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Characterization of Genetic Diversity Among *Cucumis* Accessions Based on Morphological and Phytochemical Characters

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ABSTRACT

The genus *Cucumis* L. of the Cucurbitaceae family is widely cultivated all over the world. Despite wide distribution and consumption, there is little information for the assessment of genetic diversity among Iranian *Cucumis* species. In this study, genetic diversity among 21 accessions of Iranian *Cucumis* species (*C. sativus* and *C. melo*) was assessed using 20 morphological characters of fruit and leaf and 4 phytochemical characters. High phytochemical and morphological diversity at intra- and interspecific levels were revealed among *Cucumis* species, which reflects the industrial potential of Iranian accessions for breeding and commercial usage. The grouping pattern of genetic diversity was constructed using the UPGMA dendrogram. The results of the grouping pattern revealed high efficiency of morphological and phytochemical characteristics for uncovering the genetic diversity of *Cucumis* accessions. Moreover, using morphological characters, cucumbers were separated according to their ecological zones. Considerable amounts of phytochemical properties were revealed among Iranian *Cucumis* accessions, which were comparable to those reported in other studies. Furthermore, unlike Total phenol content and total flavonoid content, melons showed more total sugar and DPPH radical scavenging activity than cucumbers. These results could be important for *Cucumis* gene bank management, agriculture, and breeding programs.

INTRODUCTION

Assessment of genetic diversity among and within species is considered essential for agricultural programs including breeding (Humphreys, 2003; Jump et al., 2008). The program is also supported by international and local organizations that emphasize the conservation and use of agricultural biodiversity as a priority (Szamosi, 2005). However, information on the extent and pattern of genetic diversity is necessary for adequate use of plant genetic resources (Kresovich & McFerson, 1992; Sheikhi et al., 2019; Pandey et al., 2021). Moreover, studies clearly showed the need to save and manage the local germplasm and wild relatives of agricultural crops (Solankey et al., 2015; Naghavi et al., 2019; Aryakia, 2020), since these materials may contain valuable genes for future breeding programs (Elbekkay et al., 2008; Chikh-Rouhou et al., 2021a).

In terms of plant breeding programs, the Cucurbitaceae family, among horticultural/vegetable crops, has a high position due to its commercially valuable species. This large plant family, also known as cucurbits, has about 130 genera and 800 species, with pumpkins (*Cucurbita* genus), watermelon (*Citrullus* genus), melon, and cucumber (*Cucumis* genus) being the most commercially important (Rolnik & Olas, 2020). However, the genus *Cucumis* L., with 32 species, is the most important genera of the Cucurbitaceae family. Some of the most well-known species in this genus are used as vegetables, medicinal plants, or ornamental plants (such as *C. dipsaceus* and *C. myriocarpus*) (Chen & Zhou, 2011). Melon (*Cucumis melo* L.) and cucumber (*Cucumis sativus* L.) are two commercial vegetable crops in the genus *Cucumis* that are extensively cultivated and consumed all over the world (Chen & Zhou, 2011). Nonetheless, there is an increasing global demand for qualitative and quantitative improvements in *Cucumis* crops, and several studies emphasize the importance of expanding the genetic basis of this genus, particularly cucumber and melon. (Chen et al., 1997; Stepansky et al., 1999a; Mangmang et al., 2016; Karakurt et al., 2020). Unfortunately, cucumber has a very narrow gene pool (Karakurt et al., 2020). It has been apparent that the genetic base of commercial cucumber germplasm is not extremely heterogeneous (Pierce & Wehner, 1990; Liu et al., 2015) which limits the development of cucumber breeding programs. On the other hand, since melon varies in leaf and fruit characters, it is considered to be the most morphologically diverse species in the genus *Cucumis* (Kirkbride, 1993; Pandey et al., 2021). In addition, knowing the relationships and characterization of the natural composition of the *Cucumis* accessions are important to improve melon (*C. melo*) and cucumber (*C. sativus*) with valuable characters during breeding programs (Deakin et al., 1971; Yu et al., 2015).

Studies on *Cucumis* species are in progress worldwide (Ismail et al., 2010; Raghami et al., 2014; Chikh-Rouhou et al., 2021a). In Tunisia, one of the rich centers of *Cucumis* genetic diversity, melon landraces have been reported as highly tolerant to many biotic stresses such as powdery mildew, fusarium wilt, aphids, and viruses. Moreover, a considerable phenotypical and molecular diversity among *Cucumis* accessions for many characters including those related to agronomical performance has been reported (Chikh-Rouhou et al., 2021a; Chikh-Rouhou et al., 2021b). Iran is also one of the richest genetic diversity resources of *Cucumis* species (Raghami et al., 2014). It is reported that the country with a total *Cucumis* production of 1,600,000 tons ranks fourth in the world (FAO, 2012). It seems that the characterization of genetic diversity could reveal their industrial potential. However, there is little information for diversity assessment of Iranian *Cucumis* species (Raghami et al., 2014). In this regard, genetic markers including morphological, biochemical, and molecular markers have been used to assay genetic diversity and germplasm characterization, monitor changes in population structure, and manage variation through concerted conservation strategies (Ghafoori et al., 2013; Chikh-Rouhou et al., 2021b).

Evaluation of genetic diversity based on morphological characters is very important to reveal valuable horticultural traits in *Cucumis* (Chikh-Rouhou et al., 2021b). It is also applied for evaluating taxonomic arrangement (Kashyap et al., 2021), for ecological studies such as the interaction between plants and pollinators (Bernhardt et al., 2008), and also the other evolutionary consequences (reviewed in Schemske, 1980). For example, assessment of the shape and size of the leaf could be an important factor influencing the success of plants as its roles in the absorption of light energy and gas exchanges (Tsukaya, 2005). However, there is no report for assessment of intra- and interspecific morphodiversity among Iranian *Cucumis* species. Besides, assessment of phytochemical diversity among them could be important in terms of nutritional, pharmacological, and breeding programs (Ismail et al., 2010; Aryakia et al., 2018; Manchali et al., 2021). In this study, we assessed genetic diversity among 21 accessions of Iranian *Cucumis* species using 20 morphological characters represented by UPOV (2019), along with 4 phytochemical characters of total phenolic content (TPC), total flavonoid content (TFC), total sugar (TS), and DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical.

MATERIAL AND METHODS

Plant Material and Experimental Design

This study was conducted at the Iranian Biological Resource Center (IBRC), Karaj, Iran. Seeds of 21 *Cucumis* accessions (12 accessions belonging to *C. sativus* and 9 accessions belonging to *C. melo*) from 9 Iranian provinces (major cultivation areas of *Cucumis*) were collected by the experts from open-pollinated accessions at the full ripening stage. The seeds were then cleaned, sorted, and stored in the seedbank before being directly examined in this study. The description of each accession is given in Table 1. The greenhouse experiment was arranged in Randomized Complete Block Design (RCBD) with three blocks (replications). Each block contained 21 *Cucumis* accessions, each accession with six plants.

Morphological Characteristics

Twenty qualitative characteristics related to fruit and leaf (Table 2) were assessed according to the list of descriptors given by the UPOV (2019). The frequency (%) of each qualitative morphological trait was calculated and is presented in Table 3.

Phytochemical Characteristics

Extraction

Plant materials including the air-dried fruit of each *Cucumis* accession were ground to a fine powder in liquid nitrogen and then extracted with 80% ethanol solvent in the ratio of 10 % w/v, filtered by Whatman filter paper no. 40, and the solvent was removed under vacuum using a rotary evaporator at 40°C.

