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Original article

Genotypic and phenotypic diversity in guava (*Psidium guajava* L.) genotypes from Iran

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Summary

Introduction – **Guava, an important fruit crop worldwide and in southern Iran, is believed to have originated from Central America. However, the extant of diversity in the most guava producing regions is poorly understood.** *Materials and methods* – **Seventy-nine morphological parameters, along with nine simple sequence repeat markers were used to characterize the genetic relations of 20 guava genotypes in Iran.** *Results and discussion* – **Principal component analysis for the first and second components explained 53.58% of the variability among the genotypes. The number of alleles per locus ranged from 2 to 4 with an average of 3 alleles per locus. The mean expected and observed heterozygosity over 8 polymorphic SSR loci were 0.62 and 0.67, respectively.** *Conclusion* – **The studied individuals were divided based on their fruit shape and pulp color, and hence, our assumption that the genotypes would be grouped according to their fruit characteristics was verified. SSR markers allowed us to monitor the studied individuals. Therefore, genotypes with pink pulp (and round fruits) were separated from those that had white pulp (with pyriform or ellipsoid fruits) by both molecular and morphological data.**

Keywords
genetic grouping, morphological diversity, molecular markers, SSRs

Abbreviations

A	Number of alleles per locus
AFLP	Amplified fragment length polymorphisms
F	Wright's fixation index
He	Expected heterozygosity
Ho	Observed heterozygosity
Ne	Effective number of alleles
PCA	Principal component analysis
PI	Probability of identity
RAPD	Random amplified polymorphic DNA
SSAP	Sequence-specific amplified polymorphism
SSRs	Simple sequence repeats

Significance of this study

What is already known on this subject?

- Guava is an important crop in tropical and sub-tropical regions, and was introduced to the southern regions of Iran about 400 years ago. Since guava is a cross-pollinated and self-incompatible crop, considerable diversity is present in this species. Recently, promising progress has been made regarding the cultivation of guava in Iran, due to the increasing range of the processed products. This expanding cultivation needs the introduction of new varieties.

What are the new findings?

- The studied individuals were divided based on their fruit shape and pulp color, and hence, the assumption that the accessions would be grouped according to their fruit characteristics was verified. Accessions with the pink pulp (and round fruits) were separated from those that had white pulp (with pyriform or ellipsoid fruits) by both molecular and morphological data.

What is the expected impact on horticulture?

- The generated information would be useful in selecting superior genotypes as parents in efficient breeding programs. The broad and distinctive range in phenotypic variation among studied guava genotypes could be connected with the outputs of other guava research centers to join both information and plant materials.

Introduction

Guava (*Psidium guajava* L., Myrtaceae, $2n = 22$), an important crop in tropical and subtropical regions, is believed to have originated from Central America (Biffin *et al.*, 2010; Marques *et al.*, 2016). Guava is a commercial crop in India, South Africa, Brazil, New Zealand, Philippines, Thailand (Tate, 2000), and Iran. About 400 years ago, guava was introduced to the southern regions of Iran (Hormozgan province), following trading routes with India. In Iran, guava trees bear fruits twice a year, which is in September–October (which is the main production period) and January–March. Based on FAO statistics (2018), the world production of guavas reaches approximately 7.25 million tons, which are consumed as fresh or frozen fruit and also processed into jelly, juice, paste, pudding, syrup and waffles (Flores *et al.*, 2015). According to the Iranian Ministry of Agriculture's latest statistics, the cultivation area and production of guava in Iran reached 1,750 ha and 4,119 tons, respectively (Center of Information and Technology, 2019).

Since guava is a cross-pollinated and self-incompatible crop, there is considerable diversity within the species. However, the focus on some main commercial parameters (*e.g.*, pulp thickness and color, fruit size, shape, TSS and aroma) (Pommer and Murakami, 2009; Sharma *et al.*, 2010; Galli

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et al., 2015; Valera-Montero *et al*., 2016; Moon *et al*., 2018) has resulted in the cultivation of a small number of varieties among the more than 400 known guava cultivars. For instance, 'Allahabad Safeda', the most popular cultivar in India, Australia, and Egypt, is characterized by its big fruit size, white flesh, sweet taste and a few seeds. 'Beaumont', which is a seedling selection, found in Oahu, Hawaii bears medium to large, round pink-fleshed fruits, which are suitable for processing objectives (Coêlho De Lima *et al*., 2002; Pommer and Murakami, 2009; Sharma *et al*., 2010). In most fruit crops, studies based on morphological traits are limited due to the long juvenility period, large individual size, perennial nature and obligate cross-pollination. Therefore, in the last two decades, molecular markers have been used to determine diversity of many woody perennial species (Larrañaga and Hormaza, 2016).

In guava, molecular diversity has been analyzed using RAPD (Dahiya *et al*., 2002; Prakash *et al*., 2002; Chen *et al*., 2007; Feria-Romero *et al*., 2009), SSR (Risterucci *et al*., 2005) and AFLP (Hernandez-Delgado *et al*., 2007) markers. Recently, promising progress has been made regarding the cultivation of guava in Iran, due to the increasing range of processed products. This expanding cultivation needs the introduction of new genotypes. As a preliminary step, we evaluate the genetic diversity of Iranian guava genotypes using morphological and SSR markers.

Materials and methods

Plant materials

This research was performed during 2017–2019 on 20 distinct guava genotypes (ten-year-old) located in Minab City, Hormozgan Province, Iran (27°N, 57°E, with an altitude of 40 m a.s.l., an average annual temperature range of 22.5 to 31.3 °C, mean RH of 60%, and a mean annual precipitation of 148 mm), which is the most important area for guava cultivation in the country. The average spacing between plants was 6 m^2. The horticultural practices included irrigation (twice a week) and fertilization (with 0.21 kg of urea, 0.18 kg of potassium sulfate, and 0.19 kg of superphosphate for each plant). The local nomenclature of guava genotypes in Iran, based on some commercial fruit parameters such as white, creamy or pink pulp color, was used in this study to describe the genotypes. We also used different code numbers tagged G-1 to G-20 to represent each of the genotypes.

