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Evaluation of Some Lime and Lemon Accessions by using Morphological Characterization in Hormozgan Province (Iran)

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ABSTRACT

In this study, some physical and chemical characteristics of lime and lemon fruits from 19 genotypes growing in Hormozgan province located in the south of Iran were investigated, including fruit length and diameter, seed number and percentage of fruit juice and peel. Some characters of fruit juice, namely pH, titration acidity, total soluble solids and antioxidant activity, were also determined. According to the results of bivariate simple correlation analysis, there were significant positive and negative correlations between some important characters. Factor analysis showed that the main factor was composed by fruit weight, fruit length and some characters of fruit juice and seed. The most effective characters were categorized into 7 main factors (with an Eigen value ≥ 1) that contributed to 85.98% of total variance. Cluster analysis was performed by using these 7 factors and genotypes were divided into 5 main clusters that included lime and lemon and unknown genotypes. Some lime genotypes were different in some characters such as fruit shape and size, and peel thickness. The lemon groups contained the cultivars 'Eureka', 'Lisbon' and 'Meyer'. Another group contained one genotype that has large fruit similar to orange but is very acid, like a lemon. Some unknown acid citrus were different from either limes or lemons.

Keywords: bivariate correlation, factor analysis, morphological traits, cluster analysis

INTRODUCTION

Citrus fruit, which are ranked among the top fruit around the world, are widely cultivated in tropical and sub-tropical regions and are grown commercially in more than 50 countries around the world (Ladaniya 2008; Hvarleva *et al.* 2008). The rapid expansion of citrus fruit cultivation in recent years is due to the importance of nutrition and improved economic conditions in consuming nations of the world and also the high demand for these crops because of the natural distinctive flavour of citrus (Kale and Adsule 1995). Citrus fruits supply nutrients that are essential for a healthy life. They are the main source of ascorbic acid (Vitamin C) whose daily consumption is necessary for humans. There is considerable variation among *Citrus* species and cultivars which is due to pollination adaptation in the genus, frequent bud mutations, inter- and intraspecific hybridization, apomixis, a long history of cultivation, seed propagation and human selection (Shahsavar *et al.* 2007; Hvarleva *et al.* 2008; Ye *et al*. 2009; Miao *et al*. 2011). Lime (*C. aurantifolia* Swing) and lemon (*C. limon* L. Burm) are two species of *Citrus* that, after oranges and mandarins, rank third in global citrus production (Khan 2007). In the classification system of Swingle, lemons and limes are represented only by two species, *C. limon* and *C. aurantifolia*, while in Tanaka's classification they comprise over 30 species, most of them being lemons (Swingle and Reece 1967). Lime and lemon are two important citrus cultivated in many countries whose fruits are consumed daily, and used for producing juice and are important components of the cosmetic and pharmaceutical industries (Prasad *et al.* 1989). Lime fruits are dried to prepare peel powder additives. In the Middle East, whole lime fruit, when dried, is also used in some cooked foods. The powdered fruit is also sprinkled over roast meat (Ezeonu *et al.* 2001). Lime and lemon, despite having differences in some characters, due to the high levels of citric acid in their fruit, people often consider them as one and the same. On the other hand, researchers believe that lemon is a result of crosses between lime and citron (Swingle 1943; Malik *et al.* 1974; Carvalho *et al.* 2005). Morphological characterization is the first and a basic step in the description and classification of germplasm, and serves as the foundation in plant breeding to select and evaluate traits of interest (i.e. superior traits) among genetic resources such that these may be combined within one new cultivar (Smith and Smith 1989; Fatahi *et al*. 2004). At the level of research, the diversity of genetic resources in collections may increase the efficiency of efforts to improve a species (Geleta *et al.* 2005). On the other hand, finding interrelationships among characteristics of a crop may be used in the contraction of selection indices and to detect some simple characteristics which may be useful as indicators for more complex ones (Johnson *et al.* 1955). Information on the differences and similarities between lime and lemon are inadequate. Since crosses between species in the *Citrus* genus can be easily achieved, and since the characters of lime and lemon are similar, it is possible to use them in breeding programs (Cooper *et al.* 1962). A high percentage of fruit juice, high citric acid (acidity) and thin fruit rind and seedless features are the most important commercial characteristics in both lime and lemon (Prasad and Rao 1989). Al-Naggar *et al*. (2009) used some characters of fruits, seed and seedlings to evaluation some interspecific citrus hybrids whose parents included Volkamer lemon and lime genotypes. Some characters such as seed number in mature fruits, percentage of germination and number of embryos per seed (i.e., polyembryony) were evaluated. Their results showed that Volkamer lemon and its hybrid had 0% polyembryony and this cultivar had monoembryonic seed.

In Iran, citrus fruits are widely cultivated in the north and south. Limes and lemons are the major citrus crop in the south of Iran. There is a high demand for lime and lemon fruits for fresh consumption, juice extract and also dried fruit. Hormozgan Province, located in south of Iran, is