Determination of Total Phenolic Content

The amount of total phenolic content was determined according to Spanos & Wrolstad (1990) who used folin-ciocalteu reagent and gallic acid as standard. A volume of 30 μl of the sample was transferred into a test tube and 500 μl of folin-ciocalteu reagent was added and mixed. The mixture was allowed to stand at a temperature of 25°C for 3 min. A volume of 500 μl of saturated sodium carbonate solution (Na_2CO_3) was added to the mixture and mixed gently. After keeping the mixture at 25°C for 60 min, absorbance was read at 725 nm using a UV–vis spectrophotometer. The TPC was expressed as milligram gallic acid equivalents (GAE) per gram dry weight (mg GAE/g DW).

Determination of Total Flavonoid Content

Total flavonoid content was calculated using the aluminum chloride colorimetric method described by Hosu et al. (2014). Treating sample, 2.5% $AlCl_3$ solution, 10% sodium acetate solution and distilled water were mixed at a ratio of 100:80:80:800 μl, respectively. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm. The results were expressed as milligrams of quercetin equivalents (QE) per gram of dry weight (mg RE/g DW).

Determination of DPPH Radical-Scavenging Activity

The ability of the extracts to scavenge DPPH radicals (inhibitory concentration at 50% activity (mg/ml)) was assessed according to the method described by Brand-Williams et al. (1995) using the 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity with some modifications. Fifty μl of the sample was mixed with 350 μl of DPPH (1 mM in methanol) and then methanol 100% was added to the final volume of 2 ml. Following incubation of the test in the dark at room temperature for 15 min, the absorbance of the reaction mixture was read at 517 nm using a UV–vis spectrophotometer. Methanol and ascorbic acid were used as controls and the inhibition ratio was calculated from the following equation (1):

$$Inhibition(\%) = \frac{Absorbance\ of\ control - Absorbance\ of\ the\ test\ sample}{Absorbance\ of\ control} \times 100 \quad (1)$$

Table 1. Accession description of the studied *Cucumis* species

Herbarium No.	Species	Ecological Zones	Address (Province-City)
IBRC P1012223	*Cucumis sativus* L.	Irano-Turanian Zone	Khorasan-Sabzevar
IBRC P1012231	*Cucumis* cf. *sativus* L.	Hyrcanian Zone	Rasht-Shajaghi
IBRC P1012625	*Cucumis sativus* L.	Irano-Turanian Zone	Hamedan-Hamedan
IBRC P1012258	*Cucumis* cf. *sativus* L.	Hyrcanian Zone	Rasht-Langerood
IBRC P1012832	*Cucumis sativus* L.	Zagros Zone	Lorestan-Brojerd
IBRC P1012280	*Cucumis* cf. *sativus* L.	Hyrcanian Zone	Rasht-Lahijan
IBRC P1012296	*Cucumis* cf. *sativus* L.	Hyrcanian Zone	Rasht-Dastak
IBRC P1012407	*Cucumis sativus* L.	Irano-Turanian Zone	Azarbayejan-Basmenj
IBRC P1012316	*Cucumis* cf. *sativus* L.	Hyrcanian Zone	Rasht-Astaneh
IBRC P1012299	*Cucumis* cf. *sativus* L.	Hyrcanian Zone	Rasht-Kiashahr
IBRC P1012710	*Cucumis sativus* L.	Zagros Zone	Ilam-Ilam
IBRC P1012250	*Cucumis* cf. *sativus* L.	Hyrcanian Zone	Rasht-Astaneh
IBRC P1012563	*Cucumis melo* var. *flexuosus* (L.) Naudin	Irano-Turanian Zone	Qom-Gazran
IBRC P1012652	*Cucumis melo* var. *flexuosus* (L.) Naudin	Zagros Zone	Kermanshah-Kermanshah
IBRC P1012564	*Cucumis melo* var. *flexuosus* (L.) Naudin	Irano-Turanian Zone	Qom-Gazran
IBRC P1012651	*Cucumis melo* var. *flexuosus* (L.) Naudin	Zagros Zone	Kermanshah-Kermanshah
IBRC P1012632	*Cucumis melo* var. *flexuosus* (L.) Naudin	Irano-Turanian Zone	Hamedan-Hamedan
IBRC P1012686	*Cucumis melo* var. *flexuosus* (L.) Naudin	Zagros Zone	Kermanshah-Sahneh
IBRC P1012468	*Cucumis melo* var. *flexuosus* (L.) Naudin	Irano-Turanian Zone	Qom-Qom
IBRC P1012793	*Cucumis melo* var. *flexuosus* (L.) Naudin	Zagros Zone	Lorestan–Dorood
IBRC P1012383	*Cucumis melo* var. *flexuosus* (L.) Naudin	Irano-Turanian Zone	Qazvin-Takestan

Table 2. List of qualitative morphological descriptors

Characteristics states (score)					Characters
		Drooping (3)	Horizontal (2)	Erect (1)	Leaf blade: attitude
Very large (9)	Large (7)	Medium (5)	Small (3)	Very small (1)	Leaf blade: ratio length of terminal lobe/length of blade
Very strong (9)	Strong (7)	Medium (5)	Weak (3)	Absent or very weak (1)	Leaf blade: blistering
		Strong (3)	Moderate (2)	Absent or weak (1)	Leaf blade: undulation of margin
Very strong (9)	Strong (7)	Medium (5)	Weak (3)	Very weak (1)	Leaf blade: dentation of margin
	Rounded (4)	Obtuse (3)	Right-angled (2)	Acute (1)	Leaf blade: shape of apex of terminal lobe
		Dark (7)	Medium (5)	Light (3)	Leaf intensity of green color
Predominantly three or four (5)	Predominantly two or three (4)	Predominantly two (3(	Predominantly one or two)2(	Predominantly one)1(	Plant: number of female flowers per node
	Other (99)	Obtuse (3)	Acute (2)	Necked (1)	Fruit predominant shape at stem end (table use)
	Other (99)	Green (3)	Yellow (2)	White (1)	Predominant fruit skin color (table use)
Very long (9)	Long (7)	Medium (5)	Short (3)	Very short (1)	Fruit: length
		Large (7)	Medium (5)	Small (3)	Fruit: diameter
Very large (9)	Large (7)	Medium (5)	Small (3)	Very small (1)	Fruit: ratio length/diameter
		Angular (3)	Round to angular (2)	Round (1)	Fruit: shape in transverse section
	Truncate (4)	Rounded (3)	Obtuse (2)	Acute (1)	Fruit: shape of calyx end
			Present (9)	Absent or weak (1)	Fruit: dots
		Evenly distributed (3)	Predominantly in bands (2)	In bands only (1)	Fruit: distribution of dots
		Strong (3)	Medium (2)	Absent or weak (1)	Fruit: ribs
			Present (9)	Absent (1)	Fruit: sutures
Very dense (9)	Dense (7)	Medium (5)	Sparse (3)	Absent or Very sparse (1)	Fruit: density of vestiture

Table 3. Frequency (%) of 20 leaf and fruit qualitative characteristics in Cucumis species

