit shape	−0.0321	0.3429	0.6926 *
Fruit hair	0.8944 *	0.1879	0.1046
Fruit hair consistency	0.9142 *	0.1458	0.0691
Fruit ribs appearance	−0.7467 *	0.3932	−0.0715
Number of ribs	−0.4288	0.2586	0.3954
Flesh color	0.1603	−0.5997 *	0.2261
Fruit sweetness	−0.2974	−0.1822	0.6126 *
Fruit size	0.1177	0.8438 *	0.0340
Fruit length/width ratio	−0.0809	0.5125 *	−0.3574
Seed mass	0.3997	−0.2435	−0.2325

*: Significant factor loading (value over 0.50).

The first and second components distribute the 70 Lebanese snake melon accessions into four main groups (Figure 4). All accessions of group I have large size fruits with hairy skin, while accessions of group II have small to medium size fruits with hairy fruits. However, group III have glabrous fruits with a striped skin color pattern, and accessions of group IV have glabrous fruits with the absence of fruit skin color pattern. Within group IV, a small subgroup of seven accessions (B5, B7, B13, B14, B18, B34, and B41), all coming from Bekaa province, could be distinguished by their long and slender fruits, strong fruit ribs, white flesh color, and slight fruit sweetness.

Also, it was noticed that the majority of the accessions collected from South Lebanon are present in group I, while accessions from North Lebanon and Mount Lebanon were mainly scattered in groups II and III. Moreover, a large number of the studied accessions coming from Bekaa and Akkar provinces are grouped in cluster IV.

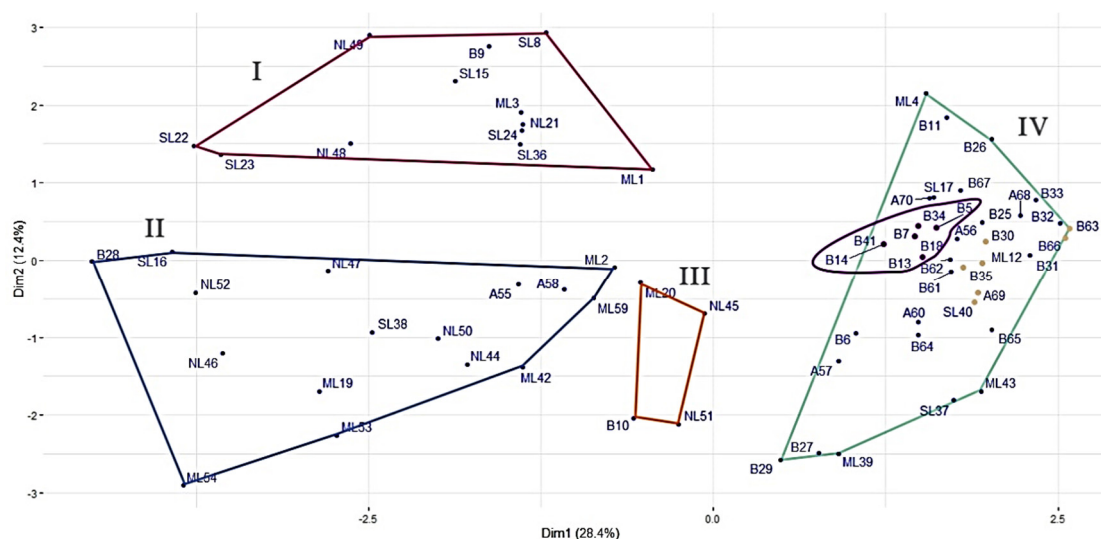


Figure 4. Cluster diagram constructed on the basis of the first two principal component axes containing 40.81% of total variation in Lebanese snake melon accessions. The small subgroup in group IV is composed of seven accessions distinguished by their long and elongated fruits, strong fruit ribs, white flesh color, and slight fruit sweetness.

3.4. Genetic Similarity

With the 10 SSRs markers used, a total of 56 alleles were detected among the 70 Lebanese snake melon accessions (Table 5). The number of alleles identified by each primer pair ranged from 2 in GMGS2-3 to 11 in CMTA134a with an average alleles number of 5.71. Size of allele fragments ranged from 59 to 340 bp. Moreover, all SSR markers were found to be polymorphic (polymorphism rate: 100%) among snake melon accessions. PIC values for SSRs ranged from 0.06 with GMGS2-3 to 0.84 with CMTA134a and the average PIC value for all the studied primer pairs was 0.38.

Table 5. Genetic variability parameters recorded for each simple sequence repeat (SSR) marker used in this study.

