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GENETIC CHARACTERIZATION OF TUNISIAN LIME GENOTYPES USING POMOLOGICAL TRAITS

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ABSTRACT

Citrus genus includes a wide number of species that have been long cultivated and well adapted in Tunisia. It is represented by small number of plantations and considered as underutilized in Tunisia. Our goal was to genetically characterize Tunisian lime genotypes to obtain data useful for gene conservation and breeding purposes. The survey of genotypes was conducted in the Cap Bon region, where citrus cultivation is the most spread. Sixteen quantitative and 19 qualitative parameters were evaluated. The observed accessions belonged to three different species: *Citrus limetta*, *Citrus latifolia* (limes Byrsa), and *Citrus limettioides* (limes of Palestine) according to Tanaka classification. Principal component analysis confirmed these classifications. Four-cell analysis (FCA) was used to determine the most threatened genotypes. Quantitative traits were evaluated and allowed the discrimination between genotypes. Many quantitative traits of fruit and juice were highly positively and significantly correlated. Phenotypic diversity was determined using Shannon–Wiener diversity index (H'). The highest value of diversity index was observed for both vesicle thickness and thickness of segment walls (H' = 0.98). Intermediate values were observed for both fruit axis (H'= 0.49) and pulp firmness (H' = 0.43). However, fruit shape (H' = 0.24), shape of fruit apex (H' = 0.24), and vesicle length (H' = 0.33) presented the lowest values of diversity index. Current findings will be useful to conserve threatened genotypes ex situ and on farm and also will guide strategic conservation on *Citrus* genetic resources for future breeding programs.

Key words: diversity, genetic resources, limes, pomology

INTRODUCTION

Citrus (Rutaceae family) is one of the most important and ancient crop species domesticated by humans (Krueger & Navarro 2007). *Citrus* taxonomy and phylogeny are very complicated, controversial, and confusing mainly due to sexual compatibility between *Citrus* and related genera, the high frequency of bud mutations, the long history of cultivation, and wide dispersion (Nicolosi et al. 2000). The taxonomy of the genus *Citrus* is controversial as two systems of classification were suggested: Swingle and Reece (1967) distinguished 156 species, whereas Tanaka (1977) only 16 species. It is believed that some *Citrus* types, including citrons, sour oranges, and lemons, were spread slowly from 500 to 1300 AD through wide areas, including Europe, by successive waves of invaders and travelers of Muslim armies, Arab traders, Crusaders, and others moving along trade routes from other populations to Europe (Moore 2001). Lemon, lime, sour orange, sweet orange, grapefruit, and other edible fruits are apomictically perpetuated biotypes with probable hybrid origin (Kumar et al. 2010).

Lime is a traditional crop in South Asia and the Middle East and comprises a varied group of types of sour and sweet cultivars, different from one to another with distinct fruit characteristics (Nicolosi et al. 2000). Limes hybridize well with other *Citrus* species.

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Hybrids could be between lime and lemon or lime and kumquat (Scora 1975), or a tri-hybrid species of citron, pummelo, and *Microcitrus* (Barrett & Rhodes 1976). In Tunisia, *Citrus* is one of the most economically important crops. The weather and soil conditions in Tunisia, particularly in the Cap Bon region, are suitable for *Citrus* production. Currently in Tunisia *Citrus* plantations extend more than 22,000 ha, and fruit production in the last 5 years increased to 323,000 tones (DGPA 2016). Although limes are classified as a major fruit crop (Mabberley 2008), it is sporadically cultivated in Tunisia. Price of fruit is very high compared with those of sweet orange. Lime is facing different increasing constraints, such as water availability and quality, weather conditions, expansion of diseases, necessity to change the old farming techniques, and urbanization. All these restrictions pose a threat to genetic resources of lime genetic resources in Tunisia. *Citrus* germplasm is very diverse with many autochthonous cultivars, and it is imperative to implement a strategy for the conservation of genetic resources. For the first time in Tunisia, collecting missions were realized in order to identify and characterize limes' accessions. In this study, pomological traits were evaluated to determine the genetic diversity of lime. Data collected allowed the establishment of passport data. These findings will enhance both ex situ and on farm genetic conservation program of *Citrus* germplasm.

MATERIALS AND METHODS

Plant material

Accessions were collected in 2013–2014 and re-collected in 2014–2015 (Table 1) throughout the Cap Bon Nord region east of Tunisia, where citrus cultivation is most widespread. Acquisitions were carried out among a wide range of stakeholders and with the presence of local governmental agencies both in farms and ex situ collections. During the collecting missions, we visited old orchards where limes were cultivated for many decades. Farmers and technical staffs from regional authorities confirmed the names of genotypes. Identification of species was performed with the help of Blondel (1978) classification.

