

## Original Research

Variability analysis of lime (*Citrus* sp) genotypes using morphological markers in the south of Iran

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## ABSTRACT:

In this study, morphological characteristics of leaves, flowers, fruit and seeds of 35 acid lime [*Citrus aurantifolia* (Christm.) Swingle] genotypes from south of Iran were evaluated during two successive years. The cluster analysis using Ward's minimum variance assigned genotypes into three groups. The majority of studied genotypes, (about 20 genotypes), were categorized in group 3 (G<sub>3</sub>). The dissimilarity matrix based on Gower coefficient showed that there was a significant difference in the range between 0.05 and 0.63 among genotypes. The maximum difference was observed between C<sub>10</sub> (Mexican lime) and TD<sub>9</sub> genotypes and the minimum difference was found among two genotypes from Minab region (MH<sub>1</sub> and MH<sub>3</sub>). The results of Kruskal Wallis Test indicated that there was a significant difference between three separated group genotypes of cluster analysis based on 25 studied characteristics. Over all, the results showed that each group based on their unique characteristics has superior breeding values than other ones and be used to produce desirable hybrids in breeding programs.

## Keywords:

Acid lime, Breeding program, Cluster analysis, Diversity.

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## INTRODUCTION

Mexican lime accessions (*Citrus aurantifolia* Swingle), from Rutaceae family and Aurantioideae sub-family, is one of the commercial citrus crops (Shrestha *et al.*, 2012), that originated in south-eastern of Asia (Win *et al.*, 2006). Researchers believe that lemon is a result of crosses between lime and citron (Barrect and Rhodes, 1976). Limes are planted in many countries such as India, Mexico, China, Argentina, Brazil, Spain, Turkey, Iran, Italy, Bangladesh, USA, Sudan, Peru and the countries having tropical climate, while Lemons are cultivated to the dry climates of Iran and Egypt. Iran is the ninth lime and lemon producer in the world ranking with 28627 hectares cultivation area and 427715 tons production (FAOSTAT, 2013). The main lime cultivation regions in Iran are Fars, Hormozgan, Kerman, Bushehr, Kohgiluyeh and Boyer-Ahmadand Sistan and Baluchestan provinces (Golein and Adouli, 2011). Lime and lemon are utilized daily or used to produce fruit juice and they are significant constituent of the beauty product, pharmaceutical and processing industries (Prasad *et al.*, 1989). The dried Lime fruits are used to prepare peel powder additives. 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 and Wichern, 1988). Furthermore, differences in some fruit characters may be due to seed propagation, differences in climate and pollination strategy (Zandkarimi *et al.*, 2011).

Al-Naggar *et al.* (2009) make use of some factors of fruits, seed and seedlings to assess some inter-specific citrus crosses whose parents included Volkamer lemon and lime genotypes. Some features such as number of seed in mature fruits, germination percent and embryos number per seed (i.e. polyembryony) were assessed. Their results confirmed that Volkamer lemon and its hybrid had no polyembryony and this cultivar had mono-embryonic seed.

In another study that was done on mandarin accessions (*Citrus reticulata* Blanco) in Bhutan, Dorji and Yapwattanaphun (2011) used morphological characteristics for the detection of mandarin accessions. They used 30 accessions from six main mandarin regions. The results of measuring leaf dimensions, fruit dimensions, epicarp thickness, and the number of seeds indicated significant differences among accessions ( $p < 0.01$ ) although there was little diversity among the trees or even the regions according to flower characteristics.

*Citrus* have considerable diversity among their varieties due to some particular characteristics such as their sexual compatibility with related genera as well as



Figure 1. One of the collection site in the south of Iran (place of sampling)