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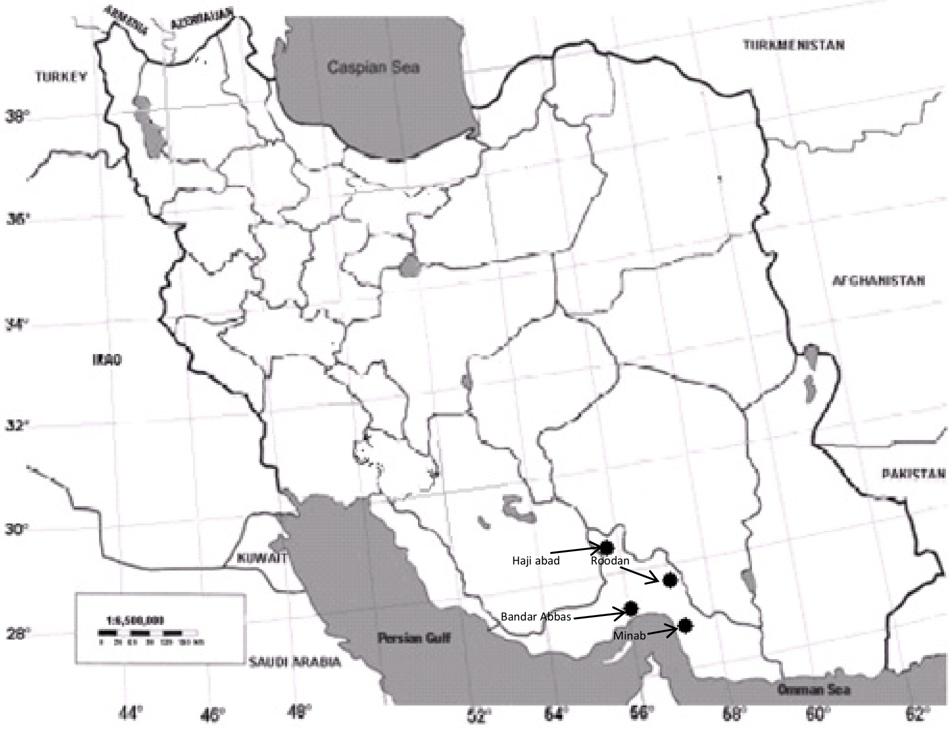

Fig. 1 Collection sites of the lime and lemon genotypes from Hormozgan province, Iran. Collection sites are indicated by black dots.

Fig. 2 Pictures of the diversity fruit size, shape and color for lime and lemon fruits collected from Hormozgan province, southern of Iran.

a major region of lime and lemon cultivation, that provides more than 60% of 700,000 tons of produced lime and lemon in Iran in 2009 (Fao Statistics 2011).

In this study we evaluated some lime and lemon genotypes cultivated in the south of Iran in terms of terms of their fruit morphological characteristics.

MATERIALS AND METHODS

Plant material

In this study, the fruits of 19 genotypes of lime, lemon and some unknown acid citrus were sampled from different regions of Hormozgan province (south of Iran; **Figs. 1, 2; Table 1**). Among these genotypes 'Eureka', 'Lisbon' and 'Meyer' lemon and 'Persian' and 'Roodan' lime (thornless genotype of lime) were used. Fruit samples (15/genotype) were harvested from mature trees (8-10 years old) at the commercial maturity stage. For seed characters, a total of 60 seeds were evaluated. Fruit juice characters were evaluated from the juice of 5 fruits. All evaluations of all characters were replicated three times.

Characteristics and method of measurement

The weight, length and diameter of fruits collected at the maturity stage was calculated (**Table 2**). Other fruit characters (percent fruit juice, fruit peel and fruit waste) were determined after these parts were separated by hand. The percentage fruit juice was determined after juicing. The number of fruit segments, rind thickness and seed number were determined, as were some seed characters (seed length, width, diameter and number of embryos).

To evaluate the number of embryos in a seed, 60 seeds were collected and washed with tap water, then disinfected in two steps. Initially, seeds were exposed to 70% ethanol for 1 min, washed with distilled water then immersed in 2.5% sodium hypochlorite solution for 30 min. After disinfection, seed testa were removed and placed on sterile Petri dishes which were kept in the dark at 27°C. After 72 h the number of embryos was counted under a microscope.

Total soluble solids (TSS), pH and electrical conductivity (EC) were evaluated with a refractometer (Atago Co., Japan), pH meter and EC meter, respectively. Titrable acidity (TA) was measured with 0.1 N NaOH until reaching a pH of 8.2 and was calculated based on citric acid. Ascorbic acid (AsA) content was evaluated according to the protocol of Redox titration using iodine solution (www.outreach.canterbury.ac.nz).

Antioxidant activity (AoA) of fruit juice was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method according to Moon and Terao (1998). In this method, 0.1 ml of fresh juice was mixed with 0.9 ml of 100 mM Tris-HCl buffer (pH = 7.4) then 1 ml of DPPH (500 μM in ethanol) was added. The mixture was shaken gently and left for 30 min in the dark. Absorbance of the final solution was measured at 517 nm on a spectrophotometer (Lambda EZ201, Perkin Elmer, USA). AoA was calculated using the following equation (Moon and Terao 1998):

$$\text{AoA (\%)} = (1 - \text{A sample (517 nm)} / \text{A control (517 nm)}) \times 100$$

Total phenol content was extracted according to Vinson *et al.* (2001). Pulp and peel samples were dried in an oven at 40°C then powdered. Then, 1 g of dried sample was mixed with 10 ml of extraction solution (methanol/H_2O, 1: 1, v/v). The mixture was kept

Table 1 List of lime and lemon accessions used in this study with their some specific characters.

Genotypes	Commercial or local name	Collection area	Peel color	Pulp color
M1	---	Minab	Yellow	Yellow
M2	---	Minab	Green-yellow	Green
M3	---	Haji Abad	Yellow	Yellow
M4	---	Minab	Yellow	Yellow
M5	---	Minab	Green-yellow	Yellow
M6	Shalili	Haji Abad	Yellow	Yellow
M7	Eureka lemon	Minab	Yellow	Yellow
M8	Lisbon lemon	Minab	Yellow	Yellow
M9	---	Minab	Yellow	Yellow
M10	Meyer lemon	Minab	Yellow	Yellow
M11	Markab	Minab	Green-yellow	Yellow-red
M12	Persian lime	Minab	Green	Green
R1	---	Roodan	Yellow	Yellow
R2	---	Roodan	Yellow	Green
R3	Roodan lime	Roodan	Yellow	Green
R4	Sangi	Roodan	Dark yellow	Yellow
B1	---	Bandar abbas	Yellow	Green
B2	---	Bandar abbas	Green	Green
B3	---	Bandar abbas	Green	Yellow

Table 2 Fruit characteristics, range of variability and means.