Phenotypic evaluation

During the 2017–2018 season, qualitative and quantitative parameters were evaluated based on the available guava descriptors (UPOV, 1987; Cárdenas-Urdaneta and Jiménez-Mendoza, 2004; Sánchez-Urdaneta and Peña-Valdivia, 2011). Data were collected during vegetative (early-summer), reproductive (early-winter and late-summer) and fruiting (late-winter and mid-autumn) stages of growth. A random sample of ten fruits, ten leaves, ten inflorescences, and ten flowers per plant were collected and transferred to the lab at the University of Hormozgan, Iran, for the following measurements. Supplemental Information – Tables S1 – S4, represent the evaluated parameters, measuring units and methods. The fruit firmness was determined using a Metrohm 744 penetrometer and expressed as kg cm^{-2}. The fruits were harvested at the mature green stage. The fruit skins were removed with a sharp knife, then every fruit was placed on a hard surface and the penetrometer was held at a right angle to the fruit surface. The force was recorded as firmness value (AOAC, 1990).

To evaluate seed germination percentage, the seeds were placed on Petri dishes and irrigated daily. The seeds with root length equal to the seed diameter were assumed as germinated. Germination percentage was determined according to Krishnasamy and Seshu (1989). The descriptive statistical analysis of qualitative and quantitative variables was done using SPSS v.22 software (IBM SPSS, 2013). The principal component analysis (PCA) was used to investigate the relationships among the studied genotypes using SPSS (IBM SPSS, 2013). The bi-plot was created based on the PC_1 and PC_2 using SigmaPlot 10.0 (SigmaPlot Inc., 2019). Moreover, the distance matrix from morphological variables was used for cluster analysis (unweighted paired group method of arithmetic average) using SPSS (IBM SPSS, 2013).

Molecular analysis

Fresh young leaves were collected during summer 2019 and total genomic DNA was extracted using the Murray and Thompson (1980) protocol. Briefly, leaf tissue (0.5 g) was ground to a fine powder in liquid nitrogen. The powder was dispersed in 100 mL extraction buffer (2% CTAB, 5 M NaCl, 2 M Tris-HCl, pH 8.0, 2% 2-mercaptoethanol and 0.5 M EDTA). The mixture was incubated at 65 °C for 30 min. The extract was emulsified by mild inversion with an equal volume of chloroform/isoamyl alcohol (24:1). The centrifugation (10,000 rpm, 15 min), was followed by removing the aqueous phase. Then one-sixth volume of the aqueous phase, cold isopropanol was added and after 5 min incubation under laboratory conditions, the tubes were re-centrifuged (10,000 rpm, 15 min). Finally, DNA was precipitated by adding 1 mL of ethanol (Murray and Thompson, 1980). A total of nine SSR loci (mPgCIR04, mPgCIR08, mPgCIR09, mPg-

Table 1. Characteristics of the nine SSR loci used to evaluate genetic variation in 20 Iranian guava genotypes.

SSR location	EMBL Accession No.	Repeat motif	Primer sequences (5'–3')	
			Forward	Reverse
mPgCIR04	AJ639755	$(GA)_{25}$	TTCAGGGTCTATGGCTAC	CAACAAGATACAGCGAACT
mPgCIR08	AJ639758	$(GA)_{12}$	ACTTTCGGTCTCAACAAG	AGGCTTCCTACAAAAGTG
mPgCIR09	AJ639759	$(GA)_{19}$	GCGTGTCGTATTGTTTC	ATTTTCTTCTGCCTTGTC
mPgCIR11	AJ639761	$(CT)_{17}$	TGAAAGACAACAAACGAG	TTACACCCACCTAAATAAGA
mPgCIR15	AJ639764	$(GA)_8GG(GA)_9$	TCTAATCCCCTGAGTTTC	CCGATCATCTCTTTCTTT
mPgCIR16	AJ639765	$(TC)_{25}$	AATACCAGCAACACCAA	CATCCGTCTCTAAACCTC
mPgCIR17	AJ639766	$(CT)_{23}$	CCTTTCGTCATATTCACTT	CATTGGATGGTTGACAT
mPgCIR20	AJ639769	$(CT)_{14}(CA)_{17}$	TATACCACACGCTGAAAC	TTCCCCATAAACATCTCT
mPgCIR26	AJ639774	$(GT)_2(GA)_{17}$	CTACCAAGGAGATAGCAAG	GAAATGGAGACTTTGGAG

CIR11, mPgCIR15, mPgCIR16, mPgCIR17, mPgCIR20 and mPgCIR26) published previously by Risterucci *et al*. (2005), were used to analyse the diversity of the 20 guava genotypes. The characteristics of those SSR loci are given in Table 1. DNA amplification was carried out following the protocol of Risterucci *et al*. (2005).

Amplification reactions were performed in 20 µL containing 50 mM KCl, 10 mM Tris-HCl (pH=8), 0.01% Tween 20, 1.5 mM $MgCl_2$, 200 µM dNTP, 0.2 µM each primer, 20 ng of genomic DNA and 0.5 U of BioTaq™ DNA polymerase (Bioline, London, UK) on a Bio-Rad (Bio-Rad Laboratories, Hercules, CA, USA) thermocycler. The temperature profile included an initial step of 5 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, and a final step of 10 min at 72 °C. The amplification products of each genotype were separated on a 6% (w/v) denaturing polyacrylamide gel. Then a 100 bp DNA ladder (GeneAll, Germany) was used as the size marker. The gel was stained with silver nitrate (Merck Company, Ger-

Table 2. List and characteristics of the 20 guava genotypes analyzed in this work.