Four-cell analysis (FCA)

The FCA was used to classify the three species under consideration based on the size of the cultivation area and on the number of households, as described by Sthapit et al. (2012).

Pomological characterization

The fully ripe fruits were taken from the four directions of the tree and from the interior and exterior layers of the canopy at the rate of 30 fruits per tree. These fruits were divided into 3 batches of 10 fruits to analyze the quantitative traits (Table 2) and the juice parameters (Table 3). For pomological characterization, analysis has been performed separately for each growing season. Sixteen quantitative traits, including seven parameters dealing with juice description, were measured (Table 4) and correlations among those traits were calculated (Table 5). Nineteen qualitative characters (Table 6) were chosen based on *Citrus* descriptors (IPGRI 1999). Fresh juice was obtained using a citrus press (Santos Classic N°11, Lyon, France). Subsequently, the juice was filtered through a 1-mm mesh sieve; weighed and volume was measured in a burette. Density was estimated in a sample of 100 ml of juice. Total soluble solids (TSS) content was determined by direct readings on a hand-held refractometer (Toledo, 30 PX) calibrated before use with distilled water. The pH was measured using a pH meter (Toledo, S22) previously calibrated. The titratable acidity (TA) of the juice was evaluated by the determination of citric acid by titration with a NaOH solution (0.1 N). The determination of vitamin C was carried out by titration with iodine solution. Data were obtained in triplicate.

Data analysis

For quantitative traits, all analyses were performed using SAS software (version 6.07, 1990). Descriptive statistics were performed and presented as minimum, maximum, mean standard deviation, and coefficient of variation (CV). One-way analysis of variance (ANOVA) was used, and data are presented as mean ± standard deviation (SD). Pearson index was calculated for quantitative traits. Principal component analysis (PCA) was carried out to examine the distribution of genotypes in the first plan of PCA for quantitative parameters. For qualitative data, frequency distributions were computed.

The numbers of phenotypic classes for qualitative parameters that differed for each trait were used to estimate the Shannon–Wiener diversity index (H'). It was used to characterize the phenotypic frequencies of the traits and was defined as $H = \sum_{i=1}^{n} pi \ln pi$,

where n is the number of phenotypic classes for a character and *pi* is the proportion of the total number of entries in the *i*th class. Each value of H was standardized by conversion to a relative phenotypic diversity index (H') by division by H_{max} = ln (n) in order to express the values of H'(H/H_{max}) in the range of 0–1 (indicating the absence of diversity and maximum of diversity, respectively). The diversity index was classified as high ($H' \geq 0.6$), intermediate ($0.40 \leq H' \leq 0.60$), or low ($0.1 \leq H' \leq 0.40$), as described by Eticha et al. (2005) and Mengistu et al. (2015).

RESULTS AND DISCUSSION

On the basis of pomological traits, we have described the characteristics and the variability of each genotype originating from different orchards. Measurements for both fruit and juice traits are presented in Tables 2 and 3, respectively. LimePal2 represented the highest value of fruit weight, diameter, length, width of skin, and width of epicarp at equatorial area. Lime10 also exhibited high caliber of fruit. The smallest fruit attributes were those of Lime17. LimePal1 had by far the most important number of segments. All genotypes held seeds varying in number from 1.2 to 5.4. The diameter of axis varied widely among the different genotypes (Table 2). Concerning juice attributes, LimPal2 and Lime8 were the juiciest. The sweetest juice was that of LimePal1, LimePal2, and Lime17. Values of pH were the highest for Lime13, Lime16, Lime17, and Lime3. All genotypes exhibited high content of vitamin C. Concentrations varied from more than 48 mg·100 mg^{-1} (Table 3) for genotypes LimePal1 and Lime8 to about 26 mg·100 mg^{-1} for Lime20 and Lime4. The recorded data were subjected to statistical analyses as described in Material and Methods that showed the utility of both quantitative and qualitative phenotypic characterizations for the identification of genetic resources of limes.