Table 1. The characteristics of different lime genotypes

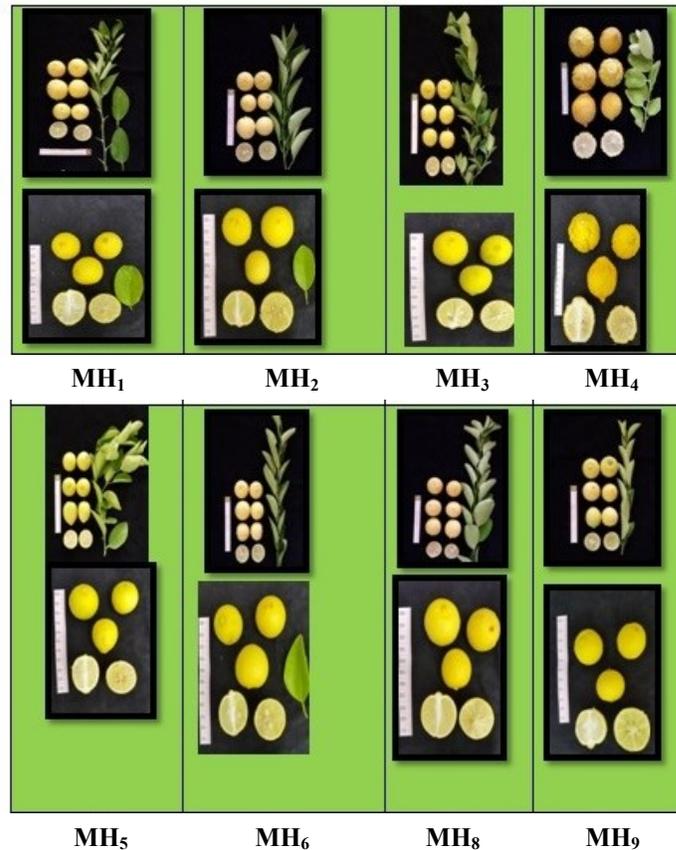
Number	Common name	Scientific name	Place of sampling	Number	Common name	Scientific name	Place of sampling
1	6 Darab(MH <sub>1</sub> )	<i>Citrus</i> sp.	Minab	19	Mesri- Kamali (nMH <sub>3</sub> )	<i>Citrus</i> sp.	Jahrom
2	1 - 1 (MH <sub>2</sub> )	<i>Citrus</i> sp.	Minab	20	Kharkolu(nMH <sub>5</sub> )	<i>Citrus</i> sp.	Jahrom
3	2 - 2 MX (MH <sub>3</sub> )	<i>Citrus</i> sp.	Minab	21	Hybrid 5 (TD <sub>1</sub> )	<i>Citrus</i> sp.	Darab
4	Binam(MH <sub>4</sub> )	<i>Citrus</i> sp.	Minab	22	Hybrid 6 (TD <sub>2</sub> )	<i>Citrus</i> sp.	Darab
5	5 - 2(MH <sub>5</sub> )	<i>Citrus</i> sp.	Minab	23	Hybrid 7 (TD <sub>3</sub> )	<i>Citrus</i> sp.	Darab
6	4 - Darab (MH <sub>6</sub> )	<i>Citrus</i> sp.	Minab	24	Limequat (TD <sub>4</sub> )		Darab
7	4 - 5(MH <sub>7</sub> )	<i>Citrus</i> sp.	Minab	25	Hybrid 8 (TD <sub>5</sub> )	<i>Citrus</i> sp.	Darab
8	1-10 (MH <sub>8</sub> )	<i>Citrus</i> sp.	Minab	26	Hybrid 2 (TD <sub>6</sub> ): <i>Citrus aurantifolia</i> × Bakraee ( <i>Citrus</i> sp.)	<i>Citrus</i> sp.	Darab
9	4 - 2(MH <sub>9</sub> )	<i>Citrus</i> sp.	Minab	27	Hybrid 3 (TD <sub>7</sub> ): <i>Citrus aurantifolia</i> × <i>Citrus sinensis</i>	<i>Citrus</i> sp.	Darab
10	1 - 13 (MH <sub>10</sub> )	<i>Citrus</i> sp.	Minab	28	Cucumber-shaped lime of Farood (TD <sub>8</sub> )	<i>Citrus</i> sp.	Darab
11	1 - 14 (MH <sub>11</sub> )	<i>Citrus</i> sp.	Minab	29	Sweet lime Forg (TD <sub>9</sub> )	<i>Citrus</i> sp.	Darab
12	1 - 7 (MH <sub>12</sub> )	<i>Citrus</i> sp.	Minab	30	Curly lime of Farood (TD <sub>10</sub> )	<i>Citrus</i> sp.	Darab
13	5 - 1 (MH <sub>13</sub> )	<i>Citrus</i> sp.	Minab	31	G <sub>6</sub>	<i>Citrus</i> sp.	Jiroft
14	1 - 4 (MH <sub>14</sub> )	<i>Citrus</i> sp.	Minab	32	G <sub>7</sub>	<i>Citrus</i> sp.	Jiroft
15	1 - 3 (MH <sub>15</sub> )	<i>Citrus</i> sp.	Minab	33	G <sub>11</sub>	<i>Citrus</i> sp.	Jiroft
16	2 - 8 (MH <sub>16</sub> )	<i>Citrus</i> sp.	Minab	34	(C <sub>4</sub> ) Persian lime	<i>Citrus latifolia</i>	Ramsar
17	2 - 10 (MH <sub>17</sub> )	<i>Citrus</i> sp.	Minab	35	(C <sub>10</sub> )Mexican lime	<i>Citrus aurantifolia</i> Swingle	Ramsar
18	1-5 (MH <sub>18</sub> )	<i>Citrus</i> sp.	Minab				

their interspecific compatibility (Cooper *et al.*, 1962).

In addition, because different varieties of lime are spermatophytic and have a long history of cultivation, there are different lime genotypes in the southern regions of Iran.

Recently, Witch's Broom Disease of Lime (WBDL) become a major limiting factor for lime production in the South of Iran and also be the main threat factor for lime industry in Iran. WBDL is a very serious disease of acid (Mexican) limes (Bove *et al.*, 2000). WBDL is a mycoplasma disease in

which it is may happen by a phytoplasma namely, *Phytoplasma aurantifolia*. In an attempt, during the past decade, to detect the varieties that are susceptible to this disease, a number of healthy lime biotypes were collected from the southern regions of Iran and now are stored in collections. No accurate horticultural evaluations, such as morphological characteristics examinations have been done on these biotypes yet. Hence, such characteristics have been studied in some of these genotypes as the first step towards a comprehensive identification of the concerned genotypes in order to use them in



**Figure 2a. Citrus genotypes used in this study**

breeding programs of traits improvement.