Characters	Melon	Cucumber
Leaf blade: attitude	Horizontal (36.4), Drooping (18.2)	Erect (45.4)
Leaf blade: ratio length of terminal lobe/length of blade	Large (36.4), Medium (4.5), Very large (13.6),	Very large (13.6), Large (31.8)
Leaf blade: blistering	Weak (31.8),Medium (18.2), Absent or very weak (4.5)	Absent or very weak (4.5), Medium (41)
Leaf blade: undulation of margin	Moderate (36.4), Absent or weak (18.2)	Strong (41), Moderate (4.5)
Leaf blade: dentation of margin	Weak (13.6), Medium (22.7), Strong (18.2)	Very strong (45.4)
Leaf blade: shape of apex of terminal lobe	Acute (18.2), Right-angled (18.2), Obtuse (18.2)	Rounded (41), Obtuse (4.5)
Leaf intensity of green color	Medium (22.7), Light (18.2), Dark (13.6)	Medium (9.1), Light (36.4)
Plant: number of female flowers per node	Predominantly one (36.4), Predominantly one or two (9.1), Predominantly three or four (4.5), Predominantly two (4.5)	Predominantly one (36.4), Predominantly one or two (9.1)
Fruit predominant shape at stem end (table use)	Necked (13.6), Obtuse (31.8), Acute (9.1)	Necked (9.1), Obtuse (36.4)
Predominant fruit skin color (table use)	Yellow (31.8), Green (22.7)	Green (18.2), White (27.3)
Fruit: length	Long (22.7), Very long (9.1), Short (9.1), Medium (13.6)	Very long (22.7), Very short (4.5), Medium (4.5), Long (13.6)
Fruit: diameter	Medium (45.4), Large (4.5), Small (4.5)	Large (22.7), Small (4.5), Large (4.5)
Fruit: ratio length/diameter	Very large (27.3), Medium (22.7), Large (4.5)	Very large (31.8), Small (4.5), Medium (4.5), Large (4.5)
Fruit: shape in transverse section	Angular (22.7), Round to angular (13.6), Round (18.2)	Round (31.8), Angular (9.1), Round to angular (4.5)
Fruit: shape of calyx end	Obtuse (50), Rounded (4.5)	Rounded (27.3), Obtuse (9.1), Truncate (9.1)
Fruit: dots	Present (54.5)	Absent or weak (45.5)
Fruit: distribution of dots	Evenly distributed (45.5), Predominantly in bands (9.1)	In bands only (27.3), Evenly distributed (18.2)
Fruit: ribs	Medium (31.8), Absent or weak (22.7)	Absent or weak (9.1), Medium (27.3), Strong (9.1)
Fruit: sutures	Present (45.5), Absent (9.1)	Present (41), Absent (4.5)
Fruit: density of vestiture	Absent or Very sparse (54.5)	Dense (4.5), Sparse (13.6), Absent or Very sparse (22.7), Very dense (4.5)

Determination of Total Sugar

Total sugars were determined according to the phenol-sulfuric acid method with minor modifications (DuBois et al., 1956). A total of 0.17 g dry weight was mixed with 10 ml of distilled water and the extract was filtered. 0.25 ml of the solution was mixed with 0.25 ml of 5% phenol. Subsequently, 1 ml of 98% sulfuric acid was added rapidly to the mixture. Following incubation of the test in the dark at room temperature for 15 min, test tubes were placed in a water bath at 30°C for 20 min for color development. The absorbance was measured at 490 nm wavelength using UV–visible spectrophotometer. A blank solution was prepared in the same way as above. Glucose solution was used for the construction of the standard curve. The content of TS was expressed as mg/gr dry weight (DW).

Data analysis

The frequency (%) of morphological variables and mean ± standard deviation of phytochemical characters were calculated using Microsoft Excel software. Dendrograms were constructed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm by SPSS 16.0. In addition, relationships among traits were determined using the Pearson correlation analysis.

RESULTS AND DISCUSSION

Leaf Characteristics

The results of the morphological descriptions and frequency (%) are given in Table 3. A high level of variation for the following leaf characteristics was found at intra- and interspecific levels: attitude, blistering, undulation and dentation of margin, the shape of the apex, and ratio length of terminal lobe/blade and leaf intensity of the green color (Table 3 and Figure 1). However, the following characteristics were only observed in melon: attitude (Erect), undulation, dentation (strong and very strong), and shape of the apex (rounded) (Table 3).

Table 4. DPPH radical scavenging activity, TPC, TFC and TS of the fruit of Cucumis species

Herbarium No.	Species	Total Phenol (mg GAE/ gr DW)	Total Flavonoid (mg QE/gr DW)	DPPH (IC50 (mg/ml)	Total sugar (mg/gr DW)
IBRC P1012231	Cucumber	3.42± 0.06	1.03± 0.04	10.37± 0.21	35.73± 1.55
IBRC P1012250	Cucumber	4.35± 0.18**	1.64± 0.05**	5.66± 0.12	32.95± 0.72
IBRC P1012258	Cucumber	2.20± 0.13	0.78± 0.02	11.01± 0.22	55.88± 3.29
IBRC P1012280	Cucumber	2.88± 0.02	0.72± 0.02	14.62± 0.20	37.75± 1.16
IBRC P1012296	Cucumber	3.00± 0.08	1.04± 0.02	10.09± 0.11	26.67± 1.39
IBRC P1012299	Cucumber	3.91± 0.17	1.58± 0.04	5.04± 0.12	45.51± 2.10
IBRC P1012316	Cucumber	2.29± 0.03	0.75± 0.03	13.92± 0.14	51.11± 1.07
IBRC P1012407	Cucumber	1.11± 0.01*	0.51± 0.02*	35.23± 1.78*	43.09± 1.32
IBRC P1012223	Cucumber	2.60± 0.07	0.69± 0.02	19.49± 0.24	20.67± 1.06*
IBRC P1012710	Cucumber	2.01± 0.04	0.60± 0.01	20.95± 0.49	34.74± 1.55
IBRC P1012832	Cucumber	2.14± 0.05	0.70± 0.01	15.14± 0.55	38.83± 0.92
IBRC P1012625	Cucumber	1.82± 0.03	0.64± 0.05	25.40± 0.96	38.10± 2.13
IBRC P1012383	Melon	1.60± 0.05	0.63± 0.01	7.94± 0.54	43.75± 3.47
IBRC P1012468	Melon	2.22± 0.04	1.04± 0.03	7.68± 0.01	51.36± 3.15
IBRC P1012563	Melon	1.53± 0.04	0.56± 0.01	7.63± 0.03	32.20± 1.45
IBRC P1012564	Melon	1.24± 0.03	0.53± 0.00	10.09± 0.16	43.39± 0.43
IBRC P1012632	Melon	1.26± 0.07	0.53± 0.02	10.1± 0.18	42.66± 1.86
IBRC P1012651	Melon	2.12± 0.06	1.05± 0.04	6.70± 0.43	54.92± 2.81
IBRC P1012652	Melon	2.26± 0.07	0.69± 0.01	10.8± 0.14	55.90± 0.85**
IBRC P1012686	Melon	1.76± 0.04	0.77± 0.05	7.48± 0.07	53.41± 1.30
IBRC P1012793	Melon	2.26± 0.10	1.42± 0.03	3.08± 0.07**	53.28± 2.38
Mean	Cucumber	**2.64± 0.92**	**0.89± 0.37**	15.58± 8.62	38.42± 9.74
	Melon	1.81± 0.42	0.80± 0.31	**7.94± 2.32**	**47.87± 7.87**

Note: *and ** represent minimum and maximum phytochemical properties, respectively.

Leaf attitude predominantly was erect (45.4), but horizontal (36.4) and drooping (18.2) of leaf attitude also were observed. Furthermore, the shape of the apex predominantly was rounded (41) and this was scarcely obtuse (4.5). Leaf blistering was classified as weak, medium, absent, or very weak. Other characters including leaf blade undulation and leaf blade dentation also were recorded. Leaf blade undulation mainly was strong (41), with leaf blade dentation of very strong (45.4) among melons.