Species classification and estimation of genetic resources status

The participatory FCA was used, while regional agricultural authorities and the farmers were interviewed. It allowed categorizing *C. limetta* as a threatened species. Although many householders cultivated this species, it was propagated in small area. *C. latifolia* and *C. limettioides* were classified as rare species because they were cultivated in small area and by few householders (Fig. 1). Thus, special attention must be paid to these species in order to conserve them and encourage their dissemination. The most cultivated species was *C. limetta* in contrast to C. *latifolia*, which is the least cultivated (Table 1). Accessions from different species showed a wide range of variability for all the pomological traits studied. According to a recent research, Curk et al. (2016) have elucidated the origins of limes and lemons based on cytoplasmic and nuclear markers. The survey highlighted that all limes and lemons descend from *Citrus medica* as the direct male parent in combination with *Citrus aurantium* for *C. limetta* and a hybrid (*Citrus maxima* × *Citrus reticulata*) for *C. limettioides*. Among triploid limes, *C. latifolia* accessions Persian lime types result from the fertilization of a haploid ovule of *Citrus limon* by a diploid gamete of *Citrus aurantifolia*. As limes and lemons were vegetatively propagated by apomixes and horticultural practices, the intra-subgroup phenotypic diversity results from asexual variations (Curk et al. 2016). Two classifications of limes have been reported: lime of Pearse known as *C. aurantifolia* hybrid by Swingle and Reece (1967) and *C. latifolia* Tan. by Tanaka (1977). Instead, sweet lime of Tunisia is classified as *C. limon* (L.) Burm. by Swingle and Reece (1967) and *C. limetta* Risso by Tanaka (1977). The results can be useful for both selection of cultivars and breeding programs aiming the improvement of fruit quality. Snoussi et al. (2012) revealed that both sexual and asexual reproductions of limes cultivated in Tunisia contributed to their genetic diversity.

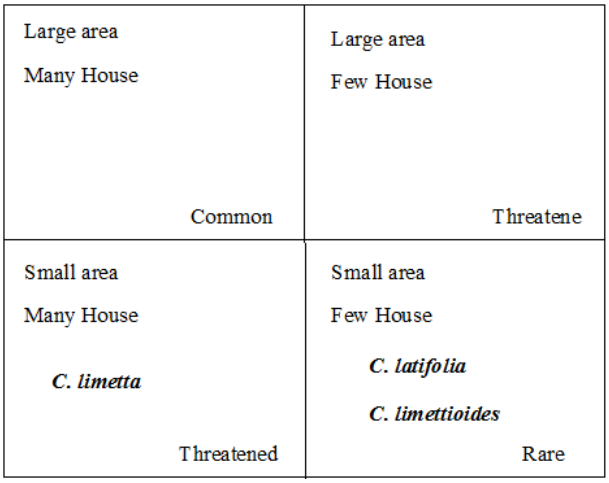

Fig. 1. Classification of limes species in Tunisia following FCA

Table 1. List of species, genotypes studied, abbreviations, acquisition date and accession numbers

Species	Abbreviations	Date of first acquisition	Accession ID
Citrus limettioides	LimePal 1	23/01/2014	NGBTUN 757 ARB
	LimePal 2	10/02/2014	NGBTUN 992 ARB
Citrus latifolia	LimeBirs	04/02/2014	NGBTUN 782 ARB
Citrus limetta	Lime1	10/02/2014	NGBTUN 1032 ARB
	Lime2	23/01/2014	NGBTUN 756 ARB
	Lime3	23/01/2014	NGBTUN 788 ARB
	Lime4	23/01/2014	NGBTUN 793 ARB
	Lime5	23/01/2014	NGBTUN 794 ARB
	Lime6	23/01/2014	NGBTUN 795 ARB
	Lime7	23/01/2014	NGBTUN 797 ARB
	Lime8	04/02/2014	NGBTUN 798 ARB
	Lime9	15/01/2014	NGBTUN 800 ARB
	Lime10	15/01/2014	NGBTUN 994 ARB
	Lime11	15/01/2014	NGBTUN 823 ARB
	Lime12	28/04/2014	NGBTUN 1033 ARB
	Lime13	30/01/2014	NGBTUN 803 ARB
	Lime14	30/01/2014	NGBTUN 808 ARB
	Lime15	30/01/2014	NGBTUN 812 ARB
	Lime16	10/02/2014	NGBTUN 815 ARB
	Lime17	10/02/2014	NGBTUN 824 ARB
	Lime18	10/02/2014	NGBTUN 759 ARB
	Lime19	30/01/2014	NGBTUN 758 ARB
	Lime20	30/03/2014	NGBTUN 993 ARB

Table 2. Mean values and significance degree of differences between lime genotypes for fruit characteristics