## MATERIALS AND METHODS

Thirty five natural lime genotypes were selected from the three main citrus research collection in the south of Iran, (Darab and Jahrom, in Fars province; Minab in Hormozgan; Jiroft in Kerman) (Figure 1). Collections were located at latitude  $27^{\circ} 06' 27''$ , longitude  $57^{\circ} 05' 39''$  and elevation of 40m.

All genotypes were grafted on the same stock and given same horticultural practices (Table 1). All genotypes were free from Witches Broom Disease of Lime (WBDL) symptoms.

At the first, all genotypes were tested for the presence of WBDL agents by specific markers ( $P_1/WB_3$ ,  $R_{16}F_{2n}/R_{16}R_2$  and  $P_1/P_7$ ) in PCR (Table 2). DNA was extracted according to Murray and Thompson (1980).

The results showed that none of the genotypes showed disease symptoms.

Thereafter, dependent on the site collection, the morphological characteristics of lime genotypes such as flowers, fruits and leaves were evaluated from June to September during the two successive years of 2015 and 2016. For this purpose, about 30 lime fruits were randomly selected from different parts of the tree based on fruit ripening index such as juice content and rind acceptable color.

About 30 flowers were gathered at different sides of each tree in the early spring during flower anthesis. Furthermore, 30 leaves were sampled from the central parts of the non-bearing vegetative shoots and immediately were transferred to the laboratory of Citrus and Subtropical Fruit Research Center of Ramsar, Iran. The morphological characteristics of the genotypes were measured based on the reference to Citrus descriptor

**Table 2. Primers used to diagnose the cause of WBDL**

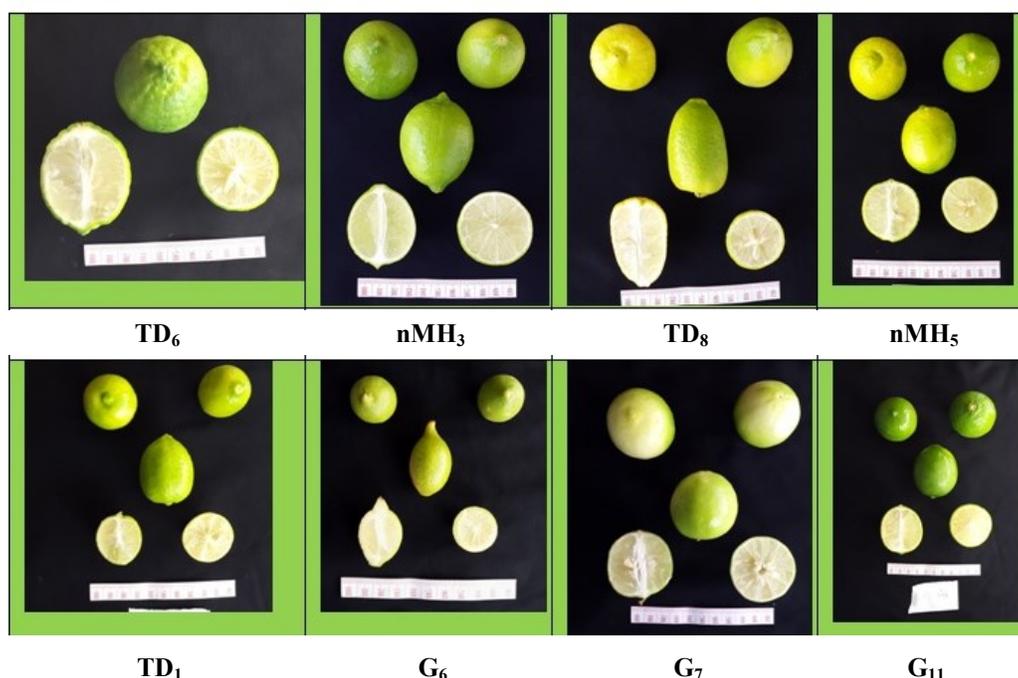
S. No	Primer	Sequences (5'-3')	Allele size (bp)
1	P <sub>1</sub> /P <sub>7</sub>	AAGAGTTTGATCCTGGCTCAGGATT CGTCCTTCATCGGCTCTT	1800
2	R <sub>16</sub> F <sub>2n</sub> /R <sub>16</sub> R <sub>2</sub>	GAAACGACTGCTGCTAAGACTGG TGACGGGCGGTGTGTACAAACCCCG	1200
3	P <sub>1</sub> /WB <sub>3</sub>	AAGAGTTTGATCCTGGCTCAGGATT GCAAGTGGTGAACCATTGTTT	1000

(IPGRI, 1999). The characteristics such as growth habit of the tree, the angle between branches and the trunk, branch density, mature tree shape, the color of shoot tip, spine density of mature trees, spine shape, leaf division, lamina shape, lamina edges, leaf tip, wing status and width, wing shape, lamina-petiol connection, anthesis, blooming start time, blooming month, type of flower, color of the opened flower, number of petals, color of anther, anther/stigma length ratio, fruiting season, fruit shape, fruit bottom and top shape, rind color, rind texture, albedo adherence to the rind, oil-gland clarity, albedo color, existence of outer ring on fruit top, the situation of the end close to the fruit style, number of segments in each fruit, adherence of segment walls to one another, thickness of segment wall, fruit axis, flesh

color of ripe fruits, flesh texture, length and thickness of vesicles, seed shape, seed surface and seed color were studied at different limes.