Genotype	Local name	Growth habit	Peak of main flowering	Peak of second flowering	Fruit shape	Pulp color	Seed per fruit
G-1	Mahali	Semi-erect	Late April	Mid November	Pyriform	White	Low
G-2	Gol	Vertical	Late April	Late November	Pyriform	White	Low
G-3	Anonym1	Extended	Late April	Late November	Round	Pink	Low
G-4	Ahmadi	Semi-erect	Late April	Late November	Round	Pink	Low
G-5	Asadi	Extended	Late May	Late November	Ellipsoid	White	Low
G-6	Abkenar	Extended	Late May	Late November	Ellipsoid	White	High
G-7	Bidaneh1	Extended	Late April	Late November	Ellipsoid	White	Low
G-8	Mikhaki	Extended	Late April	Late November	Ellipsoid	White	Low
G-9	Bidaneh2	Extended	Late April	Late November	Ellipsoid	White	Low
G-10	Zood gol	Semi-erect	Late April	Late October	Round	Pink	Low
G-11	Bidaneh3	Semi-erect	Late April	Late November	Round	Pink	Low
G-12	Too sorkh1	Extended	Late April	Late November	Round	Pink	Low
G-13	Anonym2	Extended	Late April	Late November	Round	Pink	High
G-14	Gerdeh	Semi-erect	Late April	Late November	Round	Pink	High
G-15	Khosh gol	Semi-erect	Late April	Late November	Round	Pink	High
G-16	Mokhtari	Extended	Late April	Late November	Round	Pink	Low
G-17	Too sorkh2	Semi-erect	Late April	Late November	Round	Pink	Low
G-18	Madani	Semi-erect	Late April	Late November	Round	Pink	Low
G-19	Anonym3	Extended	Late April	Late November	Round	Pink	Low
G-20	Abassi	Extended	Late April	Late November	Round	Pink	Low

Table 3. List and fruit characteristics of the 20 guava genotypes analyzed in this work.

Genotype	Fruit length (cm)	Pulp thickness (mm)	Brix (%)	Firmness (kg cm^{-2})	Epicarp thickness (mm)
G-1	4.60	6.33	11.60	0.52	0.63
G-2	4.60	6.33	11.60	0.52	0.63
G-3	5.50	10.00	9.70	2.04	0.57
G-4	5.50	10.00	9.70	2.04	0.57
G-5	5.00	7.67	12.60	0.98	0.57
G-6	5.17	10.00	12.80	0.29	0.57
G-7	5.17	10.00	11.20	0.40	0.67
G-8	4.07	7.67	12.60	0.98	0.57
G-9	5.17	10.00	11.20	0.40	0.67
G-10	6.17	9.33	12.60	1.37	0.63
G-11	5.00	10.33	11.30	1.83	0.57
G-12	6.50	10.50	16.60	0.45	0.57
G-13	5.50	10.00	9.70	2.04	0.57
G-14	6.00	8.67	9.50	0.95	0.57
G-15	6.00	10.50	3.50	1.37	0.53
G-16	5.50	9.67	5.20	0.83	0.57
G-17	4.50	9.00	4.80	1.40	0.53
G-18	5.33	9.33	3.70	0.98	0.57
G-19	5.33	8.67	8.00	1.60	0.57
G-20	4.83	9.33	7.30	0.75	0.60

many) (Creste *et al*., 2001) and finally, the presence of each amplification fragment was scored for all individuals (0: absent, 1: present).

The genetic information of each SSR locus was expressed by the number of alleles per locus (A), the effective number of alleles (Ne= 1/1-He), observed heterozygosity (Ho, direct count), expected heterozygosity (He= $1-\sum_i p_i^2$) (Nei, 1973), Wright's fixation index ($F=1-H_o/H_e$) (Wright, 1951), probability of identity (PI= $1- \sum_i p_i^4 + \sum_i\sum_j(2p_ip_j)^2$) (Paetkau *et al*., 1995) and UPGMA clustering (Nei and Li, 1979) using the Arlequin version 3.01 program (Excoffier *et al*., 2005), Popgene 1.32 software (Yeh *et al*., 1997), Identity 1.0 (Centre for Applied Genetics, University of Agricultural Sciences, Vienna, Austria), Ntsys-pc 2.11 (Rohlf, 2008) and Genepop (Raymond and Rousset, 1995). The relationships between the distance matrices, obtained with phenotypic traits and molecular data, were analyzed by the Mantel test (Mantel, 1967).

Results and discussion

Phenotypic evaluation

Some characteristics of the guava genotypes are summarized in Tables 2 and 3. The descriptive statistical analyses of the studied guava genotypes are given in Table 4. The quantitative leaf parameters including leaf length, leaf width, length of the main axis, length of the minor axis, and angle of the leaf axis were diverse among the studied guava genotypes (coefficient of variation of 11.43, 19.43, 23.52, 21.02 and 17.32%, respectively) (Table 4). Among parameters related to flower and inflorescence, some variables such as flower width and peduncle length (coefficient of variations of 19.23 and 16.32%, respectively) varied more. The average values for fruit length, width and firmness were 5.27 cm, 4.50 cm and 1.09 kg cm^{-2}. The mean value for epicarp thickness was 3.07 mm and varied between 2.90–3.33 mm. Seed germination percentage ranged between 76.67 and 94.67% (average of 87.67%) (Table 4).