Geno-types	Weight (g)	Diameter (mm)	Length (mm)	Width of skin (mm)	Width of epicarp at equatorial plane (mm)	Mesocarp thickness (mm)	Number of seg-ment	Number of seeds	Diameter of axis (mm)
LimePal 1	69.0±30e	50±0.6d	50±0.8cd	3.16±0.5bc	1.46±0.3d	1.81±0.5ab	16.46±4a	1.93±0.5de	6±1de
LimePal 2	157.9±34a	65±1.3a	66.9±0.7a	4±1a	2.75±0.6a	1.33±0.5bc	10.26±4b	3.8±0.7c	13.4±3a
Limebirs	87.1±34d	60±1a	50±0.6cd	3.09±0.5c	1.8±0.5c	1.14±0.3cd	8.6±3cd	2.26±0.6d	8.92±3b
Lime1	74.3±23ef	50±0.4d	50±0.7cd	3.1±0.4c	1.85±0.4c	1.38±0.3bc	8.46±3cd	2.46±0.6d	8±3bc
Lime2	77±17e	50±0.4d	50±0.9cd	3±0.6c	2.1±0.7ab	1±0.2d	9±3c	4.2±0.8ab	9.4±3b
Lime3	85.1±29de	55±0.6bc	60±0.2b	4±1a	2.6±0.7a	1.38±0.4bc	8.13±2d	1.2±0.2e	8.9±3bc
Lime4	87.3±39d	55±0.5bc	50±1cd	3±0.4c	1.75±0.6cd	1.4±0.3b	8.8±3cd	3.2±0.7cd	8.25±3bc
Lime5	73.8±15ef	50±0.5d	56.8±0.5c	3.77±0.7ab	2.3±0.7ab	1.42±0.4b	9.2±3c	1.93±0.5e	6.71±1d
Lime6	75.6±39e	55±0.7bc	55±0.4c	3.33±0.6b	1.81±0.4c	1.5±0.5b	9.26±3c	3.86±0.7c	7.41±2c
Lime7	64.2±32	50±0.6d	50±0.5cd	3±0.5c	1.71±0.6cd	1.25±0.1c	8.93±3cd	5.4±1a	9±3b
Lime8	106.1±21bc	59±0.8ab	60±0.6ab	3.72±0.7ab	1.7±0.5cd	2±0.6a	8±2d	3.73±0.7c	9.81±3b
Lime9	80.3±23de	55±0.8bc	52.5±1c	3.22±0.6bc	2±0.4b	1.4±0.3b	9.33±3c	5.06±0.9b	8.41±3bc
Lime10	111±24b	60±0.9a	60±0.3b	3.38±0.5b	1.88±0.4c	1.5±0.5b	8.6±3cd	4.86±1b	7.66±2c
Lime11	84.6±26de	55±0.4bc	36.3±0.2f	3.63±0.6ab	2±0.5b	1.14±0.4cd	8.13±2d	4.78±0.8ab	9.58±3b
Lime12	56.0±13f	50±0.5d	50±0.6cd	3±0.6c	1.57±0.5d	1±0.2d	10±4b	1.86±0.4e	8.25±3bc
Lime13	47.4±12f	45±0.4e	50±0.2d	3.2±0.6c	1.71±0.56cd	1.33±0.3bc	8.66±3cd	3.73±0.7c	6.12±1de
Lime14	56.6±10f	50±0.3d	32.6±0.1f	3.2±0.4c	1.66±0.3d	2±0.5a	8.93±3cd	2.4±0.6d	7.88±2c
Lime15	82.4±30de	50±0.6d	55±0.4c	3.42±0.5b	2±0.4b	1±0.2d	8.66±3cd	2.6±0.6d	6.61±1d
Lime16	41.6±12h	55±0.4bc	50±0.4d	3.16±0.4bc	2±0.5b	1±0.1d	8.88±3cd	3.11±0.7cd	7.75±3c
Lime17	41.2±16h	50±0.5d	46.6±0.3e	2.57±0.3cd	1.37±0.2e	1.4±0.3bc	8.8±3cd	3.93±0.7c	5.83±1e
Lime18	53.8±28fg	46.6±0.2ef	50±0.3d	3.66±0.8ab	2±0.4b	1.57±0.4b	8±2d	3.26±0.7cd	7±2c
Lime19	47±14g	42.5±0.8e	50±0.2d	2.75±0.1cd	1.2±0.1f	1±0.1d	8.8±3cd	3±0.7cd	7.5±2c
Lime20	90.6±21de	56.9±0.5b	60±1ab	3.6±0.6ab	1.83±0.4c	1.25±0.3c	9.2±3c	2.2±0.5de	9.66±3b

Note: Data are averaged ±SD; values in each column followed by the same letters are not significantly different according to Duncan's multiple-range test ($p < 0.05$).

Table 3. Mean values and significance degree of differences between lime genotypes for juice parameters