For this purpose, the characteristics were investigated by means of observations, digital calipers (Swiss-made, with 0.01 accuracy per millimeter) and with reference to citrus descriptor. At the end, data were categorized in to nominal, ordinal, and ratio scales. There after, Gower distance coefficient was used in order to eliminate the scale impact (Gower, 1971).

Then, together with the calculation of the dissimilarity matrix, the coefficient was used in various grouping methods such as Unweighted Pair Group Method with Arithmetic Mean (UPGMA), Complete Linkage, and Ward in SAS 9.0 software (SAS, 2002).



**Figure 2b. Citrus genotypes used in this study**

**Table 3. Results of the dominant morphological traits (criteria for the evaluation of more than 50% of both group members) in lime genotypes in three groups of cluster graph**

Number	Traits	Results			Result of Kruskal Wallis Test (P-Value)*
		Group 1(G <sub>1</sub> )	Group 2 (G <sub>2</sub> )	Group 3 (G <sub>3</sub> )	
1	Pulp color at maturity	Yellow-green	Green	Orange	0
2	Pulp texture	Crispy	Fibrous	Fleshy	0.899
3	Seed shape	Fusiform	Clavate	Ovoid	0.002
4	Seed surface	Wrinkled	Smooth	Hairy	0
5	Seed color	White	Cream	Green	0
6	Flower type	Hermaphrodite	Male	Female	1
7	Color of open flower	White	Light yellow	Yellow	0.708
8	Anther colour	White	yellow	Pale yellow	0.07
9	Fruit shape	Spheroid	Ellipsoid	Oblate	0.892
10	Shape of fruit base	Necked	Convex	Truncate	0.242
11	Fruit surface texture	Smooth	Rough	Bumpy	0.002
12	Albedo colour	White	Greenish	Yellow	0.062
13	Absence/presence of areola	Absent	Present	-	0.692
14	Number of segments per fruit	<5	5-9	10-14	0.061
15	Spine shape	Straight	Curved	-	0.703
16	Fruit stylar end	Closed	Open	Persistent style	0.462
17	Leaf lamina shape	Crenate	Dentate	Orbicular	0.892
18	leaf lamina margin	Crenate	Dentate	Entire	0
19	leaf apex	Acute	Attenuate	Rounded	0.008
20	Junction between petiole and lamina	Fused	Arcuate	-	0.239
21	Tree growth habit	Erect	spreading	Drooping	0.037
22	Tree shape	Ellipsoid	Spheroid	Obloid	0.002
23	Shoot tip color	Green	Purple	Other	0.074
24	Branch angle	Narrow	Medium	Wide	0.019
25	Density of branches	Sparse	Medium	Dense	0.767
26	Leaf lamina attachment	Sessile	Brevipetiolate	Longipetiolate	0.003
27	Petiole wing width	Narrow	Medium	Broad	0
28	Petiole wing shape	Obcordate	Obovate	Linear	0.036
29	Spine density on adult tree	Absent	Low	Medium	0
30	Flowering month	April	March	March	0.383
31	Fruiting bearing	Early	Mid	Late	0.2
32	Shape of fruit apex	Mammiform	Rounded	Acute	0.023
33	Fruit skin color	Light yellow	Green-yellow	Orange	0.241
34	Adherence of albedo to pulp	Weak	Medium	Strong	0
35	Conspicuousness of oil glands	Inconspicuous	Conspicuousness	Strongly Conspicuousness	0
36	Vesicle length	Short	Medium	Long	0

Continue....

Continue....

37	Vesicle thickness	Thin	Medium	Thick	0
38	Adherence of segment walls to each other	Weak	Medium	Strong	0
39	Thickness of segment walls	Thin	Medium	Thick	0
40	Fruit axis	Solid	Semi-hollow	Hollow	0.2
41	Number of petals per flower	4	5	5	0.29
42	Calyx diameter	Smal	Medium	Large	0.167
43	Length of anthers relative to stigma	Shorte	Medium	Longer	0.948