The assessment of genetic diversity is required for the efficient organization, conservation, and improvement of *Cucumis* germplasm (Chikh-Rouhou et al., 2021b). Among different plant parts, leaf diversity plays an important role in plant breeding programs and many other subjects including plant taxonomy, ecology, and evolution (Schemske, 1980; Bernhardt et al., 2008; Kashyap et al., 2021; Chikh-Rouhou et al., 2021b). Previous studies reported morphological traits of leaf for distinguishing *Cucumis* germplasm (Stepansky et al., 1999a; Parvathaneni et al., 2011; Chikh-Rouhou et al., 2021b). Their results revealed drastic genetic diversity among cucumbers and melons, which was consistent with our findings. This is the first report of leaf morphometric assessment of Iranian *Cucumis* accessions based on these characters. The results showed that leaf blade characters could be considered species-specific characters for breeding programs to develop and characteristics of new varieties (UPOV, 2019).

Leaf intensity of green color was mainly observed as light, but others such as medium and dark (especially in cucumber) were also observed. Variation in leaf color is a common feature between and within *Cucumis* species (UPOV, 2019; Parvathaneni et al., 2011). Diversity in leaf color was reported in other plants (De Souza et al., 2012; Aryakia et al., 2016) which may be important for evolutionary and ecological studies such as plant-insect interactions (Maskato et al., 2014).

Fruit Characteristics

There are many types of *Cucumis* fruit, different in dimensions, shape, and color (UPOV, 2019; Chikh-Rouhou et al., 2021b; Chikh-Rouhou et al., 2021c). Fruit characters are important quality factors for many plants (Grandillo et al., 1996). These characteristics are

Table 5. Correlation among morphological traits

	Leaf blade: shape of apex of terminal lobe	Leaf blade: attitude	Leaf intensity of green color	Leaf blade: ratio length of	Leaf blade: blistering	Leaf blade: undulation of margin	Leaf blade: dentation of margin	ant: number of female flowers	Fruit predominant	Predominant fruit skin color	Fruit: shape of calyx end	Fruit: dots	Fruit: distribution of	Fruit: ribs	Fruit: sutures	Fruit: length	Fruit: diameter	Fruit: ratio length/diamete	Fruit: shape in transverse
Leaf blade: attitude	-0.841**																		
Leaf intensity of green color	-0.420	0.419																	
Leaf blade: ratio length of terminal lobe/length of blade	-0.179	0.043	0.006																
Leaf blade: blistering	0.688**	-0.443*	-0.111	-0.127															
Leaf blade: undulation of margin	0.906**	-0.796**	-0.341	-0.136	0.635**														
Leaf blade: dentation of margin	0.535*	-0.727**	-0.421	0.336	0.458*	0.516*													
Number of female flowers per node	-0.536*	0.369	-0.061	0.443*	-0.463*	-0.610**	0.107												
Fruit predominant shape at stem end	0.255	-0.092	-0.371	-0.029	0.206	0.054	0.267	0.084											
Predominant fruit skin color	-0.421	0.359	0.251	0.359	-0.31	-0.0584**	-0.144	0.513*	-0.024										
Fruit: shape of calyx end	0.516*	-0.554**	-0.434*	0.335	0.289	0.518*	0.656**	0.147	0	-0.042									
Fruit: dots	-0.830**	0.886**	0.498*	-0.129	-0.500*	-0.836**	-0.862**	0.247	-0.158	0.377	-0.69**								
Fruit: distribution of dots	-0.448*	0.494*	0.307	-0.082	-0.361	-0.619**	-0.449*	0.349	-0.039	0.374	-0.392	0.584**							
Fruit: ribs	0.421	-0.341	-0.111	-0.127	0.237	0.532*	0.122	-0.0382	0.115	-0.03	0.174	-0.347	-0.361						
Fruit: sutures	0.069	0.032	-0.227	0.175	-0.151	0.303	-0.016	-0.23	-0.259	-0.421	0.101	-0.097	-0.287	0.07					
Fruit: length	0.374	-0.223	-0.036	-0.244	0.279	0.606**	-0.044	-0.529*	0.04	-0.57**	-0.062	-0.231	-0.58**	0.484*	0.530*				
Fruit: diameter	0.187	0	-0.297	-0.413	0.178	0	-0.096	0	0.127	0.131	0.162	0	0	-0.178	-0.311	-0.191			
Fruit: ratio length/diameter	0.387	-0.282	-0.124	-0.365	0.239	0.550**	-0.155	-0.64**	-0.069	-0.59*	-0.104	-0.158	-0.56	0.392	0.489*	0.856**	-0.107		
Fruit: shape in transverse section	-0.25	0.199	0.026	-0.406	-0.421	-0.216	-0.286	-0.119	0.111	0.034	-0.234	0.328	-0.032	0.008	0.068	0.105	0.241	0.201	
Fruit: density of vestiture	0.439*	-0.427*	-0.056	0.488*	0.388	0.463*	0.416	-0.026	-0.134	-0.074	0.469*	-0.482*	-0.182	0.02	0.175	0.151	-0.103	-0.012	-0.357

Note: ** and *, Significant at the probability of 0.01 and 0.05, respectively.

used to classify current cultivars and plant species into different groups (Aryakia et al., 2016; UPOV, 2019).

Among fruit morphological characters studied herein, only fruit creasing showed the monomorphic feature (data do not show). However, a high level of diversity in fruit traits was observed at intra-and interspecific levels (Table 3 and Figure 1). Fruit characteristics include white color of skin, very long size, truncate calycate, smooth texture, and strong ribs just observed in melons, while yellow skin and dense of vestiture (absent or very sparse) in cucumbers were observed.

The predominant fruit skin color was green. However other colors such as yellow and white were also observed. The fruit's green color is favorite for Iranian consumers. Variation in skin color is a common feature between and within *Cucumis* species (Parvathaneni et al., 2011; UPOV, 2019; Chikh-Rouhou et al., 2021c), which is also observed in other plants (Aryakia et al., 2016). Fruit predominant shape at stem end was classified as necked, obtuse, and acute. This character was mainly observed as obtuse. The other fruit shape criteria were the transverse section and the shape of the calyx end. Obtuse, rounded, and truncate were recorded in the calyx. Furthermore, fruit length, diameter, and ratio length/diameter were recorded to determine the fruit dimensions. These characters were classified as long, short, very long, medium, and very short. The predominant Fruit diameter was medium. In addition, fruit dots, ribs, sutures, and vestiture density were revealed in the studied species.

Although previous works have reported many classifications and evaluations of different *Cucumis* accessions based on commercial traits of fruit (Stepansky et al., 1999b; Parvathaneni et al., 2011; Raghami et al., 2014), there is little information on the morphological assessment of Iranian *Cucumis* species. Our findings show that 12 morphological characters of fruit can be accurately used to distinguish and represent potential new sources of *Cucumis* accessions.

In addition, the number of female flowers per node was variable among them. Moreover, this trait was predominantly one, but three to four female flower per node was only observed in cucumbers. It could be considered a valuable industrial potential for breeding programs because some cucumber varieties have been bred to have multiple flowers per node (Hikosaka & Sugiyama, 2004). This morphovariable trait is also applied for evaluating taxonomic arrangement in other plants (Espírito-Santo et al., 2012).

Phytochemical Characteristics

Our results revealed wide variation in DPPH, TPC, TFC, and TS among accessions of *Cucumis* species (Table 4). DPPH, TPC, TFC and TS varied from 3.08±0.07 to 35.23±1.78 mg/ml, 1.11±0.01 to 4.35±0.18 mg GAE/g DW, 0.51±0.02 to 1.64±0.05 mg RE/g DW and 20.67±1.06 to 55.90±0.85 mg/g DW, respectively. Favorable to the extreme amount of phytochemical properties were revealed among *Cucumis* accessions, which were comparable to those observed in other melons or cucumbers (Stepansky et al., 1999b; Ismail et al., 2010). Moreover, assessment of the diversity of DPPH, TPC, TFC, and TS could reveal a good source of phytochemical characteristics among plant germplasm (Chen et al., 2013; Aryakia, 2020; Chikh-Rouhou et al., 2021c; Shahrivari et al., 2022).