Genotypes	Weight (g)	Volume (ml)	Density ($g \cdot ml^{-1}$)	TA (%)	TSS (°Brix)	pH	Vit. C ($mg \cdot 100\ mg^{-1}$)
LimePal1	128.6±40h	75±8d	101.6bc	0.09c	11±2a	5.7±0.1b	48.8±4a
LimePal2	349.3±90a	150±12a	101.3c	0.07de	9.4±2b	5.7±0.1b	37.7±3d
LimeBirs	184.3±80d	117.5±10b	101bc	0.09c	8.3±.5cd	5.5±0.1b	35.7±3d
Lime1	139.3±50g	90±9c	102ab	0.1bc	7.5±1d	5.1±.01d	30.9±2de
Lime2	156.3±60f	100±9bc	100.6cd	0.08cd	8.5±1bc	5.9±0.2b	28.8±2ef
Lime3	159.6±60f	75±12cd	100.6cd	0.08cd	7.8±1d	5.8±0.1ab	27.9±2ef
Lime4	186±85d	70±10d	100.6cd	0.1bc	7.4±1d	5.5±0.1b	26.2±1f
Lime5	140.6±50g	60±7de	102.3ab	0.09c	8.5±1bc	5.6±0.1b	31.5±2d
Lime6	119.3±50h	100±10bc	101.3bc	0.08cd	8.8±1.5bc	5.6±0.1b	32.7±2d
Lime7	112.3±50h	65±5de	102.6ab	0.09c	8.7±1bc	5.6±0.1b	33.3±3d
Lime8	205±85b	152±10a	101.3bc	0.08cd	7.3±0.5de	5.3±0.1bc	48.9±4a
Lime9	163±6ef	75±9d	101bc	0.06e	7.4±0.5de	5.8±0.1b	30.6±2de
Lime10	230.6±80b	90±10c	101.3bc3	0.09c	8.2±1cd	5.6±0.1b	32.7±2d
Lime11	185±80d	85±9c	102ab	0.11ab	9.1±2b	5.84±0.1b	30.4±2de
Lime12	119.6±30i	70±9d	100.33bc	0.08cd	8.3±1cd	5.9±0.2b	45.7±3b
Lime13	94±40k	55±3f	101.3bc	0.12a	9.4±2b	6.05±0.2b	28.8±2ef
Lime14	105.3±4i	67±dde	101bc	0.09c	8.6±1bc	5.77±0.1b	34.3±3d
Lime15	173±7e	100±10bc	101.3bc	0.1bc	8.2±1cd	5.7±0.1b	32.4±2d
Lime16	97.6±3k	100±12bc	102.6ab	0.07de	7±0.5de	5.9±0.1ab	29.3±1ef
Lime17	102.6±4i	67±5de	103.6a	0.1bc	9.5±2b	6.3±0.1a	40±3c
Lime18	89.6±3l	50±5e	102ab	0.1bc	8.5±1.5cd	5.6±0.1b	31.1±2d
Lime19	77.6±1m	67.5±6de	102ab	0.11ab	8.6±1.5bc	5.3±0.1bc	27.3±2ef
Lime20	184.6±8d	95±12bc	101bc	0.1bc	7.4	5.7±0.1b	26.8±1f

Note: See Table 2

Variation among lime species for studied quantitative traits

Table 4 describes the minimum, maximum, mean, standard deviation, and CV for each variable studied. The weight of fruit varied from 41.3 g (Lime16) to 157.9 g (LimePal 2) per fruit with a mean of 77.3 g per fruit. High variability between cultivars was observed for fruit weight, which is confirmed by the relatively high CV (32%). The number of seeds per fruit varied from 1.2 (Lime3) to 5.4 (Lime8) with a mean of 3.3 and a CV of 35%. The weight of juice was highly variable (CV = 42%), ranging from 6.5 (Lime19) to 69.9 (Limpal2) with a mean of 29.9. The volume of juice varied from 50 ml (Lime18) to 152 ml (Lime8), with a mean of 80 ml (CV = 32%). These four parameters were the most discriminant between cultivars based on CVs. The fruit rind thickness (CV = 21%) range from 1 mm (Lime6 and Lime14) to 2 mm (LimPal 2 and Lime3). Diameter of fruit axis (CV = 19%) range from 26.19 (Lime4) to 48.9 $mg \cdot dm^{-3}$ (LimPal2 and Lime8).

Correlations among studied traits

In order to estimate correlation between quantitative parameters based on the data measured on Tunisian limes, Pearson's correlation coefficients were estimated (Table 5). A significant correlation among several quantitative parameters was observed. Weight of fruit was significantly and positively correlated with fruit diameter ($r = 0.81$; $p \leq 0.01$), diameter of fruit axis ($r = 0.75$; $p \leq 0.05$), weight and volume of juice ($r = 0.97$; $p \leq 0.01$; $r = 0.62$; $p \leq 0.01$, respectively),

fruit length ($r = 0.61$; $p \leq 0.05$), width of fruit skin ($r = 0.61$; $p \leq 0.05$), and width of epicarp at equatorial plane ($r = 0.60$; $p \leq 0.05$). Fruit diameter was significantly and positively correlated with the diameter of fruit axis ($r = 0.68$; $p \leq 0.01$) and weight of juice ($r = 0.84$; $p \leq 0.01$). Highly significant and positively correlations were observed between fruit skin width and width of pericarp at equatorial plane ($r = 0.77$; $p \leq 0.01$). Number of segment was significantly and positively correlated with TSS ($r = 0.63$; $p \leq 0.01$); diameter of fruit axis was significantly and positively correlated with weight of juice ($r = 0.75$; $p \leq 0.01$).