Characters	Abbreviation	Unit	Mean	Min	Max	CV%
Fruit length	FL	mm	41.41	29.48	68.66	32.29
Fruit diameter	FD	mm	38.14	29.08	66.44	28.37
Fruit length/Fruit width	FL/FD	Ratio	1.32	0.88	1.3	9.91
Fruit tip length	FTL	mm	2.3	0	5.2	58.45
Fruit tip width	FTW	mm	6.36	0	12.89	52.11
Fruit tip length/Fruit tip width	FTL/FTW	Ratio	0.35	0	0.63	55.87
Fruit rind thickness	FrT	mm	2.23	0.88	4.67	9.38
Segment number	SN	-	9.33	7.8	10.9	75.12
Seed number	SeN	-	7.98	2.3	29.29	15.65
Seed length	SeL	mm	8.39	6.33	10.77	11
Seed width	SeA	mm	4.87	4.26	5.62	21.88
Seed length/Seed width	SeL/SeA	Ratio	1.85	1.47	3.02	31.32
Seed diameter	SeD	mm	2.73	1.4	5.17	36.73
Embryo number	EN	-	1.67	1	3.2	82.57
Fruit weight	FW	g	30.18	14.04	65.33	21.75
Fruit juice	W	%	31.87	18.93	42.86	20.54
Fruit waste	R	%	36.04	20.78	53.56	38.26
Fruit peel	P	%	28.42	15.01	46.54	27.98
pH	pH	-	1.93	1.33	2.85	10.59
Electrical conductivity	EC	µmho/cm	4072.2	3500	4950	26.84
Antioxidant activity	AoA	%	36.69	19.25	65.99	17.49
Ascorbic acid	AsA	mg/100 g fw	27.1	18.8	37.5	7.77
Total soluble solids	TSS	%	7.98	6.8	9.4	33.48
Titrable acidity	TA	g/100 ml juice	5.2	1.61	7.1	64.57
Total soluble solids/Titrable acidity	TSS/TA	Ratio	1.89	1.09	5.53	25.15
Pulp ash	PuAsh	%	1.17	0.34	1.95	38.09
Peel ash	PeAsh	%	2.61	1.39	3.53	26.83
Pulp dry matter	PuDm	%	10.33	7.69	14.95	13.
Peel dry matter	PeDm	%	19.03	14.95	24.48	92
Phenolic content in pulp	PhPe	mg Gallic acid/100 g dw	38.41	24.9	58.9	24.37
Phenolic content in peel	PhPu	mg Gallic acid/100 g dw	52.11	31	81.1	26.62

in the dark at 4°C for 24 h. The supernatant was collected and replaced with an equal quantity of extraction solution, then placed in the dark at 4°C for a further 48 h. Each supernatant was mixed and added to the extraction solution to obtain a final volume of 25 ml that was used to measurement the phenol content.

The amount of total phenolic compounds was determined according to the procedure of Folin-Ciocalteu (Singleton and Rossi 1965). A diluted extract (0.05 ml of extract and 0.45 ml of deionized water) were added to 2.5 ml of a 1: 10 diluted Folin-Ciocalteu's phenol reagent, followed adding by 2 ml of 7.5% (w/v) sodium carbonate (all chemicals from Sigma-Aldrich, St. Louis, USA). After 5 min incubation at 50°C, absorbance was measured at 760 nm using a spectrophotometer (Lambda EZ201). Phenol content was estimated from a standard curve of gallic acid and results were expressed as mg gallic acid equivalents (GAE) 100 g^{-1} dry weight (DW). Evaluation of fruit parameters such as shape index was according to Descriptors for *Citrus* (1999).

Statistical analysis

Analysis of variance (ANOVA) for all traits was performed with SAS software (version 10). The mean values of parameters that showed significant differences in all genotypes were used to perform factor analysis and clustering of the genotypes. SPSS software was also used for factor analysis (Varimax rotation) and clustering of genotypes using Ward's method.

RESULTS AND DISCUSSION

Morphology of fruits

ANOVA showed significant differences in all characters between all genotypes. Differences in some fruit characters may be due to seed propagation, differences in climate and pollination strategy. In general, lime fruits are smaller than

Table 3 Means of measured fruit characters.