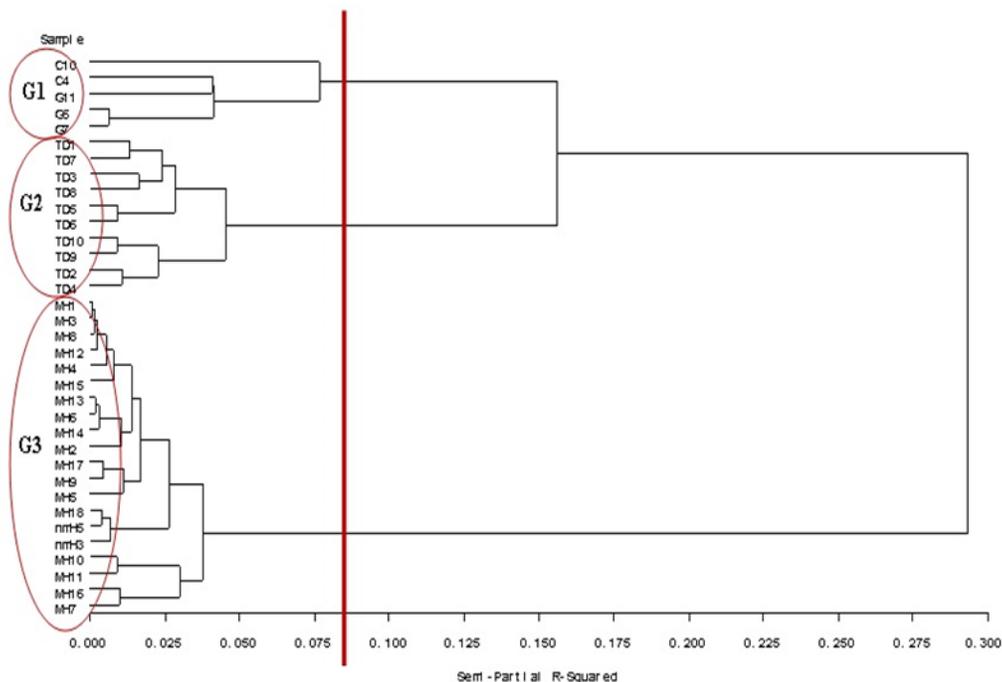
:\*P-Value less than 0.05 shows significant difference between groups based on the Kruskal-Wallis test

After building up different dendrograms by the mentioned methods, the best dendrogram was selected with respect to such criteria as lowest chaining, more compatibility with the basic genotypes information, and better sample separation. Furthermore, Kruskal Wallis Test was utilized to determine those characteristics that caused the highest variation among groups (Wallis, 1952). This non-parametric test was selected as the traits had qualitative natures.

**RESULTS AND DISCUSSION**

**Cluster analysis based on the morphological data**

After the conduction of cluster analysis based on the measured morphological traits (Figure 2a and 2b), the comparison of the resulting dendrogram from various algorithms indicated that the one obtained from Ward Method separated the different genotypes much better than the others; in addition, no chaining was observed in this method. As a result, this dendrogram was interpreted. The most appropriate dendrogram cut-off point out of the existing points was selected to divide all the studied genotypes of lime into three groups (Figure