Phenolic compounds which proved to be associated with health benefits, exhibit a wide range of physiological properties, such as antioxidant, anti-allergenic, anti-artherogenic, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective, and vasodilatory effects (Balasundram et al., 2006). Therefore, evaluation of phenolic content and antioxidant activity in plant extracts are very important issues from pharmaceutical and medical aspects. TPC was several times more than that of TFC in each *Cucumis* accession. Moreover, cucumbers showed more TPC and TFC than melons, but melons showed more TS and DPPH radical scavenging activity than cucumbers (Table 4). Previous reports showed that interspecific crossing among cucumber and melon could result in new crops (Deakin et al., 1971; Chen et al., 1997; Yu et al., 2015). So, these results could be considered for agricultural breeding programs. This research is the first report that studies DPPH, TPC, TFC, and TS of the Iranian native *Cucumis* accessions. Overall, complementary studies along with molecular and phytochemical research could help to promote *Cucumis* genetic database.

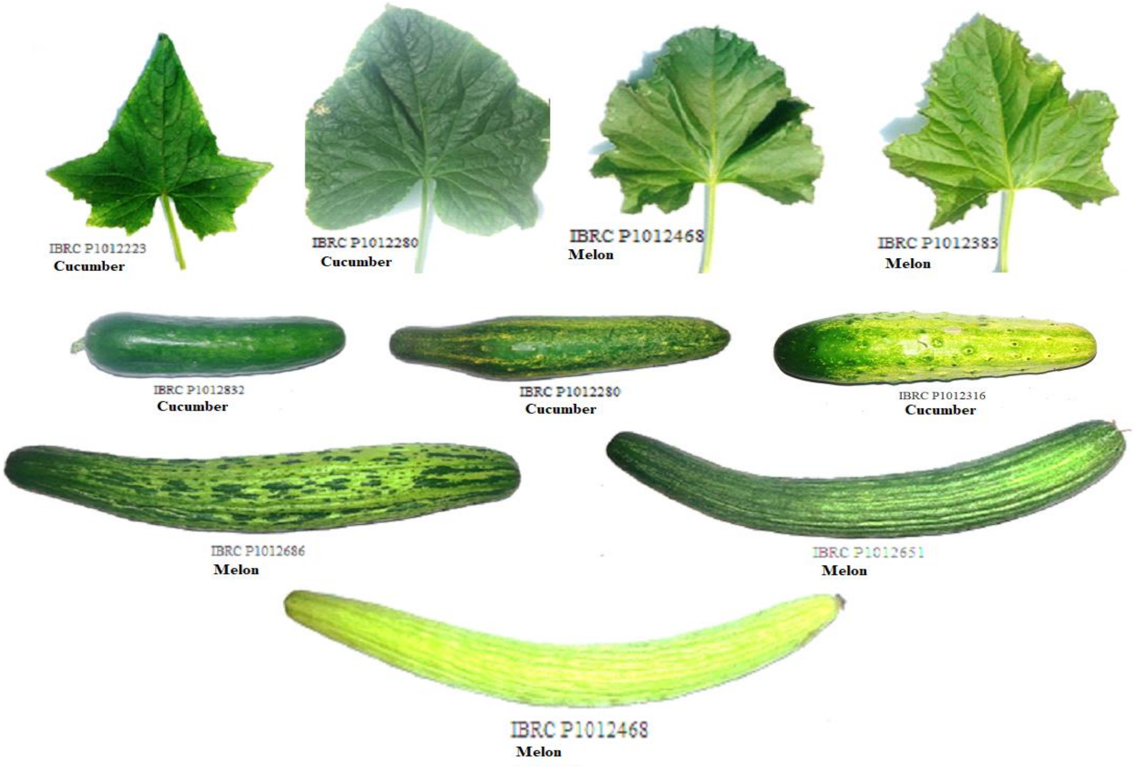

Figure 1. Morphodiversity of leaf and fruit among *Cucumis* accessions

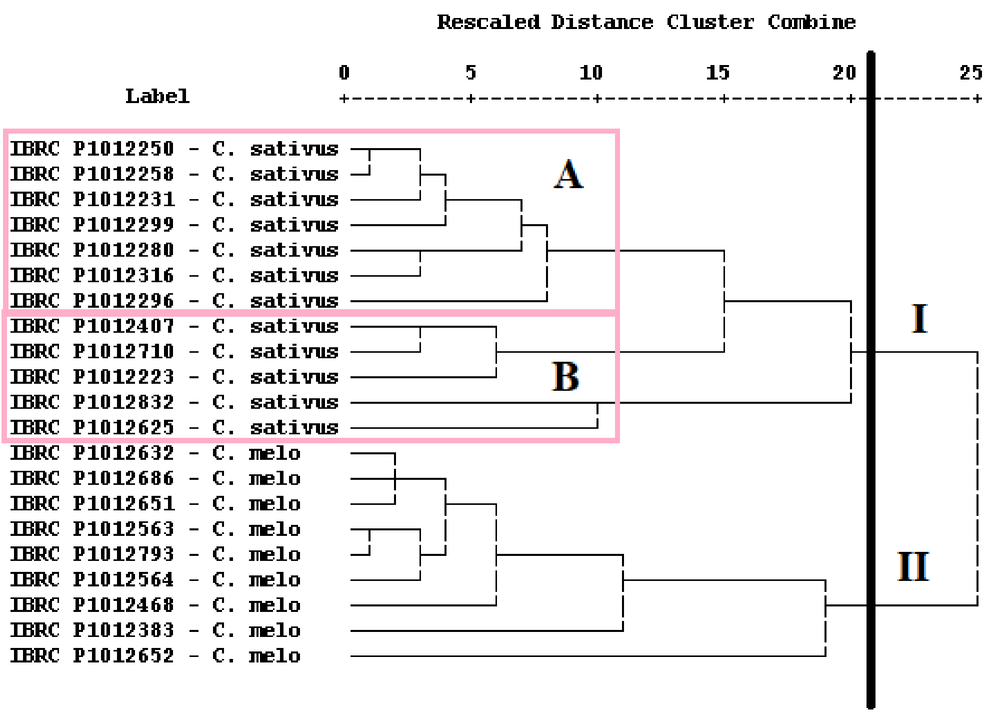

Figure 2. Relationships among 21 accessions of *Cucumis* species using 20 qualitative characters of leaf and fruit

Cluster Analysis

Analysis of the cluster divided all accessions into the two major groups, cucumber and melon (**I** and **II**) (Figure 2) which were clearly defined by twenty qualitative morphological characteristics. The first group (**I**) included cucumber accessions with two district subgroups of **A** and **B** originating from the Hyrcanian zone and other different ecological zones, respectively. It might be since accessions originated from different locations of the Hyrcanian zone have been ecologically influenced by the same climate conditions. The second group (**II**) included melon accessions. This cluster revealed a high accuracy level of intra-and interspecific morphodiversity among *Cucumis* accessions studied herein. However, similar reports in the *Cucumis* genus (Parvathaneni et al., 2011; Stepansky et al., 1999a) and other plant species (Arrieta-Espinoza et al., 2005; Aryakia et al., 2016) could confirm the classification based on morphological traits.