Characters	FL	FD	FL/FD	FW	SN	SeN	FTL	FTW	FTL/FTW	FEL	FrT
M1	30.62	29.08	1.053	14.04	9.3	6.1	1.44	5.045	0.29	0	1.17
M2	45.84	41.84	1.1	27.04	10.9	4.6	1.84	4.76	0.38	0	2.57
R1	32.2	31.54	1.021	17.54	9.2	6.5	2.12	4.56	0.46	1.4	1.85
M3	31.58	31.5	1.003	17.34	9.3	6.8	1.72	5.75	0.31	0	1.11
M4	33.49	31.8	1.05	18.85	7.8	2.3	2.63	4.17	0.63	2.5	1.4
B3	33.15	32.66	1.015	19.19	9.5	6.3	1.51	4.73	0.32	0	1.68
R2	35.3	34.3	1.03	22.89	8.6	7.2	1.54	5.88	0.26	1.2	1.11
M5	35.2	35.57	0.99	25.27	8.8	5.9	0.56	3.89	0.14	0	1.1
R4	52.89	44.07	1.2	48.59	9.7	14.7	2.85	12.89	0.22	3.1	4.33
M11	78.66	76.44	1.03	144.48	11.89	3.56	0	0	0	0	4.67
B1	35.82	34.8	1.03	24.31	9.2	5.2	2.19	6.3	0.35	0	1.12
B2	34.47	32.09	1.07	19.85	9	7.7	2.62	6.2	0.42	0	0.88
M7	58.29	45	1.3	69.18	8.7	6.1	5.2	10.39	0.5	0	3.45
M8	54.58	46.97	1.16	75.33	9.29	7.29	4.9	9.65	0.51	4.1	3.95
R3	33.31	32.18	1.035	21.62	8.7	7.4	1.53	3.98	0.38	0	1.46
M9	56.64	44.76	1.27	53.34	10.14	29.29	3.01	11.46	0.26	2.3	2.67
M6	29.48	33.47	0.88	22.08	9.5	4.3	1.62	4.14	0.39	0	2.03
M10	63.89	52.37	1.22	72.38	9.5	12.5	4.12	10.63	0.39	0	3.57
M12	42.3	35.72	1.18	32.7	9.7	5.7	1.74	4.28	0.41	0	1.57
Characters	**EN**	**TA**	**TSS/TA**	**pH**	**EC**	**AoA**	**AsA**	**TSS**	**PhPu**	**PhPe**	
M1	1.8	7.1	1.15	1.72	4950	29.46	26	8.2	24.9	31	
M2	1.9	2.52	3.25	2.34	4000	38.25	36.8	8.2	46.2	53.6	
R1	1	6.19	1.28	1.48	4400	36.98	28.6	7.9	43.4	52.3	
M3	1.3	6.84	1.15	1.38	4100	45.04	25.9	7.9	38	46.1	
M4	1.4	6.15	1.19	1.45	3800	65.99	25.4	7.3	44.6	51.6	
B3	1.5	6.21	1.09	1.53	3700	30.03	28.8	6.8	27.6	38.1	
R2	1.73	6.6	1.11	1.6	3600	35.62	25.9	7.3	31.5	37.1	
M5	1.96	6.38	1.14	1.61	4100	38.25	25	7.3	30	39.7	
R4	1.5	3.14	2.99	2.43	4000	26.55	26.5	9.4	37.8	53.2	
M11	3.4	1.61	5.53	2.85	3800	44.27	27.5	8.9	50.7	65.6	
B1	1.8	6.53	1.24	1.33	4300	40.97	23	8.1	31	57.6	
B2	1.9	6.86	1.21	1.45	4850	31.38	18.8	8.3	30	48.9	
M7	1	4.62	1.77	1.68	3650	34.26	28.2	8.2	49.6	72.5	
M8	1.3	3.81	2.12	1.73	3500	42.92	29.3	8.1	58.9	75.6	
R3	1.4	6.65	1.22	2.66	4200	19.25	35.5	8.1	28.7	34.6	
M9	1.6	5	1.46	2.75	4000	25.71	27.5	7.3	39.4	52.7	
M6	2.7	5.8	1.43	2.74	4500	33.33	37.5	8.3	32.4	46.7	
M10	1	2.2	3.72	1.94	3850	42.15	20.7	8.2	46.7	81.1	
M12	1	6.52	1.13	1.53	4870	37.23	26.9	7.4	45.2	48.7	

For abbreviations, refer to **Table 2**

Table 4 Means of measured others fruit characters.

Characters	W	R	P	PeAsh	PuAsh	PuDm	PeDm	SeL	SeA	SeL/SeA	SeT
M1	25.1	53.56	20.98	2.25	1.6	14	15	7.25	4.43	1.64	2.35
M2	20.43	37.64	36.5	2.56	1.43	8.39	15.4	7.54	4.71	1.61	1.6
R1	27.8	40.19	20.39	3.18	1.95	11.38	19.69	7.76	4.78	1.62	2.19
M3	34.27	40.26	19.59	3.19	1.47	9.33	18.05	8.25	4.85	1.71	2.45
M4	38.27	36.09	22.32	3.18	1.08	8.78	20	7.52	5.15	3.02	1.4
B3	33.05	44.21	16.74	2.66	0.89	7.69	18.75	7.39	4.65	2.18	1.4
R2	34.98	20.78	36.08	3.09	1.02	9.49	18.29	8.31	4.73	1.76	2.79
M5	42.86	34.99	15.46	3.48	0.77	10.56	21.74	7.86	5.11	1.54	3.19
R4	18.93	29.77	43.59	1.39	1.15	12.23	19.35	10.77	4.26	2.51	3.15
M11	33.72	31.79	41.1	3.16	1.94	11.42	17.04	10.21	5.54	1.84	3.34
B1	41.77	34.54	15.32	1.88	0.65	13.01	22.92	7.68	4.64	1.65	2.7
B2	38.04	35.49	15.01	2.86	1.57	11.81	24.48	8.63	5.08	1.7	2.41
M7	25.16	31.61	46.54	1.73	0.34	10.08	20.19	9.89	5.54	1.79	5.17
M8	37.43	26.03	36.54	2.02	0.92	10.2	22.94	9.32	5.62	1.66	3.04
R3	39.12	41.95	18.93	3.53	0.94	7.98	17.43	6.35	4.32	1.47	2.61
M9	26.78	44.1	28.12	3.04	1.34	9.46	18	10.59	4.39	2.41	3.12
M6	28.52	35.8	35.68	1.67	0.89	8.93	14.95	7.97	5.09	1.56	2.61
M10	27.43	29.92	42.65	2.13	1.14	11.23	18.32	7.66	4.85	1.58	3.61
M12	35.2	32.48	32.48	2.61	2.04	1.53	18.63	8.85	4.7	1.89	2.84

For abbreviations, refer to **Table 2**

lemon fruits and have a thinner rind; lime fruits can be harvested when they are green mature. The main differences between lemon and lime fruits observed in this study are differences in the length to width ratio, the shape index, seed and embryo number, and rind thickness (**Table 2**). Lemon fruits often have a high length to width ratio and the level of polyembryony in lemons fruits was lower than lime fruits.