The knowledge of the available genetic diversity is an essential step in crop breeding and germplasm conservation programs. In recent decades, morphological and molecular markers have successfully been used in the exploration of genotypic relationships in different Iranian fruit crops, including mango (Shamili *et al*., 2012), apricot (Khadivi-Khub *et al*., 2015), walnut (Ebrahimi *et al*., 2015) and stone fruits (Gharaghani *et al*., 2017). The fruit-related characteristics are closely linked to local preferences for fresh or processed guava marketing (González-Gaona *et al*., 2002; Molero *et al*., 2003; Sanabria *et al*., 2006). Intermediate sized, round fruits with white mesocarp and low seed content are promising parameters for the Mexican guava industries (González-Gaona *et al*., 2002; Hernandez-Delgado *et al*., 2007). Ovoid pink-mesocarp fruits are the most frequent in Colombian guavas (Sanabria *et al*., 2006). Furthermore, the fruits with white-colored pulp and globose shape are regular in the Indian guava markets (Kanupriya *et al*., 2011). In Pakistan, the diameter of fruit cavity, seed weight, epicarp thickness, presence and prominence of longitudinal ridge and fruit skin color are the most critical commercial parameters (Mehmood *et al*., 2014, Kareem *et al*., 2018). In Iran, pyriform and round guavas, with high soluble solids, soft, juicy and seedless pulp are desired for the fresh market, while fruits with pink pulp

Table 4. The descriptive statistical analyses of quantitative parameters of the studied guava genotypes.

Variables	Descriptive statistics					
	Mean	Minimum	Maximum	Kurtosis	Skewness	Coefficient of variation
Leaf length	10.58	6.00	14.00	1.50	-0.11	11.43
Leaf width	0.97	0.25	2.00	1.81	1.45	19.43
Length of the main axis	11.58	9.00	13.17	-0.14	-0.70	23.52
Length of the minor axis	3.59	2.00	4.17	1.50	-1.60	21.02
Angle of the leaf axis	44.23	41.67	45.00	1.61	-1.52	17.32
Flower length	11.52	11.00	11.67	0.18	-1.00	12.43
Flower width	6.63	4.50	7.90	-0.39	1.14	19.23
Inflorescence length	5.43	5.00	6.00	-1.66	0.24	14.32
Inflorescence diameter	3.34	2.50	4.17	0.33	0.68	15.80
Calyx diameter	11.47	10.33	11.67	1.72	-1.36	11.23
Peduncle length	1.61	0.90	2.00	-1.33	-0.63	16.32
Flower disk length	3.19	2.50	3.50	0.14	-1.28	14.21
Petal length	1.70	1.50	1.83	-1.48	-0.61	9.23
Petal number	4.88	4.00	5.00	1.89	-1.65	8.21
Sepal length	10.75	8.33	12.00	0.25	-0.79	5.23
Sepal number	4.45	4.00	5.00	-1.47	0.39	4.32
Stigma length	9.65	8.67	10.00	0.48	-1.13	7.36
Mesocarp thickness	0.59	0.53	0.67	-0.02	1.01	56.32
Epicarp thickness	3.07	2.90	3.33	1.48	1.05	76.23
Fruit length	5.27	4.07	6.50	-0.04	0.12	20.12
Fruit width	4.50	3.57	5.73	-0.28	0.45	18.67
Fruit firmness	1.09	0.29	2.04	-1.12	0.39	37.32
Seed weight	0.49	0.33	0.63	-0.50	-0.03	9.29
Germination percentage	87.67	76.67	94.67	0.29	-0.57	25.32

Table 5. The principal component analysis of guava genotypes using vegetative, reproductive and bearing parameters.

Principal components		Absolute variation	Accumulated variation (%)
1	Fruit shape, mesocarp color, fruit length, fruit width, mesocarp thickness, epicarp thickness, fruit cavity, fruit firmness	35.58	35.58
2	Young leaf length, angle of the leaf axis, seed weight, germination percentage, peduncle length, petal number, sepal length, petiole length	18.00	53.58
3	Shape of the fruit apex, shape of the fruit base	9.35	62.93
4	Growth habit, stem cortex texture, stem color, offspring	7.98	70.91
5	Inflorescence length, calyx diameter	6.17	77.08
6	Stigma shape, inflorescence position	5.45	82.53

Absolute variation and accumulated variation state the total variance explained by each component and accumulated variance explained by components, respectively.

are chosen for processing. In addition, according to our data, a wide range in fruit-related commercial characteristics was found among the studied Iranian guava germplasm.

According to the results, some qualitative variables, for example branch distribution, upper and back color of young leaves, semi-rough fruit surface texture, intermediate maturity period, high productivity, elliptical canopy, elliptic leaf and pyramidal inflorescence, were monomorphic. The axillary position of inflorescence (80%) was more frequent than the terminal position (20%). The white inflorescence color (75%) was dominant in comparison with white-creamy (25%). The stem cortex texture in most of the genotypes was smooth. The growth habit of 55% of the genotypes was extended and the rest were semi-erect (25%) or vertical (20%). Oval seed shape (45%), light fruit cavity (50%), round fruit shape (65%), convex fruit base shape (50%) and truncated fruit apex shape (50%) where the frequent variables. About 90% of genotypes had five stamens and capital stigma shape, but the rest had mace-form stigma shape and four stamens.

Several works reported the success of phenotypic data in distinguishing guava genotypes (Sanabria *et al*., 2006; Kanupriya *et al*., 2011; Mehmood *et al*., 2014; Kareem *et al*., 2018). Fruit weight and the number of seeds showed the highest coefficient of variation in guava accessions (Campos-Rivero *et al*., 2017). Guava fruits with white pulp had lower longitudinal to transversal diameter ratio, larger pulp thickness and higher fruit weight, total soluble sugars and vitamin C content compared to those with pink pulp (Coêlho De Lima *et al*., 2002). However, some guava populations did not display a wide morphological variation (Valdés-Infante *et al*., 2006; Hernandez-Delgado *et al*., 2007; Padilla-Ramírez and Gonzalez-Gaona, 2010; Aranguren *et al*., 2010). According to our findings, the epicarp thickness had the highest coefficient of variation (76.23%), while the lowest value (4.32%) was related to sepal number (Table 4). The traits with a high coefficient of variation value have a wide range of selection opportunities for breeders.