In addition, analysis of clusters based on four phytochemical characters (including TPC, TFC, TS, and DPPH) could also cluster most of the *Cucumis* accessions according to their genetic pattern (cucumber and melon) (Figure 3). These results suggest that clustered relative accessions have similar bioactive compounds responsible for their phytochemical characters. Chikh-Rouhou et al. (2021c) used phytochemical characters including TFC and TPC for the classification of different *Cucumis* species. Their results showed that the cluster analysis could segregate *Cucumis* species into four different groups. Moreover, TFC and TPC were affected by the botanical group and genotype within the same group. Maietti et al. (2012) found similar results, demonstrating that two distinct clusters were clearly identified in melons belonging to the same cultivar, confirming a significant effect on the chemical composition of the fruit.

This valuable diversity observed among *Cucumis* accessions revealed a good industrial potential for commercial exploration of them. In addition, Iran is one of the most cultivated areas of Cucurbitaceae species comprising drastic variable accessions. Therefore, determination of the distribution and the level of genetic diversity using morphological and phytochemical characters could provide basic data for designing conservation and breeding programs for *Cucumis* species (Xiao et al., 2004; Hao et al., 2006).

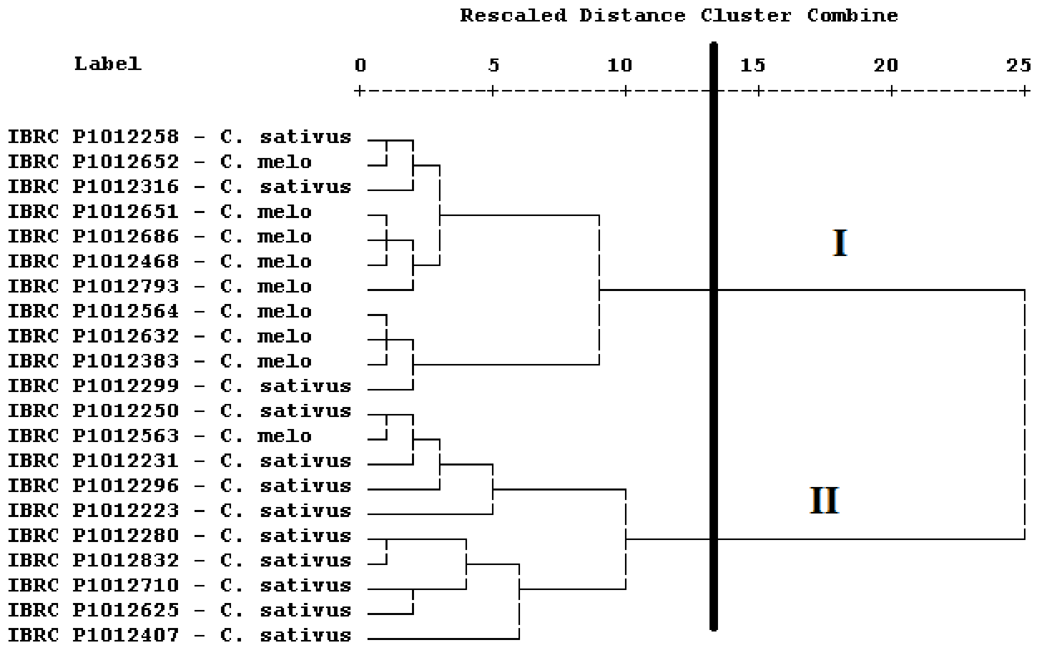

Figure 3. Relationships among 21 accessions of *Cucumis* species using 4 phytochemical characters

Correlation Among Characters

Pearson's correlation analysis could find significant relationships among characters (Tables 5 and 6). A significant correlation among all leaf characters was observed except for leaf color and terminal lobe/blade. No similar results were observed among fruit characters. However, some significant correlations were revealed, such as fruit length/diameter and fruit length with ribs and sutures. In addition, the correlation between fruit and leaf characters was also observed. Correlation among morphological characters was reported by previous studies (De Souza et al., 2012; Aryakia et al., 2016). These correlations could be due to the evolutionary mechanism (Davis, 2001). For example, correlation among fruit and leaf characters might be the consequence of some allometric relationship affecting all plant parts (Herrera, 2002). The correlation between different organs can be useful for breeders to predict expected morphological traits, so the breeding process can be shortened. For farmers, they can observe the shape and size of other organs to predict relative traits of fruit and to harvest commodity fruit (Cui et al., 2020).

Table 6. Correlation among phytochemical traits

	TPC	TFC	DPPH
TFC	0.787**		
DPPH	-0.358	- 0.553**	
TS	-0.258	0.079	-0.292

Note: ** Significant at the probability of 0.01.

Correlation between phenolic content and antioxidant activity was revealed in this study (Table 6) according to previous reports (Aryakia et al., 2015). However, no correlation was found between TS and phenolic content or antioxidant activity. It has been demonstrated that hydroxyl functional groups of phenolic compounds, are responsible for antioxidant activity and flavonoids also stabilize the reactive oxygen species (Nijveldt et al., 2001). These compounds interrupt the propagation of the free radical autoxidation chain by contributing a hydrogen atom from a phenolic hydroxyl group, followed by the formation of a relatively stable free radical that does not initiate or propagate further oxidation processes (Bahramikia et al., 2009). These correlations might be useful as a powerful tool for the selection and breeding of economically valuable traits with low heritability.

CONCLUSION

This study increased our knowledge about the intra- and interspecific diversity of Iranian *Cucumis* germplasm. Both morphological and phytochemical properties can be used effectively in the identification and classification of *Cucumis* accessions. These results might also be considered in the characterization and distinguishing of other *Cucumis* species. Further morphological and phytochemical studies, especially the extraction of different parts using different solvents, as well as the evaluation of other phytochemical properties, might be necessary for a better understanding of the *Cucumis* breeding program.

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Compliance with Ethical Standards

Authors' Contributions

Both authors have contributed equally to this paper.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

REFERENCES

Arrieta-Espinoza, G., Sanchez, E., Vargas, S., Lobo, J., Quesada, T., & Espinoza, A. M. (2005). The weedy rice complex in Costa Rica. I. Morphological study of relationships between commercial rice varieties, wild *Oryza* relatives and weedy types. *Genetic Resources and Crop*

Evolution, *52*, 575–587. https://doi.org/10.1007/s10722-004-6109-x

Aryakia, E. (2020). Study on anti-acetylcholinesterase, anti-tyrosinase, and antioxidant activities, and total phenolic content of nine Apiaceae species. *Iranian Journal of Medicinal and Aromatic Plants Research*, *36*, 560-571. https://dx.doi.org/10.22092/ijmapr.2020.342448.2756

Aryakia, E., Karimi, H. R., Naghavi, M. R., Yazdanfar, N., & Fazeli, S. A. S. (2018). Evaluating essential oil composition of four *Allium* species (Subgen. & Sect. *Allium*). *Iranian Journal of Field Crop Science*, *49*(1), 115-123.

Aryakia, E., Karimi, H. R., Naghavi, M. R., & Fazeli, S. A. S. (2016). Morphological characterization of intra-and interspecific diversity in some Iranian wild *Allium* species. *Euphytica*, *211*(2), 185-200. https://doi.org/10.1007/s10681-016-1729-8

Aryakia, E., Naghavi, M. R., Farahmand, Z., & Shahzadeh Fazeli, S. A. H. (2015). Evaluating allelopathic effects of some plant species in tissue culture media as an accurate method for selection of tolerant plant and screening of bioherbicides. *Journal of Agricultural Science and Technology*, *17*(4), 1011–1023.