An important character in breeding programs of lime and lemon fruits is high TA, thin peel fruit and a high quantity of fruit juice. In these genotypes the highest level of TA was observed in M1 and the least in M11 (**Table 3**). M11, however, had the thickest fruit peel, which was thinnest in B2 (**Table 4**). The highest percentage of fruit juice was

Table 5 Bivariate simple correlation among studied characters.

	FL	FA	FL/FA	FTL	FTA	FTL/ FTA	FrT	SN	SeN	SeL	SeA	SeL/ SeA	SED	EN	FW
FL	1														
FD	0/95**	1													
FL/FD	0/61**	0/35	1												
FTL	0/31	0/06	0/72**	1											
FTA	0/30	0/05	0/76**	0/79**	1										
FTL/FTA	-0/30	-/46*	0/18	0/63**	0/22	1									
FrT	0/89**	0/84**	0/52*	0/39	0/40	-0/19	1								
SN	0/62**	0/73**	0/08	-0/31	-0/17	-0/60**	0/56*	1							
SeN	0/32	0/15	0/58**	0/29	0/68**	-0/19	0/25	0/16	1						
SeL	0/74**	0/68**	0/51*	0/21	0/37	-0/39	0/69**	0/49*	0/44	1					
SeA	0/54*	0/67**	-0/07	0/02	-0/33	-0/13	0/45	0/31	-0/41	0/43	1				
SeL/SeA	0/05	-0/05	0/28	0/16	0/27	0/20	0/11	-0/19	0/33	0/29	-0/14	1			
SED	0/59**	0/48*	0/57*	0/46*	0/46*	-0/17	0/50*	0/05	0/25	0/57*	0/34	-0/24	1		
EN	0/19	0/44	-0/52*	-0/61**	-0/53*	-0/62**	0/15	0/56*	-0/20	0/25	0/48*	-0/17	-0/08	1	
FW	0/96**	0/98**	0/39	0/17	0/09	-0/39	0/85**	0/61**	0/13	0/75**	0/73**	-0/04	0/59*	0/37	1
W	-0/30	-0/19	-0/41	-0/25	-0/44	0/03	-0/49*	-0/39	-0/34	-0/29	0/23	-0/13	-0/14	0/08	-0/13
R	-0/38	-0/38	-0/19	-0/29	-0/22	-0/02	-0/38	0/05	0/12	-0/36	-0/39	0/03	-0/41	-0/01	-0/39
P	0/72**	0/65**	0/52*	0/42	0/39	-0/06	0/81**	0/38	0/14	0/57**	0/34	0/04	0/58**	0/06	0/64**
PH	0/46*	0/52*	0/06	-0/17	0/02	-0/42	0/54*	0/58**	0/38	0/36	0/08	-0/02	0/17	0/53*	0/46*
EC	-0/46*	-0/44	-0/24	-0/33	-0/27	0/02	-0/50*	-0/01	-0/09	-0/26	-0/34	-0/26	-0/27	0/06	-0/44
AoA	0/07	0/14	-0/14	0/06	-0/25	0/34	0/05	-0/15	-0/44	-0/04	0/49*	0/32	-0/19	0/01	0/17
AsA	-0/13	-0/04	-0/25	-0/17	-0/25	0/07	0/13	0/24	-0/15	-0/17	-0/05	-0/17	-0/18	0/21	-0/07
TSS	0/43	0/46*	0/12	0/12	0/20	-0/21	0/59**	0/40	-0/03	0/40	0/21	-0/19	0/35	0/32	0/44
TA	-0/87**	-0/86**	-0/42	-0/25	-0/26	0/24	-0/94**	-0/66**	-0/17	-0/52*	-0/45	-0/03	-0/38	-0/24	-0/79**
TSS/TA	0/84**	0/95**	0/23	-0/02	-0/05	-0/42	0/84**	0/76**	0/02	0/49*	0/53*	-0/08	0/27	0/45	0/84**
PeAsh	-0/21	-0/11	-0/34	-0/53*	-0/56*	-0/19	-0/47	-0/07	-0/04	-0/25	0/05	-0/01	-0/35	0/07	-0/14
PuAsh	0/09	0/16	-0/06	-0/45	-0/36	-0/23	0/01	0/49*	0/02	0/16	0/05	-0/03	-0/31	0/13	0/11
PeDm	0/15	0/15	-0/08	0/09	0/22	-0/22	0/16	0/02	0/08	0/09	0/09	-0/13	0/16	0/26	0/14
PuDm	-0/02	-0/09	0/17	0/38	0/25	0/25	-0/1	-0/40	-0/02	0/12	0/17	0/08	0/16	-0/27	-0/01
PhPu	0/69**	0/62**	0/51*	0/52*	0/22	0/26	0/71**	0/31	-0/01	0/48*	0/58*	0/10	0/34	-0/16	0/66**
PhPe	0/75**	0/66**	0/56*	0/69**	0/44	0/22	0/73**	0/25	0/12	0/46*	0/53*	-0/02	0/54*	-0/13	0/69**

**Significant at %1 prob. *Significant at 5% probability
For abbreviations, refer to **Table 2**

Table 5 (Cont.)