**Figure 3. Dendrogram of 35 citrus genotypes based on Ward's minimum variance method**

**Table 4. Dissimilarity matrix based on the morphological data**

Sample	C10	C4	G11	G6	G7	MH1	MH10	MH11	MH12	MH13	MH14	MH15	MH16	MH17	MH18	MH2	MH3	MH4	MH5	MH6	MH7	MH8	MH9	nMH3	nMH5	TD1	TD10	TD2	TD3	TD4	TD5	TD6	TD7	TD8	TD9				
C10	0																																						
C4	0.513	0																																					
G11	0.5861	0.4335	0																																				
G6	0.4607	0.4463	0.4302	0																																			
G7	0.5136	0.313	0.3197	0.1706	0																																		
MH1	0.5269	0.3707	0.41	0.3754	0.3996	0																																	
MH10	0.4804	0.4518	0.5529	0.424	0.5294	0.267	0																																
MH11	0.4951	0.4252	0.4604	0.3792	0.4596	0.1606	0.046	0																															
MH12	0.512	0.3784	0.4634	0.4184	0.4181	0.1205	0.3405	0.2213	0																														
MH13	0.5535	0.406	0.4833	0.3512	0.3965	0.1395	0.2883	0.2133	0.1263	0																													
MH14	0.4868	0.3827	0.5654	0.3931	0.4363	0.1706	0.3085	0.2581	0.1971	0.0993	0																												
MH15	0.5088	0.3796	0.5037	0.4033	0.4072	0.1603	0.3088	0.2647	0.1437	0.1651	0.1201	0																											
MH16	0.5448	0.3827	0.483	0.4501	0.4812	0.1963	0.2651	0.2491	0.2526	0.2079	0.2716	0.3115	0																										
MH17	0.5181	0.3735	0.4832	0.4176	0.465	0.1645	0.2224	0.2346	0.161	0.1489	0.2126	0.1873	0.2496	0																									
MH18	0.5746	0.3467	0.4025	0.3976	0.3632	0.1194	0.3147	0.2047	0.1976	0.1072	0.1831	0.1712	0.2619	0.1487	0																								
MH2	0.5737	0.3233	0.5173	0.4029	0.3954	0.2057	0.3304	0.2529	0.17	0.2069	0.1649	0.2237	0.313	0.305	0.196	0																							
MH3	0.5117	0.3348	0.4521	0.4175	0.4317	0.0536	0.3119	0.1758	0.0791	0.1416	0.1293	0.1625	0.2194	0.159	0.1218	0.1521	0																						
MH4	0.5673	0.383	0.4192	0.4159	0.4155	0.0166	0.3509	0.2228	0.1882	0.1872	0.2204	0.208	0.2151	0.1871	0.1673	0.2265	0.1179	0																					
MH5	0.4367	0.4452	0.5494	0.3625	0.4896	0.2031	0.3357	0.2349	0.1802	0.1638	0.184	0.2675	0.2832	0.2108	0.2246	0.2626	0.1935	0.2653	0																				
MH6	0.5904	0.3472	0.4813	0.3837	0.4319	0.1438	0.2947	0.2487	0.1726	0.0948	0.126	0.2462	0.1456	0.2212	0.1837	0.1933	0.1452	0.1719	0.2196	0																			
MH7	0.6102	0.4283	0.5377	0.4002	0.4878	0.2183	0.3865	0.2391	0.2425	0.2367	0.2506	0.287	0.2156	0.3413	0.3083	0.2836	0.2335	0.2609	0.2315	0.176	0																		
MH8	0.4737	0.3365	0.4304	0.3863	0.401	0.0973	0.2791	0.2033	0.0884	0.1688	0.1151	0.1283	0.2698	0.1829	0.131	0.1322	0.056	0.1595	0.2106	0.1976	0.286	0																	
MH9	0.5089	0.3561	0.4815	0.3599	0.4507	0.1314	0.2116	0.1876	0.1707	0.1586	0.2005	0.2622	0.1953	0.1454	0.1825	0.2813	0.1392	0.2008	0.2108	0.1215	0.293	0.1613	0																
nMH5	0.5738	0.3454	0.5222	0.454	0.4579	0.2255	0.3549	0.3079	0.2648	0.231	0.2947	0.2911	0.3494	0.2451	0.1679	0.2309	0.2277	0.2732	0.2953	0.2852	0.3698	0.2555	0.2415	0															
nMH3	0.5662	0.3302	0.4469	0.4176	0.4216	0.1676	0.3498	0.172	0.1666	0.1734	0.2579	0.1895	0.2971	0.1704	0.135	0.286	0.1662	0.2153	0.2814	0.2534	0.3049	0.179	0.203	0.1679	0														
TD1	0.5904	0.4108	0.4364	0.4184	0.3773	0.4462	0.3941	0.3901	0.3552	0.3733	0.3376	0.3865	0.4316	0.4089	0.3495	0.4107	0.396	0.397	0.3334	0.3531	0.4104	0.4442	0.3905	0.4237	0														
TD10	0.5556	0.3989	0.4301	0.3077	0.4255	0.4297	0.4532	0.4536	0.4184	0.4331	0.4687	0.53012	0.3083	0.4151	0.4734	0.4984	0.4489	0.3892	0.4716	0.368	0.3806	0.4873	0.4067	0.5464	0.4772	0.3672	0												
TD2	0.6089	0.3949	0.4323	0.3107	0.4115	0.3628	0.4286	0.3535	0.3322	0.3302	0.4157	0.3627	0.3737	0.3617	0.3271	0.4227	0.3615	0.4	0.3588	0.4113	0.3222	0.3742	0.4147	0.3566	0.342	0.3038	0.3052	0											
TD3	0.5475	0.4887	0.4629	0.5232	0.4704	0.4192	0.4973	0.4034	0.357	0.4223	0.3949	0.4471	0.4273	0.4474	0.4095	0.4487	0.3744	0.4597	0.4194	0.4557	0.4676	0.3872	0.4335	0.5694	0.4712	0.3725	0.2938	0.3051	0										
TD4	0.6251	0.3657	0.4705	0.4823	0.3983	0.4691	0.5408	0.4904	0.385	0.3851	0.3938	0.4393	0.4177	0.4514	0.421	0.3895	0.4191	0.4483	0.4064	0.3659	0.3156	0.4133	0.4644	0.4759	0.4459	0.3523	0.2838	0.2251	0.3188	0									
TD5	0.607	0.4597	0.429	0.479	0.387	0.3308	0.4141	0.3658	0.3337	0.2665	0.3474	0.3999	0.299	0.3542	0.3241	0.4289	0.3678	0.3535	0.353	0.2908	0.4335	0.3837	0.3457	0.466	0.3721	0.3049	0.258	0.2291	0.2593	0.2491	0								
TD6	0.5022	0.4155	0.45	0.3808	0.3645	0.3232	0.4051	0.3581	0.3127	0.289	0.3047	0.4007	0.365	0.3238	0.3245	0.3676	0.3131	0.3676	0.2952	0.2699	0.4095	0.3128	0.2476	0.4113	0.3827	0.3363	0.3744	0.3672	0.3442	0.282	0.2088	0							
TD7	0.5534	0.4718	0.3984	0.4008	0.4307	0.4153	0.4197	0.4191	0.3847	0.4044	0.4246	0.4684	0.4052	0.41	0.3971	0.4495	0.4139	0.4524	0.407	0.3985	0.4974	0.4049	0.3542	0.4688	0.4588	0.2479	0.3117	0.1852	0.2583	0.3543	0.283	0.2689	0						
TD8	0.5705	0.4865	0.49	0.4978	0.4008	0.4138	0.5384	0.4277	0.3796	0.3679	0.3831	0.4178	0.4198	0.4966	0.4173	0.4336	0.4159	0.4825	0.3781	0.4019	0.3988	0.4232	0.4649	0.4917	0.4554	0.258	0.4514	0.2983	0.274	0.3894	0.2348	0.3177	0.2923	0					
TD9	0.6297	0.4115	0.4465	0.5234	0.4512	0.4478	0.489	0.435	0.3717	0.3631	0.421	0.4797	0.3405	0.4285	0.3605	0.4323	0.4233	0.4074	0.4293	0.3695	0.4085	0.4396	0.4415	0.4704	0.4375	0.3145	0.2034	0.222	0.2561	0.2784	0.219	0.3823	0.2622	0.3611	0				