Bahramikia, S., Ardestani, A., & Yazdanparast, R. (2009). Protective effects of four Iranian medicinal plants against free radical-mediated protein oxidation. *Food Chemistry*, *115*(1), 37–42. https://doi.org/10.1016/j.foodchem.2008.11.054

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry*, *99*(1), 191–203. https://doi.org/10.1016/j.foodchem.2005.07.042

Bernhardt, C. E., Mitchell, R. J., & Michaelsy, H. J. (2008). Effects of population size and density on pollinator visitation, pollinator behavior, and pollen tube abundance in *Lupinus perennis*. *International Journal of Plant Sciences*, *169*(7), 944–953. https://doi.org/10.1086/589698

Chen, J. F., & Zhou, X. H. (2011). *Cucumis*. In C. Kole (ed.), *Wild Crop Relatives: Genomic and Breeding Resources* (pp. 67-90). Springer.

Chen, J. F., Staub, J. E., Tashiro, Y., Isshiki, S., & Miyazaki, S. (1997). Successful interspecific hybridization between *Cucumis sativus* L. and *C. hystrix* Chakr. *Euphytica*, *96*, 413–419. https://doi.org/10.1023/A:1003017702385

Chen, S., Shen, X., Cheng, S., Li, P., Du, J., Chang, Y., & Meng, H. (2013). Evaluation of garlic cultivars for polyphenolic content and antioxidant properties. *PLoS ONE*, *8*(11), e79730. https://doi.org/10.1371/journal.pone.0079730

Chikh-Rouhou, H., Gómez-Guillamón, M. L., González, V., Sta-Baba, R., & Garcés-Claver, A. (2021a). *Cucumis melo* L. germplasm in Tunisia: Unexploited sources of resistance to fusarium wilt. *Horticulturae*, *7*(8), 208. https://doi.org/10.3390/horticulturae7080208

Chikh-Rouhou, H., Mezghani, N., Mnasri, S., Mezghani, N., & Garcés-Claver, A. (2021b). Assessing the genetic diversity and population structure of a Tunisian melon (*Cucumis melo* L.) collection using phenotypic traits and SSR molecular markers. *Agronomy*, *11*(6), 1121. https://doi.org/10.3390/agronomy11061121

Chikh-Rouhou, H., Tlili, I., Ilahy, R., R'him, T., & Sta-Baba, R. (2021c). Fruit quality assessment and characterization of melon genotypes. *International Journal of Vegetable Science*, *27*(1), 3-19. https://doi.org/10.1080/19315260.2019.1692268

Cui, M., Yang, Y., Cheng, Z., & Meng, H. (2020). Dynamic changes of fruit shape traits and its correlation to the morphology of other organs in cucumber. *Agronomy*, *10*(8), 1111. https://doi.org/10.3390/agronomy10081111

Davis, S. L. (2001). Phenotypic and genetic correlations among floral traits in two species of *Thalictrum*. *Journal of Heredity*, *94*(4), 361–366. https://doi.org/10.1093/jhered/92.4.361

De Souza, E. H., De Carvalho Costa, M. A. P., Souza, F. V. D., De Oliveira, S. S., & Dos Santos-Serejo, J. A. (2012). Genetic variability of banana with ornamental potential. *Euphytica*, *184*(3), 355–367. https://doi.org/10.1007%2Fs10681-011-0553-4

Deakin, H. R., Bohn, G. W., & Whitaker, T. W. (1971). Interspecific hybridization in *Cucumis*. *Economic Botany*, *25*, 195–211.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350-356. https://doi.org/10.1021/ac60111a017

Elbekkay, M., Hamza, H., Haddad, M., Ferchichi, A., & Kik, C. (2008). Genetic erosion in melon (*Cucumis melo*): A case study from Tunisia. *Proceedings of the IXth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae*, France. pp. 295–300.

Espírito-Santo, M. M., Neves, F. S., Fernandes, G. W., & Silva, J. O. (2012). Plant phenology and absence of sex-biased gall attack on three species of *Baccharis*. *PLoS ONE*, *7*(10), e46896. https://doi.org/10.1371/journal.pone.0046896

FAO. (2012). FAOSTAT agricultural database. http://ref.data.fao.org.

Ghafoori, H., Sariri, R., Naghavi, M. R., Aryakia, E., Dolatyari, A., Fazeli, S. S., Ramazani, H., & Farahmand, Z. (2013). Analysis of artemisinin isolated from *Artemisia annua* L. by TLC and HPLC. *Journal of Liquid Chromatography & Related Technologies*, *36*(9), 1198-1206. https://doi.org/10.1080/10826076.2012.685916

Grandillo, S., Ku, H. M., & Tanksley, S. D. (1996). Characterization of *fs8.1*, a major QTL influencing fruit shape in tomato. *Molecular Breeding*, *2*(3), 251-260. https://doi.org/10.1007/BF00564202

Hao, B., Li, W., Linchun, M., Li, Y., Rui, Z., Mingxia, T., & Weikai, B. A. (2006). Study of conservation genetics in *Cupressus chengiana*, an endangered endemic of China, using ISSR markers. *Biochemical Genetics*, *44*(1-2), 29-43. https://doi.org/10.1007/s10528-006-9011-8

Herrera, C. M. (2002). Correlated evolution of fruit and leaf size in bird-dispersed plants: Species-level variance in fruit traits explained a bit further? *Oikos*, *97*(3), 426-432.

Hikosaka, S., & Sugiyama, N. (2004). Characteristics of flower and fruit development of multi-pistillate type cucumbers. *The Journal of Horticultural Science and Biotechnology*, *79*(2), 219-222. https://doi.org/10.1080/14620316.2004.11511751

Hosu, A., Cristea, V. M., & Cimpoiu, C. (2014). Analysis of total phenolic, flavonoids, anthocyanins and tannins content in Romanian red wines: Prediction of antioxidant activities and classification of wines using artificial neural networks. *Food Chemistry*, *150*, 113–118. https://doi.org/10.1016/j.foodchem.2013.10.153

Humphreys, M. O. (2003). Utilization of plant genetic resources in breeding for sustainability. *Plant Genetic Resources: Characterisation and Utilisation*, *1*(1), 11–18. https://doi.org/10.1079/PGR20032

Ismail, H. I., Chan, K. W., Mariod, A. A., & Ismail, M. (2010). Phenolic content and antioxidant activity of cantaloupe (*Cucumis melo*) methanolic extracts. *Food Chemistry*, *119*(2), 643–647. https://doi.org/10.1016/j.foodchem.2009.07.023

Jump, A. S., Marchant, R., & Penuelas, J. (2008). Environmental change and the option value of genetic diversity. *Trends in Plant Science*, *14*(1), 51–58. https://doi.org/10.1016/j.tplants.2008.10.002

Karakurt, Y., Güvercin, D., Önder, S., & Işler, Ö. (2020). Assessment of genetic diversity in cucumber (*Cucumis sativus* L.) genotypes using morphological characters and AFLP analysis. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi*, *23*(3), 577-585. https://doi.org/10.18016/ksutarimdoga.vi.583844

Kashyap, S., Sahu, C. K., Verma, R. K., & Chaudhary, L. B. (2021). Taxonomic application of macro and micro morphological characters of seeds in *Astragalus* L. (Galegeae, Fabaceae) in India. *Phytotaxa*, *502*(2), 191-207. https://doi.org/10.11646/phytotaxa.502.2.8

Kirkbride, J. H. (1993). *Biosystematic monograph of the genus Cucumis (Cucurbitaceae): Botanical identification of cucumbers and melons*. Parkway Publishers, Inc.