	W	R	P	PH	EC	AoA	AsA	TSS	TA	TSS/ TA	PeAsh	PuAs h	PuDm	PeDm	Phpu	Phpe
W	1															
R	-0/16	1														
P	-0/58**	-0/57*	1													
PH	-0/40	0/06	0/45	1												
EC	0/02	0/48*	-0/43	-0/11	1											
AoA	0/29	-0/29	0/05	-0/39	-0/28	1										
AsA	-0/29	0/17	0/20	0/56*	-0/09	-0/26	1									
TSS	-0/43	-0/18	0/47*	0/44	0/11	-0/16	0/05	1								
TA	0/49*	0/37	-0/75**	-0/59*	0/49*	-0/17	-0/26	-0/51*	1							
TSS/TA	-0/36	-0/31	0/64**	0/54*	-0/34	0/15	0/08	0/67**	-0/98**	1						
PeAsh	0/49*	0/22	-0/52*	-0/07	-0/04	0/13	-0/04	-0/49*	0/32	-0/18	1					
PuAsh	-0/17	0/22	-0/08	0/07	0/49*	0/05	-0/09	0/12	-0/06	0/26	0/38	1				
PuDm	-0/15	0/11	-0/07	-0/01	-0/03	-0/03	-0/35	0/48*	-0/17	0/26	-0/26	-0/17	1			
PeDm	0/56*	-0/37	-0/30	-0/56*	-0/09	0/19	-0/66**	-0/08	0/18	-0/27	-0/01	-0/26	0/19	1		
Phpu	-0/19	-0/47*	0/64**	0/10	-0/46*	0/47*	0/07	0/22	-0/67**	0/55*	-0/16	0/15	-0/18	0/10	1	
Phpe	-0/17	-0/54*	0/66**	0/07	-0/44	0/36	-0/19	0/38	-0/78**	0/59**	-0/49	-0/12	0/14	0/29	0/85**	1

**Significant at %1 prob. *Significant at 5% probability
For abbreviations, refer to **Table 2**

measured in M5 (42.86%) while the least juice was observed in R4 (18.93%). M11 had the longest and widest fruits followed by M10 and M7 (i.e., 'Meyer' and 'Eureka' lemon); M6 and M3 had the smallest fruits. FL/FD is the parameter that indicates fruit shape. Fruits with a high FL/FD value are longer while those with a lower FL/FD are more spherical. Among the genotypes assessed, M6 had the lowest FL/FD value and was thus round. M10 had the highest FL/FD value and thus had a stretched appearance.

M5 and B1 had the heaviest fruit (**Table 3**). The highest level of TSS (8.9%) in fruit juice was measured in R4 and the least in B3 (**Table 3**). The nature of the ratio of TSS/TA gave a characteristic fruit flavor. M11 had the highest TSS/TA value (5.53) and therefore was less sour in taste. B3, R2, M5, M1 and M3 with a TSS/TA ratio of 1.09, 1.11, 1.14 and 1.15 had the most sour-tasting fruit juice among all the genotypes (**Table 4**). The highest AsA content was observed in M6 and then in M2, and the least in B2 (**Table 4**). Citrus fruit juices have a high level of antioxidant activity (Zvaigzne *et al.* 2009). In this study, the evaluation of AoA in all lime and lemon genotypes showed that the fruit juice of both have high AoA values, ranging from 19.25 to 65.99% in R3 and M4, respectively.

Evaluation of phenolic content in peel and pulp of these genotypes showed that they have a high level of phenolic compounds ranging from 24.9 to 81.1 mg gallic acid per 100 g dry matter. M8 ('Lisbon' lemon) had the highest level of phenolic acids in the pulp of fruit among all genotypes; the highest level of phenolic compounds in the peel of fruit was observed in M10 ('Meyer' lemon).

Table 6 Eigen values accepted (≥1), variance and cumulative variance for 10 factors.

Factor	1	2	3	4	5	6	7
Eigen value	10.78	5.41	3.63	2.33	2.15	1.66	1.56
% of variance	33.68	16.92	11.34	7.28	6.71	5.21	4.86
Cumulative variance%	33.68	50.60	61.94	69.22	75.93	81.12	85.99
FL	0.95**	-0.06	0.06	0.07	0.16	0.03	-0.15
FD	0.90**	-0.32	0.20	0.07	0.12	-0.03	-0.07
FL/FA	0.57	0.58**	-0.29	0.02	0.22	0.19	-0.29
FTL	0.40	0.84**	-0.08	-0.04	-0.21	0.09	-0.02
FTW	0.40	0.74**	-0.47	0.19	0.04	-0.06	0.06
FTL/FTW	-0.23	0.68**	0.13	-0.45	-0.28	0.24	0.09
FrT	0.96**	-0.01	-0.09	-0.09	-0.03	-0.04	0.09
SN	0.58	-0.66**	-0.17	-0.05	0.15	0.19	-0.05
SEN	0.28	0.27	-0.62**	0.18	0.54	-0.14	-0.11
SEL	0.76**	0.01	-0.06	0.19	0.37	-0.04	0.01
SEA	0.53	-0.26	0.70**	0.03	-0.09	-0.02	-0.02
SEL/SEA	0.06	0.35	-0.06	-0.31	0.58**	-0.14	0.43
SED	0.60**	0.24	-0.04	0.43	-0.23	-0.10	-0.44
EN	0.19	-0.80**	0.15	0.18	-0.04	-0.25	0.26
FW	0.90**	-0.22	0.24	0.11	0.09	-0.03	-0.11
W	-0.43	0.01	0.69**	0.24	0.14	-0.24	-0.19
T	-0.47	-0.27	-0.44	-0.02	0.15	0.23	0.11
P	0.84**	0.07	-0.14	-0.24	-0.24	-0.04	-0.09
PH	0.49	-0.58**	-0.47	-0.16	0.01	-0.31	0.01
EC	-0.49	-0.25	-0.28	0.24	-0.07	0.61**	-0.04
AoA	0.08	0.14	0.76**	-0.32	0.09	0.17	0.32
AsA	0.02	-0.35	-0.32	-0.63**	-0.37	-0.32	-0.07
TSS	0.58**	-0.21	-0.21	0.28	-0.37	0.24	0.36
TA	-0.91**	0.15	0.02	0.16	0.05	-0.03	-0.11
TSS/TA	0.84**	-0.42	0.09	-0.02	-0.02	0.11	0.12
PeAsh	-0.39	-0.33	0.32	-0.09	0.54	-0.10	-0.27
PuAsh	0.02	-0.45	-0.03	-0.14	0.44	0.69**	-0.02
PuDm	0.16	-0.02	-0.06	0.63**	-0.16	-0.01	0.59**
PeDm	-0.05	0.56**	0.49	0.48	0.14	0.01	0.04
PhPu	0.74**	0.23	0.34	-0.40	0.02	0.23	-0.08
PhPe	0.79**	0.35	0.28	-0.01	-0.16	0.20	-0.03