Primer	Sequences	Size (bp)	Total Allele Number	Major Allele Frequency	PIC	Ref
CMCTT144	CAAAGGTTTCGATTGGTGGG AAATGGTGGGGTTGAATAGG	215, 195, 192, 187, 183	5	0.68	0.29	[27]
GMAGN68	GGAAGGAAATTAGCATGCAC GCCACTCTGCTTCTTCC	192, 190, 187, 184, 180, 170, 168	7	0.51	0.37	[28]
GMGS2-3	CTCTTTTGCAATAATAATTAACC GGGCAACGAAATCCAGTCATAGA	320, 315	2	0.95	0.06	[28]
GMAGN73	ATCCAACGACCAAGAAAC CAGCTCTACAACAACATCTC	82, 78, 72, 66, 63, 59	6	0.5	0.31	[28]
CMTCN9	CCCCATATTCATCAAAACT CTTCTTTTTTTCACACCCT	240, 225, 213, 209, 205	5	0.68	0.15	[28]
TJ24	AAACACGGGCTTGAAGAAAA CCCAGAAGGTGAGAGACCT	178, 170, 165, 160, 140, 137	6	0.56	0.24	[28]
CMTCN41	CCCCAAGATTCGTATTAATC TGGTAGTAGAGATGATATAC	80, 76, 73	3	0.79	0.34	[28]
CMAGN79	CTTCACTAAAATAACAAGAG TTCCAACCTATTCATCCAC	192, 190, 173, 170, 165, 156, 152	6	0.47	0.69	[28]
CMTA134a	ACGTGCTTCAGTAAACATG CCGACATTGAAAACCACTTC	150, 146, 142, 138, 128, 125, 117, 105, 98, 90, 78	11	0.24	0.84	[27]
CMSR08180	TATGTCTCTCTTGGTCGCC AGTCGATTGGCAAATTACCG	340, 300, 298, 290	4	0.56	0.59	[29]
Total	-	-	56	-	-	-
Mean	-	-	5.6	0.59	0.38	-

In all accessions tested more than one allele was observed in at least three SSR loci analyzed. Generally multiple alleles were observed in four or five SSR loci (in 36 and 18 accessions, respectively). The individual SSR alleles occurred in between one and 67 of the tested accessions. Overall, 11 alleles were rare (occurred in less than 5% of the accessions), 27 were common (in 5 to 25% of accessions) and 18 alleles were frequent (over 50% of accessions).

3.5. Cluster Analysis

Cluster analysis (UPGMA) engaging data collected from SSR markers resulted in a dendrogram with two main groups (Figure 5), although these accessions are coming from different agroclimatic areas within the country. The first main group was divided into two subgroups G1 and G2 from the second node. G1 comprised 22 accessions, NL45, ML54, NL51, B65, NL50, B63, B66, B5, B41, A56, B33, A60, B34, B26, B61, B64, SL36, SL38, SL8, NL44, SL40, and A69. G2 consists of eight accessions, ML42, B29, ML39, A58, B7, SL17, B11, and B13. G1 and G2 were partitioned into several sub-clusters. Two cases of close similarity of about 0.94 were found between B63 and B66 accessions (both from Bekaa), and between SL40 (from South Lebanon) and A69 (from Akkar) accessions, although these two accessions are coming from different agroclimatic areas.

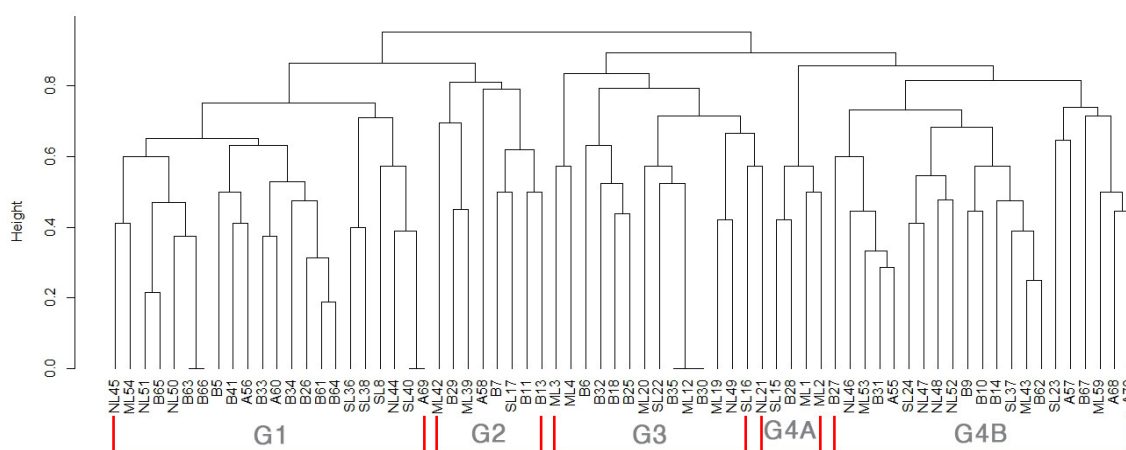


Figure 5. Cluster analysis of 70 snake melon accessions by unweighted pair-group method with arithmetic mean (UPGMA) using Jaccard's similarity coefficient.