The PCA analysis of guava genotypes using vegetative, reproductive and bearing parameters accounted for 82.53% of the total variation (Table 5). The first component explained 35.58% of the total variation. It included fruit shape, mesocarp color and thickness, fruit length and width, epicarp thickness, fruit cavity and firmness. The second component explained 18.00% of the total variation, including young leaf length, angle of leaf axis, seed weight, germination percentage rate, peduncle length, petal number, sepal length and petiole length. The third component explained 9.35% of the total variance, including the shape of the fruit apex and fruit base. Additional fourth, fifth and sixth components explained 7.98% (growth habit, stem cortex texture, stem color and offspring), 6.17% (inflorescence length and calyx diameter) and 5.45% (shape of stigma and inflorescence position) of the total variance (Table 5.). Bi-plot based on PC_1 and PC_2 for the studied guava genotypes is shown in Figure 1.

According to the principal component analysis (PCA), the mesocarp color, mesocarp and epicarp thickness, along with some fruit parameters (shape, length, width, cavity and firmness), were the main variables which explained 35.58% of the total variance. The second PC was connected to some characteristics of leaves, flowers and seeds (18.00% of the total variation). In guavas of Pakistan, the first component (24.21% of the total variation) related to fruit weight, diameter and length, seed weight and pulp thickness. The second component (12.38% of the total variation) was associated with leaf length and internal fruit contents. According to Hernandez-Delgado *et al*. (2007), the leading PC (explaining 30% of the total variation) included fruit and leaf parameters in Mexican guavas. Sanabria *et al*. (2006) reported yield, growth characteristics and fruit quality as the first PCA (explaining 72% of the variation) in Colombian guava accessions. In addition, the PCA analysis using 18 qualitative characteristics in guava accessions of Pakistan considered the longitudinal ridges, fruit skin color and shape, longitudinal grooves, leaf variegation as the first component (13.81% of the total variation), but included leaf shape, pulp color and young shoot color as PC_2 (61% of the total variation) (Mehmood *et al*., 2014).

Molecular analysis

Table 6 illustrates diversity indices of the studied guava genotypes using eight SSR loci. A total of 24 polymorphic alleles were obtained (with an average of 3 alleles per locus). Only one locus, mPgCIR15, generated monomorphic alleles. The maximum number of alleles (4 alleles) were obtained with mPgCIR04 and mPgCIR09, whereas mPgCIR08 and mPgCIR16 showed the lowest polymorphism with two alleles. The observed and expected heterozygosities among the studied genotypes varied from 0.58 to 0.84 and from 0.48 to 0.73. The average fixation index was -0.12, implying a high genetic substructure within guava genotypes or a high inbreeding rate. The probability of identity index ranged between 0.26 and 0.77.

Successful diversity analysis of guavas using molecular markers was already reported (Padilla-Ramírez *et al*., 2002; Sanabria *et al*., 2006). High variability obtained in Venezuelan guavas (Aranguren *et al*., 2010) and China (Zehua *et al*., 2019), whereas low variability was reported in guavas of Cuba (Valdés-Infante *et al*., 2006). Zehua *et al*. (2019) reported 65

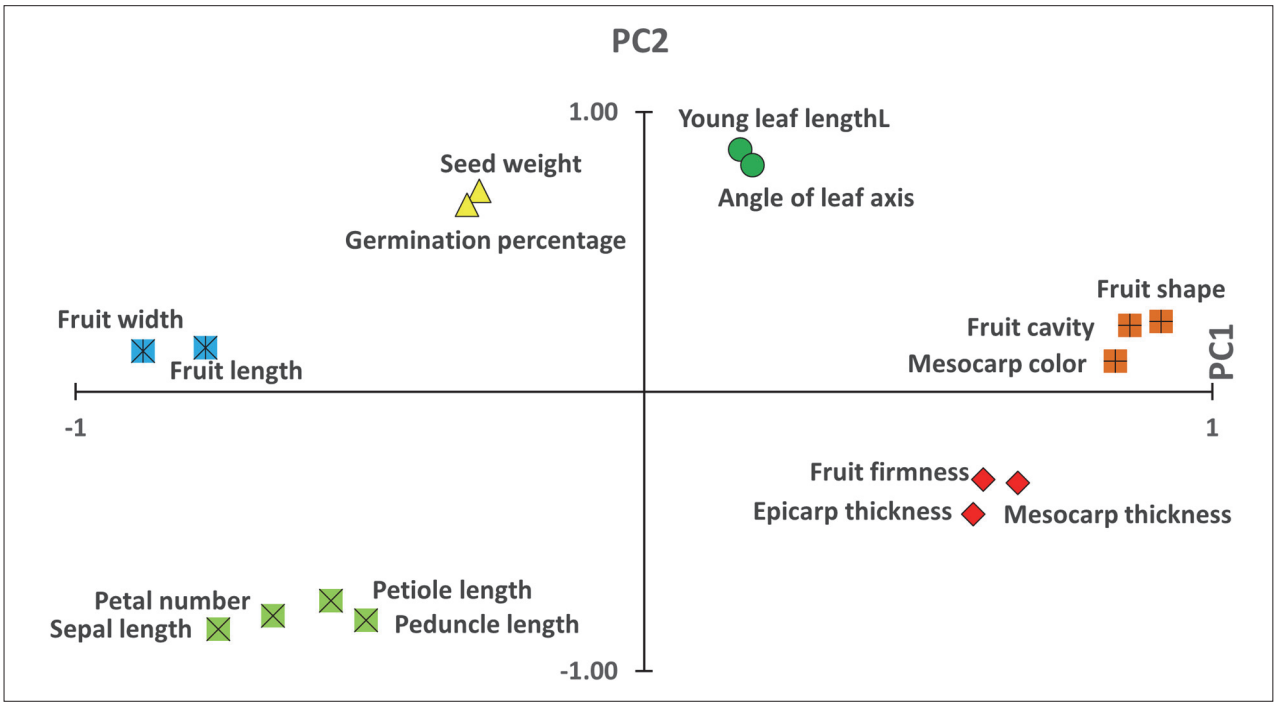

Figure 1. Bi-plot for the guava genotypes evaluated based on PC_1 and PC_2 (the first two principal components).