**Significant factor loadings (considered values above 0.58)
For abbreviations, refer to **Table 2**

One of the important characters in lime, lemon and other citrus crops is seedlessness or low seed. In citrus fruit a range of 1 to 6 seeds per fruit was determined as seedless, a range between 6 to 10 seeds per fruit is considered to be low seed and fruits with > 10 seeds per fruit are considered as high seed (Khan 2007). In these genotypes, M9 had the highest average of 29.29 seeds per fruit while R4 and M10 ('Meyer' lemon) ranked next. The seedless genotypes included M4, M11, M6 and M2 with 2.3, 3.6, 4.3 and 4.6 seeds/fruit, respectively (**Table 4**).

In terms of seed size, we observed that the length of seed in all genotypes ranged from 6.35 mm to 10.77 in R3 and R4, respectively. The width of seed ranged from 4.26 to 5.62 mm in R4 and M8 ('Lisbon' lemon), respectively (**Table 5**).

R1, M7 ('Eureka' lemon), M10 ('Meyer' lemon) and M12 ('Persian' lime) were monoembryonic genotypes, while M11, with an average of 3.4 embryos per seed, had the highest level of polyembryony. The number of embryos in other genotypes ranged from 1.3 to 2.7 embryos per seed.

Bivariate simple correlation

In this study 31 characters of lime and lemon fruit were evaluated. Bivariate simple correlation analysis showed significant positive and negative correlation among some characters (**Table 6**). Significant relationships among some fruit characteristics of lime have been reported (Prasad and Rao 1989). Simple correlation analysis is beneficial, especially when the determined character is expensive, complex or difficult. It is possible to use characters that have a high correlation for indirectly measuring parallel characters (Sarkhosh *et al.* 2008).

We found a positive correlation among fruit length and fruit diameter, and also among fruit length and diameter with seed size (length and width) and fruit weight, as also reported by Krezdorn (1967). A positive correlation between the FL/FW ratio and seed number was observed. Embryo number was positively correlated with seed width. Rodríguez *et al.* (2004), in a study on polyembryony in 'Volkamerian' lemon, observed a positive correlation between seed size and embryo number. There was a negative correlation between fruit rind thickness and fruit peel percentage with fruit juice percentage. A positive correlation between the pH of fruit juice and vitamin C was observed. Since vitamin C is kind of organic acid, this correlation was expected (Pereira *et al.* 2008). On other hand, a negative correlation between pH and TA was also observed, which was also predictable and reported in other studies (Sarkhosh *et al.* 2008). A positive correlation between fruit rind thickness and fruit weight mirrored the results of Prasad and Rao (1989) in lime fruits. A reported positive correlation between high level of TA and fruit juice percentage in lime fruits by Prasad and Rao (1989) was also observed in this study. We observed a negative correlation between TSS and TA while in Prasad and Rao (1989) this correlation was positive, although the negative correlation between TA and fruit weight and fruit ring thickness was observed in both experiments. We observed a positive correlation between fruit diameter with TA, TSS and TSS/TA, which was also reported by Ketsa (1988) in tangerine fruit.

A significant positive correlation was observed between mg gallic acid in pulp and peel and also between mg gallic acid in pulp and percent antioxidant activity in fruit juice that is due to the AoA of phenolic compounds (Gorinstein *et al.* 2001).

Factor analysis

Table 6 shows the results of factor analysis. Factor analysis could decrease the 31 characters to 7 main factors (**Table 6**). The amount of variance for each factor shows the importance of that factor in justifying total variance of the studied characters, which are explained as percentages. Seven factors with an Eigen value of ≥1 accounted for 85.99% of the total variance. The characters with a threshold level of > 0.5 (Johnson and Wichern 1988) were chosen to be significant for each factor. The first factor accounted for 33.69% of the overall variance including variables such as fruit length, fruit diameter, fruit length/diameter, fruit weight, segment number, seed length, seed diameter, TSS, TA, TSS/TA, peel and pulp phenolic compounds. In the second factor, the largest scores were due to characters such as fruit shape, fruit index, embryo number and pH. High loading characters on factor three included seed number, seed diameter, fruit juice percent and fruit juice AoA.

Cluster analysis

Cluster analysis has been used to identify morphological variability in different crop species (Decker and Wilson 1986; Cartea *et al.* 2002). According to **Fig. 3**, cluster analysis based on morphological traits grouped all genotypes within 25 to 20 relative distances into 3 main groups: the lime group (containing 'Persian' and 'Roodan' lime), the lemon group (containing 'Lisbon', 'Eureka' and 'Meyer') and an unknown group. The lime group was divided two sub-groups containing large fruits (i.e., 'Persian' lime) and small fruits (i.e., 'Roodan' lime). The small fruits contained 6 genotypes that were small and had a low length to width ratio. The small sub-group also has smaller seeds and a higher level of polyembryony. The large fruit lime contained 7 genotypes. This sub-group had larger fruits the rind was thinner. The lemon group generally had stretched fruits, and the fruits index was quite prominent. The fruits from this group had larger seed but a low level of polyembryony. The lemon cluster was divided into two clusters 22 distance points apart one of which contained three commercial lemons and another contained two genotypes that were similar to lemon.