The second main group was also portioned into two subgroups as the second node. Fifteen accessions coming from Bekaa, Mount Lebanon, South and North Lebanon, namely ML3, ML4, B6, B32, B18, B25, ML20, SL22, B35, ML12, B30, ML19, NL49, SL16, NL21, were found in the first subgroup G3, of which B35, ML12, and B30 accessions were clustered very closely together having genetic similarity of 0.94. The second subgroup G4 was subdivided into two main sub-groups G4A and G4B of the third node containing the remaining studied accessions. The first sub-group G4A constituted of SL15, B28, ML1, and ML2 accessions, and the second sub-group G4B constituted from 21 accessions, B27, NL46, ML53, B31, A55, SL24, NL47, NL48, NL52, B9, B10, B14, SL37, ML43, B62, SL23, A57, B67, ML59, A68, and A70.

4. Discussion

This is the first report on the assessment of the genetic diversity of the Lebanese germplasm of snake melon by means of both morphological descriptors and SSR markers. The morphological characterization of the Lebanese snake melon accessions was done using 18 quantitative and qualitative morphological descriptors. All accessions were monoecious and had a dull and ribbed fruit skin. Overall, all traits revealed intermediate to high H' diversity index ranging from 0.57 to 0.96 for the qualitative traits and between 0.51 and 0.99 for quantitative traits. These relatively high diversity indices are mainly due to the existence of more than two phenotypic classes as the case in fruit hair

consistency, or to the almost even distribution of individuals in the different classes of a studied trait as the case in fruit shape. The principle component analysis (PCA) indicated that fruit pubescence and its consistency, fruit size, along with skin color pattern were the most relevant traits to explain the variability within the studied accessions. The percent of the total variability found in our study was 51.51% as accounted by the first three components. This result was higher than that previously obtained in Jordanian snake melon accessions (39.9%) [13]. However, higher percentages were observed for Palestinian snake melon landraces (69.9%) [9] and in Tunisian snake melon accessions (89.8%) [37].

The PCA scatter diagram clustered the accessions into four main groups according to the presence (group I and II) or absence (group III and IV) of hair. However, the seven accessions collected from farmers in the Bekaa area present in the small subgroup of the major group IV produced elongated, ribbed, hairless, white skinned and flesh fruits, with a Brix value less than 4.2°, and length-to-width ratio higher than 8. These characters agree well with the description of snake melon varieties by Pitrat et al. [4]. Thus, these accessions are considered to be typical and original flexuous landraces. It has become very clear that the method of seed-saving practiced by local farmers since antiquity has maintained genetically diverse crops where farmers continuously selected better snake melon features to overcome the change in the agroclimatic conditions and/or to meet the consumers' preferences. As to the remaining 63 studied accessions growing in the different provinces, the large variability revealed within this set is probably the result of outcrossing nature of the snake melon. Similarly, Jordanian snake melon germplasm exhibited a genetic variation among populations for only 18.52% of the total variation, while more than 80% occurred within populations due to the outcrossing nature of this crop [13].

In addition, it was noticed that accessions coming from the same province have similar morphological characteristics. Majority of accessions collected from Bekaa and Akkar were characterized by their white color and hairless skin, while a significant number of accessions growing in the North, South, and Mount Lebanon are glabrous. Although the consumer's preference of snake melon differs among Lebanese regions, accessions from different geographic sources were found to be clustered in the same group. This could be the result of seeds dissemination that is actively practiced for landraces through the informal farmers' network and the traditional seed stores [21].

The molecular analysis of the 70 snake melon accessions performed through the 10 SSR markers generated a total of 56 alleles with two to 11 alleles per primer pair and an average number of 5.6 alleles per primer pair. This result is in line with the one previously obtained for Turkish snake melon accessions by Solmaz et al. [15], while Henane et al. [18] had a much lower value of 2.54 alleles per primer when studying the diversity of the Tunisian snake melon genotypes. Also, our average number of alleles per primer was higher than the one obtained by Ning et al. [20] through 36 SSR markers (4.03 alleles/primer) used to characterize the Chinese snake melon and its relationship with melon germplasms of diverse origins. Conversely, higher diversity was detected in melon germplasms for which the primer pairs were initially developed. Monforte et al. [38] identified 6.3 alleles on a collection of 27 accessions of wild and cultivated melons. Likewise, Kacar et al. [19] reported 6.55 alleles on 96 Turkish melon genotypes belonging to various subspecies. On the other hand, the polymorphism percentage obtained here with the Lebanese snake melon accessions was 100%, similarly to the findings of Solmaz et al. [15] for the Turkish snake melon germplasm and the ones of Tzitzikas et al. [16] for the Greek/Cypriot germplasm. Nevertheless, Tzitzikas et al. [16] reported a polymorphism information content (PIC) average value of 0.44, which is higher than the 0.38 value obtained here. This difference might be due to the different germplasms studied, or to the selected set of SSR markers used that might be more informative when compared to our study. Also, the multiple number of alleles per locus observed in this study is suggested to be linked to the heterogeneity and/or heterozygosity of the plants sampled to represent the respective accessions that may be caused by the outcrossing nature of this crop [13].