polymorphic bands analyzing genetic diversity of 45 guava germplasm using SSR markers. The averages of expected and observed heterozygosity in guava germplasm of Kenya were 0.31 and 0.63, respectively, which are similar to our findings (Chiveu *et al.*, 2019). The same authors reported a mean of 6.5, and 3.1 for the number of alleles and the effective allele numbers, respectively. Likewise, the average of 0.74 for the Nei heterozygosity index was reported in guava accessions of Venezuela (Aranguren *et al.*, 2010). The characterization of Indian guavas using microsatellite markers produced 6.39 alleles per locus and the means of 0.82 and 0.14 obtained for expected heterozygosity and probability of identity, respectively (Kanupriya *et al.*, 2011).

Wright's fixation index is a useful tool to understand the evolution rate acting on populations (Wright, 1969). A heterozygote excess, shown by negative F values, might be the consequence of small reproductive population size and asexual reproduction or mutation over generations (Ruggiero *et al.*, 2005). According to our findings, the fixation index for most of the SSR loci had negatives values due to the small population size. Similar F values have been reported earlier in mango (Shamili *et al.*, 2012). Besides, the probability of identity (PI) estimates the genetic diversity levels in populations (Waits and Leberg, 2000). Based on our data, the least and the most PI values belonged to mPgCIR17 and mPgCIR11 loci (0.26 and 0.77, respectively).

In our work, mPgCIR11 and mPgCIR09 loci were the most suitable primers to distinguish the 18 guava genotypes. Those two loci also proved to be adequate for the differentiation of guava genotypes in India and Cuba (Kanupriya *et al.*, 2011; Rodriguez *et al.*, 2004). According to Kherwar *et al.* (2018), the mPgCIR03, mPgCIR05 and mPgCIR251 loci displayed high genetic diversity. Also, in our work, the genotypes G-7 and G-9, which were not distinguishable with molecular markers, were differentiated with morphological markers, probably due to being closely related genotypes (Wünsch and Hormaza, 2002).

Cluster analysis

Two dendrograms created using morphological (Figure 2a) and molecular (Figure 2b) data are represented in Figure 2. Clustering by the morphological data separated the guava

Table 6. Diversity indices of 20 Iranian guava genotypes using 8 polymorphic SSR loci.

SSR location	Number of alleles per locus (A)	Number of effective alleles (Ne)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)	Fixation index (F)	Probability of identity (PI)
mPgCIR04	4	2.32	0.84	0.73	-0.15	0.47
mPgCIR08	2	1.87	0.79	0.58	-0.35	0.48
mPgCIR09	4	2.77	0.74	0.48	-0.54	0.66
mPgCIR11	3	2.92	0.63	0.66	0.04	0.77
mPgCIR16	2	1.98	0.58	0.68	0.14	0.33
mPgCIR17	3	3.00	0.58	0.51	-0.14	0.26
mPgCIR20	3	2.46	0.58	0.68	0.15	0.53
mPgCIR26	3	2.47	0.74	0.61	-0.12	0.45
Mean	3	2.51	0.67	0.62	-0.12	0.51