Kresovich, S., & McFerson, J. R. (1992). Assessment and management of plant genetic diversity: Consideration of intra- and interspecific variation. *Field Crops Research*, *29*(3), 185-204. https://doi.org/10.1016/0378-4290(92)90025-5

Liu, J., Qu, J., Hu, K., Zhang, L., Li, J., Wu, B., Luo, C., Wei, A., Han, Y., & Cui, X. (2015). Development of genome-wide simple sequence repeat fingerprints and highly polymorphic markers in cucumbers based on next-generation sequence data. *Plant Breeding*, *134*(5), 605-611. https://doi.org/10.1111/pbr.12304

Maietti, A., Tedeschi, P., Stagno, C., Bordiga, M., Travaglia, F., Locatelli, M., Arlorio, M., & Brandolini, V. (2012). Analytical traceability of melon (*Cucumis melo* var *reticulatus*): Proximate composition, bioactive compounds, and antioxidant capacity in relation to cultivar, plant physiology state, and seasonal variability. *Journal of Food Science*, *77*(6), C646-C652. https://doi.org/10.1111/j.1750-3841.2012.02712.x

Manchali, S., Chidambara Murthy, K. N., & Patil, B. S. (2021). Nutritional composition and health benefits of various botanical types of melon (*Cucumis melo* L.). *Plants*, *10*(9), 1755. https://doi.org/10.3390/plants10091755

Mangmang, J. S., Deaker, R., & Rogers, G. (2016). Germination characteristics of cucumber influenced by plant growth–promoting rhizobacteria. *International Journal of Vegetable Science*, *22*(1), 66-75. https://doi.org/10.1080/19315260.2014.938850

Maskato, Y., Talal, S., Keasar, T., & Gefen, E. (2014). Red foliage color reliably indicates low host quality and increased metabolic load for development of an herbivorous insect. *Arthropod-Plant Interactions*, *8*, 285-292. https://doi.org/10.1007/s11829-014-9307-2

Naghavi, M. R., Aryakia, E., Hadi, S., Ghafoori, H., Mousavi, H., Ramazani, H., Feyzbakhsh, M., Ajani, Y., Farahmand, Z., Poorhosseini, L., & Shahzadeh Fazeli, S. A. H. (2019). Characterization of morphological, phytochemical and molecular diversity of *Artemisia annua* accessions in Hyrcanian area of Iran. *Journal of Agricultural Science and Technology*, *21*(5), 1265-1276.

Nijveldt, R. J., van Nood, E., Van Hoorn, D. E. C., Boelens, P. G., Van Norren, K., & Van Leeuwen, P. A. M. (2001). Flavonoids: A review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition*, *74*(4), 418–425. https://doi.org/10.1093/ajcn/74.4.418

Pandey, A., Ranjan, P., Ahlawat, S. P., Bhardwaj, R., Dhariwal, O. P., Singh, P. K., Malav, P. K., Harish, G. D., Prabhu, P., & Agrawal, A. (2021). Studies on fruit morphology, nutritional and floral diversity in less-known melons (*Cucumis melo* L.) of India. *Genetic Resources and Crop Evolution*, *68*(4), 1453-1470. https://doi.org/10.1007/s10722-020-01075-3

Parvathaneni, R. K., Natesan, S., Devaraj, A. A., Muthuraja, R., Venkatachalam, R., Subramani, A. P., & Laxmanan, P. (2011). Fingerprinting in cucumber and melon (*Cucumis* spp.) Genotypes using morphological and ISSR markers. *Journal of Crop Science and Biotechnology*, *14*, 39-43. https://doi.org/10.1007/s12892-010-0080-1

Pierce, L. K., & Wehner, T. C. (1990). Review of genes and linkage groups in cucumber. *HortScience*, *25*(6), 605-615.

Raghami, M., López-Sesé, A. I., Hasandokht, M. R., Zamani, Z., Moghadam, M. R. F., & Kashi, A. (2014). Genetic diversity among melon accessions from Iran and their relationships with melon germplasm of diverse origins using microsatellite markers. *Plant Systematics and Evolution*, *300*, 139-151. https://doi.org/10.1007/s00606-013-0866-y

Rolnik, A., & Olas, B. (2020). Vegetables from the Cucurbitaceae family and their products: Positive effect on human health. *Nutrition*, *78*, 110788. https://doi.org/10.1016/j.nut.2020.110788

Schemske, D. W. (1980). Evolution of floral display in the orchid *Brassavola nodosa*. *Evolution*, *34*(3), 489-493. https://doi.org/10.2307/2408218

Shahrivari, S., Alizadeh, S., Ghassemi-Golezani, K., & Aryakia, E. (2022). A comprehensive study on essential oil compositions, antioxidant, anticholinesterase and antityrosinase activities of three Iranian *Artemisia* species. *Scientific Reports*, *12*, 1-12. https://doi.org/10.1038/s41598-022-11375-6

Sheikhi, A., Arab, M. M., Brown, P. J., Ferguson, L., & Akbari, M. (2019). Pistachio (*Pistacia* spp.) breeding. In J. M. Al-Khayri, S. M. Jain, & D. V. Johnson (Eds.), *Advances in plant breeding strategies: Nut and beverage crops* (pp. 353-400). Springer, Cham. https://doi.org/10.1007/978-3-030-23112-5_10

Solankey, S. S., Singh, P. K., & Singh, R. K. (2015). Genetic diversity and interrelationship of qualitative and quantitative traits in sweet potato. *International Journal of Vegetable Science*, *21*(3), 236-248. https://doi.org/10.1080/19315260.2013.867295

Spanos, G. A., & Wrolstad, R. E. (1990). Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. *Journal of Agricultural & Food Chemistry*, *38*(7), 1565–1571. https://doi.org/10.1021/jf00097a030

Stepansky, A., Kovalski, I., & Perl-Treves, R. (1999a). Intraspecific classification of melons (*Cucumis melo* L.) in view of their phenotypic and molecular variation. *Plant Systematics and Evolution*, *217*, 313-332. https://doi.org/10.1007/BF00984373

Stepansky, A., Kovalski, I., Schaffer, A. A., & Perl-Treves, R. (1999b). Variation in sugar levels and invertase activity in mature fruit representing a broad spectrum of *Cucumis melo* genotypes. *Genetic Resources and Crop Evolution*, *46*, 53–62. https://doi.org/10.1023/A:1008636732481

Szamosi, C. (2005). The importance of Hungarian melon (*Cucumis melo* L.) landraces, local types and old varieties. *International Journal of Horticultural Sciences*, *11*(4), 83-87. https://doi.org/10.31421/IJHS/11/4/611

Tsukaya, H. (2005). Leaf shape: genetic controls and environmental factors. *International Journal of Developmental Biology*, *49*(5-6), 547–555. https://doi.org/10.1387/ijdb.041921ht

UPOV. (2019). International union for the protection of new varieties of plants: Guidelines for the conduct of tests for distinctness, uniformity and stability. Cucumber, Gherkin, UPOV code: Cucum_Sat (*Cucumis sativus* L.) Geneva, Switzerland.

Xiao, L., Ge, X. G., Gong, X., Hao, G., & Zheng, S. X. (2004). ISSR Variation in the endemic and endangered plant *Cycas guizhouensis* (Cycadaceae). *Annual Botany*, *94*(1), 133-138. https://doi.org/10.1093/aob/mch119

Yu, X., Hyldgaard, B., Rosenqvist, E., Ottosen, C. O., & Chen, J. (2015). Interspecific hybridization in Cucumis leads to the divergence of phenotypes in response to low light and extended photoperiods. *Frontiers in Plant Science*, *6*, 802. https://doi.org/10.3389/fpls.2015.00802