In addition, the polymorphism per locus in the used markers varied between our results and previous findings. For instance, in marker GMAGN68, 11 different alleles were detected by

Solmaz et al. [15], while only seven alleles were observed in our study, of which four alleles (168, 180, 184, and 187 bp) were found to be the same in both studies. In marker GMGS2-3 there were six different alleles observed while assessing the Turkish germplasm, while only two alleles were detected in our study. However, these two alleles were found at higher molecular weights with 320 and 315 bp as compared to the Turkish alleles ranging between 190 and 221 bp. Marker CMTA134a identified six alleles while assessing the Greek/Cypriot germplasm versus a total of 11 different alleles detected in our study. These findings show that the Lebanese snake melon germplasm have some distinctive characters as compared to other germplasms in the region.

The SSR markers used here allowed us to differentiate the studied Lebanese snake melon accessions into five main clusters. The similarity among accessions according to Jaccard's similarity distance coefficient ranged from 0.25 to 0.96. This proved the existence of wide diversity among Lebanese snake melon accessions studied.

Although both SSR markers and morphological descriptors revealed considerable variation among the Lebanese snake melon accessions, the clustering generated by SSRs was poorly connected to the one made by the morphological traits from one side, and to the geographic origin of studied accessions from the other side. Such weak connection could be due to the absence of linkage between SSR markers chosen for this study and loci that control the traits [38]. Similar results were also observed in the reports of Kacar et al. [19] and Solmaz et al. [15] indicating no correlation between molecular clustering and major morphological traits from one side and geographical origin of melon and snake melon genotypes from the other side. It is worthy to note that Kacar et al. [19] analyzed 12 snake melon accessions, along with other melon accessions, that were fairly clustered together but did not have any relation to geographic origin of the studied accessions, and Solmaz et al. [15] did not identify the geographic region and source of the used Turkish snake melon accessions.

Nevertheless, the three cases of similar accessions commonly named 'Abyad' as revealed by SSR analysis at 0.96 level of similarity, had also similar fruit characteristics and were very closely related in the morphological scatter plot. Specifically, the similar ML12, B30, and B35 accessions had white, semi curved, hairless, medium length fruits. B63 and B66 had white, elongated, hairless, short fruits, and SL40, and A69 had white, curved, hairless, long fruits. It is worthy to note that these 'Abyad' accessions that sound similar are sold in farmers' seed stores and disseminated in different agroclimatic areas in terms of altitude and precipitations where they are cultivated under different agricultural practices.

The crop vegetable diversification in the Mediterranean area is a complex matter [39]. The morphological assessment showed that some classes among studied characteristics were dominant. These include the white primary (71%) and secondary (74%) color, absence of pubescence (57%), the elongated to semi curved fruit shape (88%) and the medium sized fruits with respect to weight (63%) and dimensions (71%). Interestingly, the majority of the traits observed in this study are of various interest to consumers, growers, and breeders. For instance, some desirable phenotypic criteria of the fruit including elongated shape, sweet taste, white skin, and crisp or soft texture are critical for fresh snake melon consumption, while growers are mainly interested in marketable characters related to fruit dimensions and weight [26]. Breeders tend to search in the existing germplasm for desirable traits to improve the fruit appearance and plant resistance to biotic and abiotic stresses [40].

The overall analysis proves the existence of a wide diversity in plant material that may have important implications for genetic resources management. This high diversity could be linked to the old history of snake melon cultivation in Lebanon and around the Mediterranean [1]. Moreover, cultural practices like seed conservation and the necessity to protect rain-fed crops may have increased diversification of snake melon

5. Conclusions

This study highlighted the diversity of the Lebanese germplasm of snake melon as revealed by both morphological descriptors and SSR markers. Snake melon accessions assessed here represent a

valuable gene pool that needs to be further evaluated for its agronomic traits and its performance in changing environments. Attention should be addressed to the preservation and sustainable utilization of snake melon genetic resources within the growing consideration for agrobiodiversity and its vital importance in feeding populations.

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