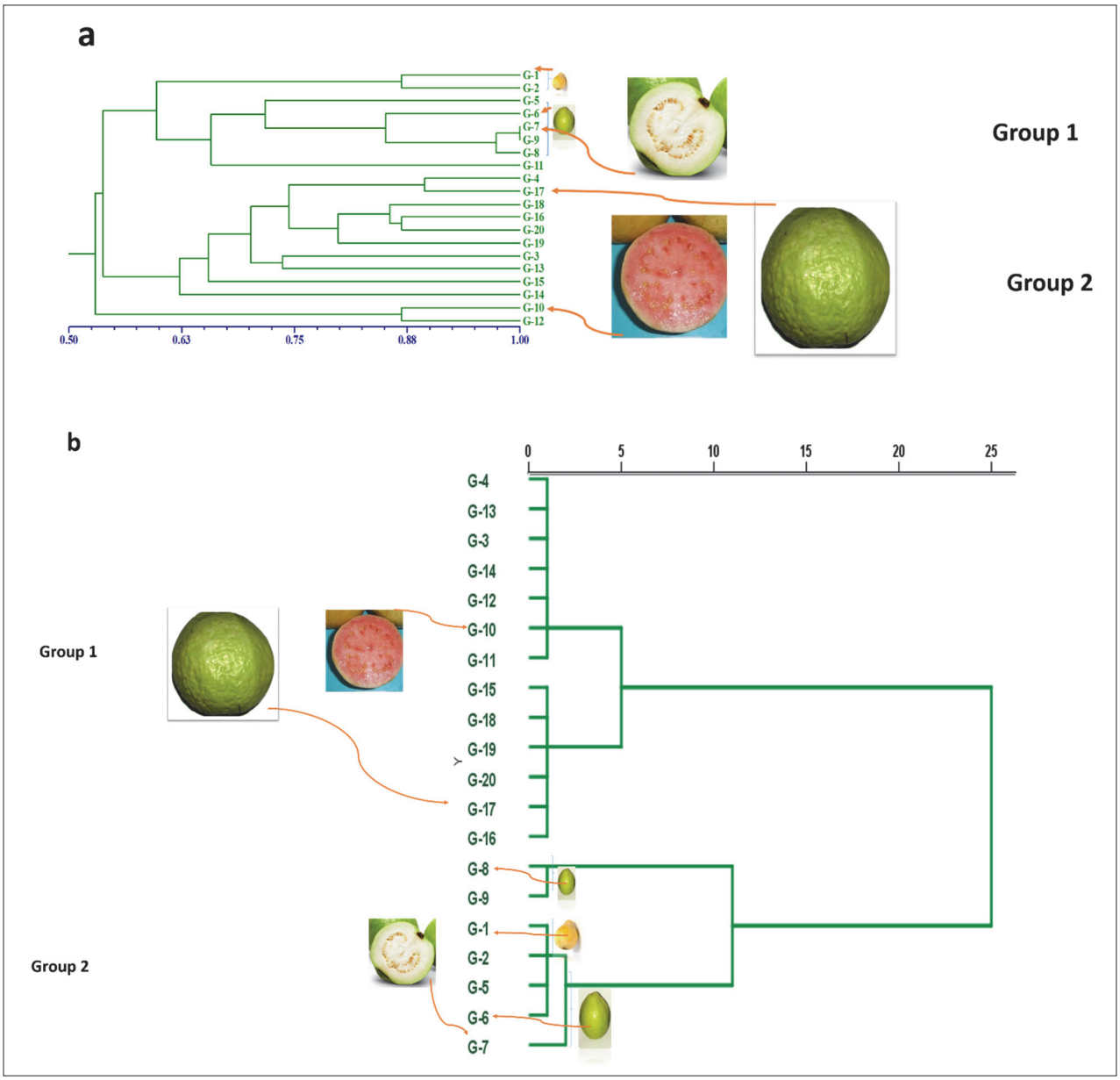

Figure 2. Dendrogram of the 20 guava genotypes evaluated based on morphological (a) and molecular (b) data.

genotypes into two groups; Group one included genotypes with the pink pulp and round fruits (G-3, G-4, G-10, G-11, G-12, G-13, G-14, G-15, G-16, G-17, G-18, G-19 and G-20). Group two included white pulp color genotypes (G-1, G-2, G-5, G-6, G-7, G-8 and G-9). G-1 and G-2 had pyriform fruit shape, but the rest had ellipsoid shape (Figure 2a). The molecular cluster divided the genotypes into two groups. G-11 was placed in group 1 next to G-8. Moreover, G-7 and G-9 were not distinguished by molecular data (Figure 2b).

The Mantel test was used regularly to evaluate the significance of the associations between the matrixes of phenotypic and genotypic measurements (Mantel, 1967). The distance matrixes of both data were compared using the Mantel test to evaluate the agreement between two dendrograms derived from phenotypic and SSR data. The estimated correlation was significant ($r = 0.85$, $p = 0.002$), which confirms the accuracy of dendrograms.

The pulp color, along with fruit shape and size, have proved to be useful traits in the identification of guava from different geographical populations (Mehmood *et al.*, 2014; Kareem *et al.*, 2018). Our graphic illustration of genetic similarity clustered the genotypes evaluated into white and pink-pulp guava; genotypes found in the separated groups, which confirmed previous reports (Chen *et al.*, 2007; Kanupriya *et al.*, 2011). Similar results were reported by Kareem *et al.* (2018), who evaluated 37 genotypes of guava and divided them into six groups based on fruit shape, size, and seed contents. The sequence-specific amplified polymorphism (SSAP) clustered guava genotypes based on their pulp color and fruit shape (Campos-Rivero *et al.*, 2017). Also, guava genotypes have been grouped into two groups based on the pulp color (Kanupriya *et al.*, 2011; Kherwar *et al.*, 2018).

The potential of germplasm selection based on morphological parameters is a tool for different breeding objectives. The findings of the present study supported the efficiency of pomological variables as a reliable tool for identification and differentiation among the guava genotypes and cultivars, as reported earlier by Mehmood *et al.* (2014), Hernandez-Delgado *et al.* (2007), and Padilla-Ramírez and Gonzalez-Gaona (2010).

Conclusion

Determination, maintenance and preservation of genetic diversity in edible plant species are the universal requirements for current and future food supply. In the present research, a combination of morphological and molecular data was used to determine the genetic diversity of guava genotypes in Iran. The studied individuals were divided based on their fruit shape and pulp color, and hence, our assumption that the genotypes would be grouped according to their fruit characteristics was verified. The loci mPgCIR09 and mPgCIR11 were the most informative to distinguish the studied genotypes. Both molecular and morphological data allowed us to differentiate genotypes with the pink pulp (and round fruits) from those with white pulp (with pyriform or ellipsoid fruits). The broad and distinctive range in phenotypic variation among the studied guava genotypes could be connected with the outputs of other guava research centers to join both information and plant materials.

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Supplemental Information

Supplemental Information – Table S1. Parameters of tree and stem used to analyze 20 guava genotypes.

Parameter		Measuring unit	Measuring method
Tree	Canopy shape	1. Elliptical, 2. Globose, 3. Flat, 4. Pyramidal, 5. Rectangular, 6. Irregular	Numeric codes
	Growth habit	1. Erect, 2. Semi-erect, 3. Extended	Numeric codes
	Plant height	m	Meter
	Canopy diameter	cm	Meter
	Branch distribution	1. Ascending, 2. Axial, 3. Irregular, 4. Horizontal, 5. Vertical	Numeric codes
Stem	Stem cortex texture	1. Smooth, 2. Smooth, a little flaky, 3. Very flaky	Numeric codes
	Stem diameter	cm	Meter
	Stem color	1. Light dotted green, 2. Green with dotted brown, 3. Dotted brown, 4. Grayish brown, 5. Light brown	Numeric codes
	Offspring	0. Absent, 1. Present	Numeric codes

Supplemental Information – Table S2. Parameters of the leaf used to analyze 20 guava genotypes.