Estimation of variation among qualitative traits using the Shannon–Wiener diversity index

Seed color was the most discriminant trait with four different phenotypes (Table 6). Similarly, fruit skin color, fruit surface texture, adherence of albedo to pulp, adherence of segment walls, vesicle length and thickness, fruit axis, and cotyledon color were also more discriminant compared with all the other parameters. Uniformity of pulp color and cross-section shape of axis were monomorphic for all the accessions studied (Table 6). H' ranged from 0 for both cross-section shape and pulp color uniformity to 0.98 for both thickness of segment walls and vesicle thickness (Table 6) with a mean value of 0.61. Moreover, other parameters showed high values of H': fruit skin (epicarp) color (H' = 0.96), shape of fruit base (H' = 0.93), seed color (H' = 0.84), pulp (flesh) color (H' = 0.82), and fruit surface texture (H' = 0.8). According to the Shannon diversity index, we assume that shape of fruit apex, fruit skin epicarp color, fruit surface texture, thickness of segment walls, pulp (flesh color), vesicle thickness, seed shape, and seed color were the most discriminant qualitative parameters. Pulp (flesh color), fruit skin epicarp color, and fruit surface texture are definitely used as selection criteria throughout the supply and consumption chain. It is well known that genetics, environment, and cultural practices interact to define the eventual main fruit traits (weight, diameter, length, and width of skin).

Principal component analysis

PCA was performed based on fruit and juice quantitative parameters. The results showed that 60.3% of the total variability is accounted for the first three principal components (PCs). The first two PCs account 35.5% and 15.5% of the total variability (Fig. 2 and Table 7). The PC1 positively correlated with weight and diameter of fruit, diameter of axis, and weight of juice. The PC2 positively correlated with fruit rind (mesocarp) thickness, number of segments, and vitamin C content. The projection of lime cultivars in the plan 1–2 of the PCA allows the discrimination of the species *C. limettioides* and Lime8 from the other genotype (Fig. 2). Regarding *C. latifolia* and *C. limetta*, we did not observe any significant discrimination. Accessions from these cultivars were grouped together. Regarding TSS and content of vitamin C, both LimPal1 and LimPal2 exhibited the highest values. Moreover, PCA has also distinguished *C. limettioides* (LimPal 1 and LimPal 2) and Lime 8 from all the other cultivars. Lime8 genotype, which belongs to *C. limetta* species, is characterized by large fruit. For this reason, it has been clustered with genotypes LimPal1 and LimPal2, which belongs to *C. limettioides* species. This species can be selected for breeders in order to improve fruit size (weight and diameter of fruit) and yield of juice. On the basis of the same descriptors of *Citrus* (IPGRI 1999), similar findings were recorded, referring to Saddoud Debbabi et al. (2013) for the main parameters correlated with the first axis of PCA (weight, diameter, and length of fruit). Phenotypic characterization have shown their efficiency for many crops, such as carrot (Mezghani et al. 2014) and wheat (Mengistu et al. 2015), and for many fruit trees such as fig (Saddoud et al. 2008; Gaaliche et al. 2012), olive (Hannachi et al. 2008), apricot (Ruiz & Egea 2008), apple (Mratinić & Fotirić Akšić 2011), and cornelian cherry (Moradi et al. 2019). This evaluation is necessary to achieve the developmental program and genetic improvement of the lime species. The outcomes of this study will be very useful for Tunisian Gene Bank and for a good identification and documentation of *Citrus* genetic resources. Although pomological characterization is low cost method and has many advantages, it remains limited in the number of characters and is limited in use. The characterization could be improved through the involvement of molecular markers, for example Simple Sequence Repeat (SSR) that allows the study of molecular diversity and the establishment of fingerprints of the cultivars studied.