3). Using this cut-off point, the genotypes in each of the groups had distinct features that distinguished them from one another. According to the results, the largest cluster was group 3 that held 20 genotypes: MH<sub>1</sub>, MH<sub>3</sub>, MH<sub>8</sub>, MH<sub>12</sub>, MH<sub>4</sub>, MH<sub>15</sub>, MH<sub>13</sub>, MH<sub>6</sub>, MH<sub>14</sub>, MH<sub>2</sub>, MH<sub>17</sub>, MH<sub>9</sub>, MH<sub>5</sub>, MH<sub>14</sub>, MH<sub>2</sub>, MH<sub>17</sub>, MH<sub>9</sub>, MH<sub>5</sub>, MH<sub>18</sub>, nMH<sub>5</sub>, nMH<sub>3</sub>, MH<sub>10</sub>, MH<sub>11</sub>, MH<sub>16</sub>, and MH<sub>7</sub>. Furthermore, all genotypes of group 3 (G<sub>3</sub>) belong to Minab, Hormozgan province in Iran. The formation of a single group for the specimens of Minab may be the result of a common evolutionary path for these genotypes. Because limes cover the largest portion of area under cultivation among citrus in this region, gene doping seems to be lower. Moreover, as limes are propagated by seed in this region, the protection of the genotypes would be more likely. In a cluster analysis of the diversity and family relations of some lime genotypes using measured morphological traits.

Group 2 (G<sub>2</sub>) was the second- largest group into population. It included genotypes TD<sub>1</sub>, TD<sub>7</sub>, TD<sub>3</sub>, TD<sub>8</sub>, TD<sub>5</sub>, TD<sub>6</sub>, TD<sub>10</sub>, TD<sub>9</sub>, TD<sub>2</sub>, and TD<sub>4</sub>. As the Table 1 showed, these genotypes were belonging to Darab, Fars Province (in Iran).

Genotypes C<sub>10</sub>, C<sub>4</sub>, G<sub>11</sub>, G<sub>5</sub>, and G<sub>7</sub> were the members of group 1 (G<sub>1</sub>). G<sub>5</sub>, G<sub>7</sub>, and G<sub>11</sub> belonging to Jiroft, Kerman province, and made up of a group together with the Mexican lime (C<sub>10</sub>), and Persian lime (C<sub>4</sub>). According to the results of this study, the morphological grouping of the examined genotypes was considerably consistent with geographical and climatic conditions of their cultivation regions. This fact may point to the distinct evolution path of each of the groups. Zandkarimi *et al.* (2011) evaluated morphological characteristics of some lime and lemon genotypes in Hormozgan province, they found that lime genotypes were different in some of their traits such as shape, size, and rind thickness. The morphological specifications of the groups are provided in Table 3. With regard to the non-parametric Kruskal-Wallis test, which analyzes the difference among genotypes with regard to qualitative variables, the three groups were significantly different in 21 traits.

Group 1 genotypes showed some noticeable morphological characteristics. Their seeds had crinkly surfaces, while the other groups' genotypes had soft-skin seeds. Their seeds were green that was distinct from the creamy color of the seeds in the other groups.

They produced oval-shaped fruits, their laminas had crenate edges, the trees had a vertical and erect growth habit, their wings had a narrow width, they had low spine density, they started flowering in March a month later than the other groups, and their fruits had mammiform tips.

Group 2 members were also characterized in particular ways as well. They had crispy flesh texture, coarse rind texture. They had ellipsoid-shaped laminas with dentate edges. The trees had a hanging growth habit. The bearing trees were oval shape. Their shoot tips were green, while the other groups were green-violet. The branches formed acute angles from the trunk. The majority of the genotypes lacked laminas contrary to the other groups. The glands were not observable, and their fruits' tips were spherical.

Among the noticeable characteristics of group 3 genotypes are as follows: fleshy and juicy fruits, oval and spherical fruits, spreading and expanding growth habit; soft skin texture, oval-stretched laminas with crenate edges, heart-shaped wings, medium spine density, strong segment wall adherence, and thin segment walls. Such information indicates the fact that each group may have some superior breeding value over others with regard to their unique characteristics. For example, group 3 genotypes have fleshy and juicy fruits which are favourite for processing industry. The fruit juice content and acidity are much important than other morphological, quantitative, and qualitative characteristics in determining the lime quality (Shrestha *et al.*, 2012). Further, Group-3 fruits were typically spherical shape which is a more valuable characteristic in processing industries (Ghazvini and Moghadam, 2010; Barta, 2006).