Parameter	Measuring unit	Measuring method
Leaf length	cm	Ruler
Leaf width	cm	Ruler
Leaf margin	1. Soft, 3. Low dented, 5. High dented	Numeric codes
Petiole length	cm	Ruler
Shape of the leaf	1. Elliptic, 2. Oblong, 3. Lanceolate, 4. Oval, 5. Obovate, 6. Trapezoid	Numeric codes
Leaves orientation	1. Erect, 2. Flat, 3. Falls	Numeric codes
Shape of the leaf apex	1. Obtusa, 2. Apiculate, 3. Acuminate, 4. Acute, 5. Round	Numeric codes
Shape of the leaf base	1. Round, 2. Oblique, 3. Acute, 4. Attenuated, 5. Cord	Numeric codes
Back color of young leaves	1. Yellowish green, 2. Light green, 3. Greenish brown, 4. Reddish brown, 5. Brilliant reddish, 6. Brown	Numeric codes
Back color of mature leaves	1. Light green, 3. Green, 5. Dark green, 7. Intense brilliant green	Numeric codes
Upper color of young leaves	1. Yellowish green, 2. Light green, 3. Greenish brown, 4. Reddish brown, 5. Brilliant reddish, 6. Brown	Numeric codes
Upper color of mature leaves	A. Light green, B. Green, C. Dark green, D. Intense brilliant green	Numeric codes
Leaf texture	1. Rough, 2.Soft	Numeric codes
Leaf thickness	1. Thin, 2. Thick	Numeric codes
Disposition of the leaves on stem	1. Opposites, 2. Decisive opposites	Numeric codes
Pubescent on the leaf back	0. Absent, 1. Present	Numeric codes
Pubescent on the upper leaf surface	0. Absent, 1. Present	Numeric codes
Leaf surface curve	0. Absent, 1. Present	Numeric codes
Vein surface curve	0. Absent, 1. Present	Numeric codes
Vertical leaf cavity	1. Low, 2. Moderate, 3. High	Numeric codes
Main vein color	1. Green, 2. Green-creamy, 3. Pale green, 4. Green-yellow	Numeric codes
Length of the main axis	cm	Ruler
Length of the minor axis	cm	Ruler
Angle of the leaf axis	°	Protractor

Supplemental Information – Table S3. Parameters related to inflorescence and flowers used to analyze 20 guava genotypes.

Parameter		Measuring unit	Measuring method
Inflorescence and flowers	Disposition of flowers	1. Solitary, 2. Together	Numeric codes
	Flower length	mm	Caliper
	Flower width	mm	Caliper
	Peduncle length	mm	Caliper
	Petal length	mm	Caliper
	Petal number	Number	Counting
	Sepal length	cm	Ruler
	Calyx diameter	mm	Caliper
	Inflorescence length	cm	Ruler
	Inflorescence diameter	mm	Caliper
	Flower disk length	mm	Caliper
	Stigma length	mm	Caliper
	Main flowering period	1. Summer, 2. Winter	Numeric codes
	Main flowering intensity	1. Low, 3. Moderate, 5. High	Numeric codes
	Second flowering period	1. Summer, 2. Winter, 3. Not second flowered	Numeric codes
	Inflorescence position	1. Axillary, 2. Terminal	Numeric codes
	Inflorescence shape	1. Conical, 2. Pyramidal	Numeric codes
	Flowering regularity	1. Regular, 2. Intermediate, 3. Irregular, 4. Extremely irregular	Numeric codes
	Stomata number	Number	Counting
	Stigma shape	1. Capital, 2. Mace form, 3. Flattened, 4. Lobed	Numeric codes
	Style shape	1. Duplicate, 2. Fimbria, 3. Cylindrical	Numeric codes
	Position of sepals	1. Straight, 2. Convex, 3. Concave	Numeric codes

Supplemental Information – Table S4. Parameters related to fruit and seed used to analyze 20 guava genotypes.

Parameter		Measuring unit	Measuring method
Fruit	Shape of the mature fruit	1. Spherical, 2. Round, 3. Ovoid, 4. Pyriform, 5. Ellipsoid	Numeric codes
	Shape of the fruit apex	1. Angular, 2. Truncated, 3. Depressed or sunken, 4. Concave, 5. With belly button	Numeric codes
	Shape of the fruit base	1. With neck, 2. Convex, 3. Concave, 4. Convex with neck	Numeric codes
	Insertion of the peduncle in fruit	1. Oblique, 2. Vertical or central	Numeric codes
	Peduncle base shape	1. Truncated, 2. Round, 3. Curved	Numeric codes
	Fruit cavity	1. Absent, 2. Light, 3. Shallow, 4. Deep	Numeric codes
	Longitudinal rib	0. Absent, 1. Present	Numeric codes
	Rib prominence	1. Strong, 2. Intermediate, 3. Weak, 4. No rib	Numeric codes
	External color	1. Yellow, 2. Green, 3. Yellow with pink spot, 4. Green with yellow spot	Numeric codes
	Surface texture	1. Rough, 2. Semi-rough	Numeric codes
	Mesocarp thickness	mm	Caliper
	Epicarp thickness	mm	Caliper
	Mesocarp color	1. White, 2. Creamy, 3. Red, 4. Pink	Numeric codes
	Sandy texture in mesocarp	0. Absent, 1. Present	Numeric codes
	Maturity period	3. Early, 5. Intermediate, 7. Late	Numeric codes
	Productivity	3. Low, 5. Intermediate, 7. High	Approximate yield of tree
	Duration of the harvest period	Day	Counting
	Fruit length	mm	Caliper
	Fruit width	mm	Caliper
	Fruit firmness	kg cm^{-2}	Penetrometer
Seed	Seeds per fruit	1. Low, 2. Moderate, 3. High	Numeric codes
	Seed shape	1. Semi-oval, 2. Oval, 3. Triangle	Numeric codes
	Seed weight	g	Digital balance
	Germination percentage	%	Germination test