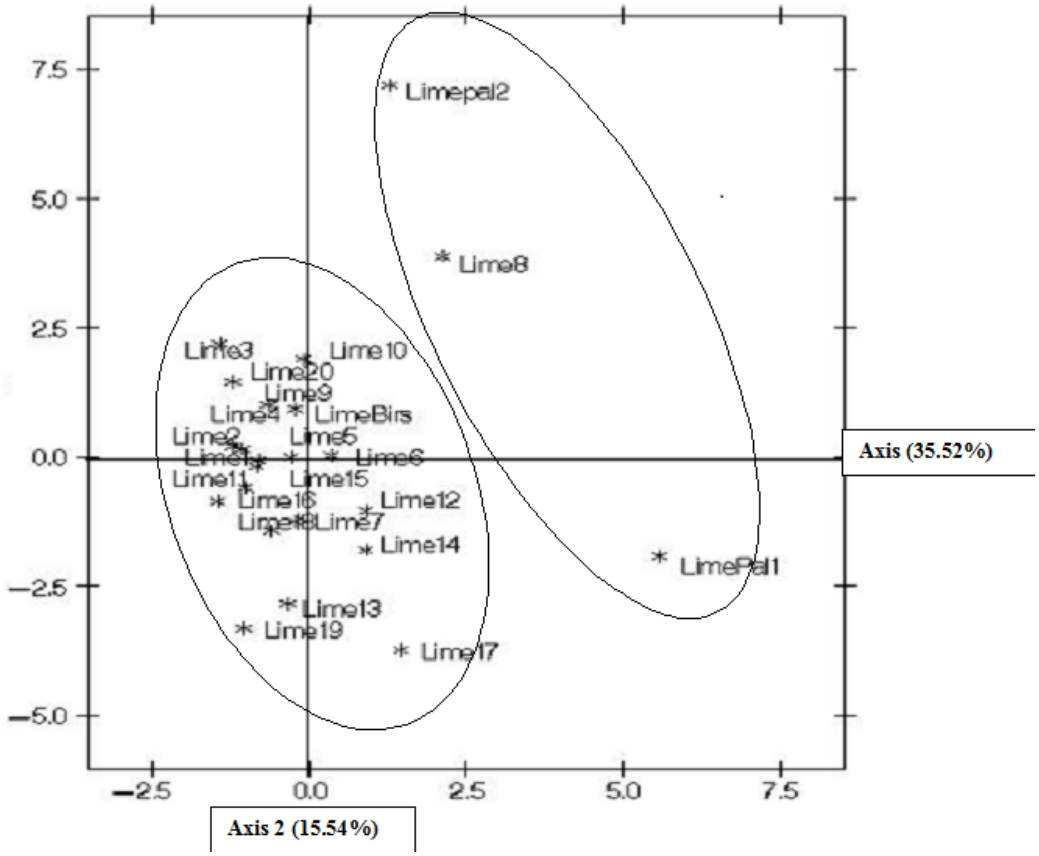

Fig. 2. PCA biplot of lime cultivars based on quantitative traits

Table 4. Descriptive statistics of quantitative traits studied for Tunisian lime genotypes

Variable	Minimum	Maximum	Mean	Standard deviation	CV (%)
Weight (g)	41.26	157.9	77.3	25.1	32
Fruit diameter (mm)	42.5	65	52.8	52	9
Fruit length (mm)	32.6	66.9	51.7	74	14
Width of fruit skin (mm)	2.5	4.0	3.3	0.3	11
Width of epicarp at equatorial plane (mm)	1.2	2.7	1.8	0.34	18
Fruit rind (mesocarp) thickness (mm)	1.0	2.0	1.3	0.3	21
Number of segments	8	16.4	9.1	1.6	18
Number of seeds	1.2	5.4	3.2	1.1	35
Diameter of fruit axis (mm)	583	1340	817	164	20
Weight of juice (g)	6.5	69.8	29.9	12.71	42
Volume of juice (ml)	50	152	80	26	32
Density of juice ($g \cdot ml^{-1}$)	100.3	103.6	101.5	0.8	0.7
TSS (°Brix)	7.00	11	8.4	0.9	10
pH	5.1	6.2	5.7	0.2	4
TA (%)	0.06	0.1	0.09	0.01	15
Vitamin C ($mg \cdot 100\ mg^{-1}$)	26.2	48.9	33.5	6.57	19

Table 5. Correlation matrix based on Pearson index for the quantitative parameters studied