In this study R4 with local name 'Sangi' is a popular genotype in the south of Iran such as Hormozgan and Fars provinces was located in the lemon group together with 'Lisbon', 'Eureka' and 'Meyer' lemon, which corresponded with a grouping offered by Shahsavar *et al.* (2007) using ISSR markers for evaluation genetic relationships between some citrus genotype in Fars province.

A comparison between commercial lime and lemon showed that the juice of lemon fruits had the lowest TA and highest pH and TSS, and the level of phenolic compounds in lemon peel and pulp was higher than in limes. However, some genotypes in one of these groups may be hybrids of lime and lemon or with other species of the *Citrus* genus. The third group with a distance of 25 units includes one genotype that did not belong to either lime or lemon. It has large fruits like sweet orange, has a yellow peel like a lemon, a thick rind and a low TA. In addition, the fruit juice of this genotype was almost red. It is most likely a hybrid of lime or lemon but containing other *Citrus* genes.

Some genotypes had useful characters that could be used in breeding programs. For example, genotypes B2, B1, M3, R2, and M5 in the lime groups have a thinner rind. On the other hand, M4, B1, M2, and M6 also have seedless fruits. The percentage of juice fruit in M5, B1 and R3 was highest and these genotypes had the lowest percent of peel and waste. M6 and M2 fruit juice had the highest level of AsA. The high level of TA that was the most important economic characteristic observed among lime genotypes, especially M1 and B2. Hybridization between parents with high levels of acidity produce progeny whose acid levels are mostly higher than that of either parent (Soost and Cameron 1961).

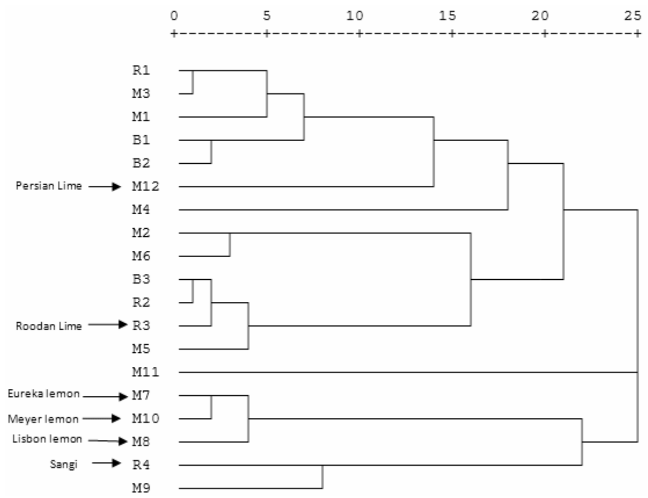

Fig. 3 Dendrogram of grouping 19 genotypes of lime and lemon based on 7 main factors using Ward's method.

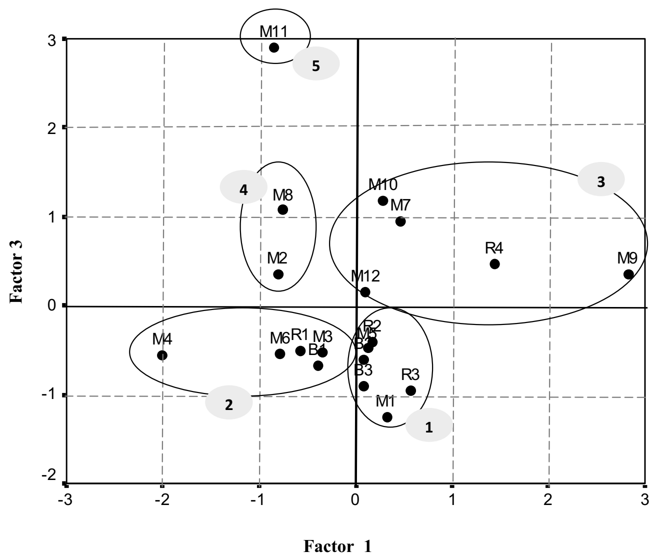

Fig. 4 Position of lime and lemon genotypes on the scatter plot produced by first and third factors.

Finally, it seems that B1 and B2 lime genotypes are useful for use as breeding plant materials to improve economic characters such as thinner rind, seedlessness, high level of juice fruit and TA. R3 is the only genotype that has thornless branches that are useful in terms of ease of harvest. Lemon genotype M8 ('Lisbon') has better characters such as high juice fruit percent than other lemon genotypes.

Scatter plot

A scatter plot was prepared according to the two main factors such as first and third factors by using SPSS software. The genotypes were approximately separated by a scatter plot. Results of the scatter plot supported the results of cluster analysis. Lime and lemon genotypes were clearly separated in the scatter plot. M11, an unknown genotype that was separated from others, was obviously separated in the scatter plot (**Fig. 4**).

CONCLUSIONS

Lime and lemon are two major species of the *Citrus* genus that are used in many applications such as fresh and dry consumption, the processing, cosmetic and drug industries.
Description of morphological characters is a usual method accepted for evaluation and registration of varieties. Genetic studies and variety characterization of lime and lemon

are used in breeding programs focusing on introducing some commercial traits such as fruit size and shape, fruit juice percentage, high TA in fruit juice, seedlessness and rind thickness.

The evaluation of several characters showed a significant difference between limes and lemons, such as the ratio of length to width, TSS/TA, fruit index shape, pH of fruit juice and phenolic contents. On the other hand, evaluation of morphological traits can be used to identify superior genotypes of each region, which were adapted to the condition of those regions and can be widely cultivated by farmers. The primary conclusion of this study is that the conservation of autochthonous lime and lemon cultivars is highly recommended.

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