The fruits in group 3 had soft rinds. Thus, they are more market-favored than the other groups with coarse rinds. The angles between the branches and the trunk were medium in group 3 genotypes, which it is the same as group 2 and different to group 1. According to

previous studies, lime trees with smaller branch-trunk angle ratios showed more vegetative growth habit than productive habits. It could be a negative trait for the tree. On the other hand, among the superior characteristics of group 1 and group 2 trees were their lower spine densities than that of group-3 trees. Therefore, the desirable traits of each genotype can be used in breeding programs.

#### **Analysis of dissimilarity matrix of morphological data**

The dissimilarity matrix based on Gower coefficient (Table 4) showed that the genotypes were different in a range between 0.05 and 0.63. The minimum difference was observed between two genotypes of Minab region (MH<sub>3</sub> and MH<sub>1</sub>). As the dendrogram showed MH<sub>3</sub> and MH<sub>1</sub> had the highest level of similarity. On the other hand, C<sub>10</sub> genotype from Minab region and TD<sub>9</sub> genotype, known as local lemon, from Forg, Fars province, had the maximum difference. Such a big difference was not surprising at all if we consider the morphological characteristics of their fruit, seed, flower and leaf. These two genotypes were distinct from each other regarding many traits such as flesh texture, seed shape, seed surface, color of opened flower, anther color, fruit shape, rind texture, fruit's bottom and top shape, lamina and lamina's edge shape. Lamina-pedicel connection, shape of mature tree, color of shoot top, wing status, spine density, fruiting season, gland clarity and length, and many others. According to the results, the Mexican lime had the highest level of morphological similarity to MH<sub>5</sub> genotype locally known as 2-5 seeded fruits.

In group 1 (G<sub>1</sub>), the highest level of difference (0.58) occurred between G<sub>11</sub> and G<sub>10</sub> genotypes, the former belonging to Jiroft, Kerman, and the latter C<sub>10</sub> (Mexican lime) to Minab. On the other hand, the lowest level of difference (0.17) was observed between G<sub>6</sub> and G<sub>7</sub>, both belonging to Jiroft. In group 2 (G<sub>2</sub>), the highest level of difference (0.45) was between TD<sub>10</sub> (curly lime of Farood) and TD<sub>8</sub> (cucumber-shaped lime of Farood).

Such a big difference was due to the high level of morphological difference between the fruits of these two genotypes. Further, the smallest difference (0.2) occurred between TD<sub>5</sub> (No. 8 lemon hybrid) and TD<sub>6</sub> (No. 2 lemon × Bakraee hybrid). Moreover, the maximum difference (0.38) was between MH<sub>7</sub> (5-4 seedy) and MH<sub>10</sub> (13-1 seedy) genotypes in group 3 and the minimum difference (0.17) occurred between MH<sub>3</sub> (2-2 seedy) and MH<sub>1</sub> (6 seedy) genotypes.

Malik *et al.* (2012) found a similarity range of 0.18 to 0.64 in their investigation of the genetic diversity of varieties of orange in India. Likewise, Kumar *et al.* (2010) observed the minimum and the maximum level of calculated morphological similarity in mandarins between 0.17 and 0.45. Pal *et al.* (2013) carried out an investigation on mandarins based on 33 morphological indexes and their result of similarity range was between 0.15 and 0.73. Campos *et al.* (2005) studied the genetic diversity of mandarins using morphological markers with 20 quantitative and 10 qualitative traits and reported a 0.15-0.73 similarity coefficient. The mentioned studies (Pal *et al.*, 2013).

The results of the test of Mexican lime's similarity to the study genotypes showed that the similarity varied from 37% (to genotype TD<sub>6</sub>) up to 56% (to genotype MH<sub>3</sub>), which suggests the noticeable affinity between these genotypes and Mexican lime. As a result, we can infer significant diversity among these genotypes regarding their morphological markers. The existing diversity in the germplasm of the study limes can be useful in breeding programs or the introduction of the desirable variety after the evaluation of tolerant to witch's broom and a thorough analysis of the quantitative and qualitative characteristics of the fruit.

Moreover, because of the adequate diversity among lime genotypes of this study, it is possible to select the proper vegetative material among the groups to promote breeding programs with multiple goals. The change in the morphological traits of the fruit, flower,

seed, and leaf is influenced by genetic factors and environmental conditions. Accordingly, we can expect that the hybrids from G<sub>1</sub>, G<sub>2</sub>, and G<sub>3</sub> prove superior over the existing genotypes concerning the analyzed traits such as trees' spinelessness, fruits' sphericity and juiciness, rind's softness, and so on.

## CONCLUSION

The results of the current study showed that there was a significant morphological difference between 35 selected lime genotypes. The most appropriate dendrogram cut-off point could divide the lime genotypes into three groups. The results of cluster analysis showed that 20 lime genotypes fell into group 3 (G<sub>3</sub>). Furthermore, the test of the dissimilarity matrix based on Gower's coefficient indicated a range of genotypes difference between 0.05 and 0.63. The largest difference was observed between C<sub>10</sub> (Mexican lime) and TD<sub>9</sub> genotype, while the lowest difference was found between two genotypes from Minab region (MH<sub>3</sub> and MH<sub>1</sub>). Therefore, there was a considerable diversity among the studied genotypes based on the morphological traits of fruits. The existence diversity among lime germplasm can be the first step of breeding programs for introducing desirable cultivars which were tolerant to witch's broom disease of lime.

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