	Fruit weight	Fruit diameter	Fruit length	Width of fruit skin	Width of epicarp at equatorial area	Fruit rind (mesocarp) thickness	Number of segment	Number of seeds	Diameter of fruit axis	Weight of juice	Volume	Density	TA	TSS	pH	Vit. C
Fruit weight (g)	1.0000															
Fruit diameter (mm)	**0.8183****	1.0000														
Fruit length (mm)	**0.6129***	0.4769	1.0000													
Width of fruit skin (mm)	**0.6149***	0.4755	0.4908	1.0000												
Width of epicarp at equatorial area (mm)	**0.6025***	0.5052	0.4736	**0.7756**	1.0000											
Fruit rind (mesocarp) thickness (mm)	0.1439	0.1300	-.0565	0.2476	-.1151	1.0000										
Number of segment	0.0187	-0.046	0.0322	-.1099	-.2007	0.2365	1.0000									
Number of seeds	0.1437	0.1465	-.0711	-.1708	-.0551	-.0379	-.2409	1.0000								
Diameter of fruit axis (mm)	**0.7554****	**0.6867****	0.3484	0.4592	0.5531	-.0981	-.1671	0.1889	1.0000							
Weight of juice (g)	**0.9735****	**0.8405****	0.5593	0.5459	0.5953	0.0446	0.0089	0.1582	**0.7593****	1.0000						
Volume (ml)	**0.6208****	0.5572	0.4609	0.4207	0.2159	0.3570	-.0635	0.1301	0.5907	0.5951	1.0000					
Density ($g \cdot ml^{-1}$)	-.3851	-.2664	-.1799	-.2582	-.2307	-.0187	-.0311	0.2690	-.3394	-.3357	-.1086					
TA (%)	-.3412	-.5322	-.3556	-.2083	-.4321	-.0898	-.1527	-.0749	-.4175	-.3276	-.3394	0.2334	1.0000			
TSS (°Brix)	-.0684	-.2438	-.2031	-.1186	-.1954	0.1768	**0.6380****	0.0464	-.2266	-.0631	-.1352	0.2360	0.2749	1.0000		
pH	-.2784	-.0593	-.1700	-.0979	0.0577	-.1669	0.1209	0.1338	-.1677	-.1419	-.2572	0.1517	-.1032	0.3129	1.0000	
Vit. C (mg per 100 mg)	0.1211	0.1635	0.0633	-.0395	-.2831	0.4372	0.5253	-.1120	0.0001	0.1241	0.5019	0.0362	-.2619	0.4061	0.0555	1.0000

* Correlations are significant at $P \leq 0.05$ according to Pearson correlation

** Correlations are significant at $P \leq 0.01$ according to Pearson correlation

Table 6. Qualitative descriptors used for estimating pomological trait diversity in lime genotypes, their number of classes, proportion (%) of occurrence of each class, and estimated phenotypic diversity index (H') for each trait

Pomological trait	Observed phenotypic class	Class*	Proportion (%)	Shannon–Wiener index (H')
Fruit shape	2	1 spheroid	96	0.24
		2 ellipsoid	4	
Shape of fruit apex	2	1 mammiform	96	0.24
		3 rounded	4	
Shape of fruit base	2	2 convex	65	0.93
		3 truncate	35	
Fruit skin (epicarp) color	3	2 green-yellow	35	0.96
		4 yellow	22	
		5 dark yellow	43	
Fruit surface texture	3	1 smooth	30	0.8
		2 rough	9	
		4 pitted	61	
Adherence of albedo (mesocarp) to pulp (endocarp)	3	3 weak	4	0.75
		5 medium	44	
		7 strong	52	
Adherence of segment walls to each other	3	3 weak	4	0.72
		5 medium	35	
		7 strong	61	
Thickness of segment walls	3	3 thin	35	0.98
		5 medium	39	
		7 thick	26	
Fruit axis	3	1 solid	4	0.49
		2 semi-hollow	83	
		3 hollow	13	
Cross-section shape of axis	1	1 round	100	0
Pulp (flesh) color	2	2 green	26	0.82
		3 yellow	74	

Pulp color uniformity	1	1 uniform	100	0
Pulp firmness	2	5 intermediate	9	0.43
		7 firm	91	
Vesicle length	3	3 short	5	0.33
		5 medium	4	
		7 long	91	
Vesicle thickness	3	3 thin	35	0.98
		5 medium	26	
		7 thick	39	
Seed shape	2	2 clavate	74	0.82
		4 ovoid	26	
Seed surface	2	1 smooth	78	0.76
		2 wrinkled	22	
Seed color	4	2 cream	13	0.84
		3 yellowish	26	
		4 green	52	
		5 green (medium)	9	
Cotyledon color	4	1 white	13	0.65
		2 light yellow–cream	4	
		3 light green	13	
		5 green (medium)	70	

* observed class defined based on IPGRI Manual (1999)

Table 7. Eigenvectors, the main eigenvalues, and variation in percentage of the two first principal components of PCA

Principal components	PC1	PC2
Cumulative (%)	35.52	51.06
Proportion (%)	35.52	15.54
Eigenvalues	Weight of fruit (g) (+0.39)	Fruit rind (mesocarp) thickness (mm) (+0.37)
	Diameter of fruit (mm) (+0.36)	Number of segments (+0.49)
	Diameter of axis (mm) (+0.34)	Vitamin C mg·100 g^{-1} (+0.55)
	Weight of juice (g) (+0.38)	

CONCLUSION

The accessions Lime8, LimePal2, and Lime10 had good genetic potential for the important characters: weight and diameter of fruit, diameter of axis, and weight of juice. The collected data point to the need to protect endangered lime species and may help further efforts to portray the diversity of this species in Tunisia.

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