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Evaluation of genetic diversity in acid lime (*Citrus aurantifolia* Swingle) genotypes using AFLP markers

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Abstract

Citrus is one of the most important crops of sub-tropical regions worldwide, including acid lime (*Citrus aurantifolia*). This includes a large group of citrus family. AFLP analysis was done using four primer combinations to monitor genetic relationships between 30 local genotypes and six foreign cultivars. AFLP data analysis revealed the presence of 126 scorable bands which revealed % 69.84 polymorphism. The number of amplified bands for each primer combination was recorded as 26-37 (with an average of 22 bands for each primer combination). The measured polymorphic information content (PIC) had an average of 0.48. The range of similarity was between 0.24 and 0.96. Minimum similarity was found between sweet lime (*Citrus limetta*) and D8 genotype, while the maximum observed between two genotypes of Minaab region. Samples were not separated using cluster analysis regionally. Instead, samples of Minaab region revealed high level of genetic similarity with one another, in comparison to samples of Manoojan and Darab regions. This is due to limited citrus varieties throughout the region. Using these markers, remarkable genetic diversities between genotypes of acid lime were found. Results showed that Iranian acid lime genotypes have high level of genetic diversity because they are sexually propagated.

Keywords: *Citrus*; Primer Combination; Polymorphic Information Content (PIC); Cluster Analysis; genetic similarity; Molecular Markers.
Abbreviations: AFLP_amplified fragment length polymorphism; ISSR_inter simple sequence repeat; PIC_polymorphism information content; RAPD_random amplified polymorphic DNA; RFLP_restriction fragment length polymorphism; SNP_single nucleotide polymorphism; SSR_simple sequence repeat; UPGMA_unweighted pair group method with arithmetic average.

Introduction

Citrus, which includes some of the most important fruits worldwide, belongs to the family Rutaceae. This family contains 140 genera and 1300 species. It is a long-lived perennial crop and is grown in more than 100 countries (Singh and Rajam, 2009). In Iran, citrus has an economic role in horticultural industry. The weather conditions in Iran, particularly in the southern provinces, represent a suitable condition for citrus production, especially acid lime (*Citrus aurantifolia*, Swingle).

Citrus plants are normally diploid, with the chromosome of 2n=2x=18. Flowers are the natural hybrids produced through cross pollination. In some cases, bud mutation is occurred leading to diversity in morphological characters in different branches of a very plant (Nicolosi et al., 2000). Limes hybridize freely with other citrus species, like lime and lemon or lime and kumquat (Scora, 1975), or a tri-hybrid species of citron, pummelo and micro citrus (Barret and Rohds, 1976). Issues like breeding and conservation of genetic resources require assessment of genetic diversity in plant species. Having knowledge of genetic diversity is crucial for gaining maximum relative benefits in breeding programs from germplasms (Vinu et al., 2013).

Genetic diversity within and among different populations or argo-ecological regions can be assessed using morphological, biochemical and molecular approaches (Vinu et al., 2013). In scientific researches, almost all citrus cultivars and related genera have been investigated (Fang et al. 1997; Campos et al., 2005; EL-Mouei et al. 2011; Golein et al., 2012; Al-Sadi et al., 2012; Nematolahi et al., 2013; Pal et al. 2013; Al-Anbari et al., 2014; Sharma et al., 2015). However, genetic relationships in acid lime were evaluated in only a few studies (Robles- Gonzalez et al. 2008; Al-Sadi et al., 2012; Munankarmi et al., 2014). Therefore, there would be a lack of comprehensive knowledge concerning genetic diversity of acid limes in the body of scientific studies.

Various molecular markers, including isozymes (Fang et al. 1997), RFLP (Fang et al. 1997), ISSR (Fang et al. 1997), RAPD (EL-Mouei et al. 2011; Al-Anbari et al., 2014), SSR (EL-Mouei et al., 2011; Golein et al., 2012; Nematolahi et al., 2013; Sharma et al., 2015) and AFLP (Pang et al. 2007; Nartvaranant and Nartvaranant, 2011; Al-Sadi et al., 2012), have been employed to evaluate genetic diversity of *Citrus* and related genera. AFLP method had not been tried in the phylogenetic analysis of citrus family until 2007 (Pang et al. 2007). This method has been demonstrated to be a powerful technique in estimating genetic diversity and phylogenetic relationships in population of *Citrus* and related genera (Pang et al. 2007; Robles- Gonzalez et al. 2008; Nartvaranant and Nartvaranant, 2011; Al-Sadi et al., 2012).

The present study tried to estimate genetic diversity in acid lime. The objectives of the present investigation were as follows: (1) to characterize genetic diversity within acid

limes in Iran using AFLP method, (2) to manifest genetic differentiation of acid limes in different regions of Iran, and, (3) to show relations of acid limes in Iran to other acid lime cultivars.

Results

AFLP primer combinations

In the present investigation, nine primer combinations of AFLP were tested on six random samples. Then, four primer combinations, including ECGC/MAGA, ECCA/MAGA, ECCA/MAGT and CGC/MAAG, were chosen, due to both higher percentage of polymorphic fragment and polymorphic information content (PIC). The genetic diversity in 36 acid lime samples was investigated. Table 2 shows the name of primer combinations, total number of fragments, number of polymorphic fragments, polymorphic percent and PIC for each combination. Totally, 126 fragments were amplified, among which 88 (69.84%) were polymorphic. Number of amplified fragments for each combination was 26 to 37, with an average of 22 for any combination. Maximum and minimum numbers of fragments were observed in C3 and C2 combinations, which are 37 and 26, respectively. Furthermore, maximum polymorphic percent was found in C1 with 75%, and minimum in C4 with 64.52%.

Phylogenetic relationships among genotypes

It was found in the similarity matrix that minimum similarity was between sweet lime cultivar (S55) and one of Darab genotypes (D8) (0.24%). Also, two genotypes from Sandark village of Minaab showed maximum similarity (96%). Jaccard similarity coefficients matrix among 36 acid lime samples are shown in Table 3. According to the calculated similarity, three lime populations of this study did not show high levels of genetic distances, when compared to four lime and lemon cultivars (Mexican lime, Persian lime, Rough lemon and Lisbon lemon). Averages of similarity for these four cultivars were 0.57, 0.54, 0.48 and 0.48, respectively.

At first, for cluster analysis, three different similarity coefficients (Jaccard, Dice and Simple matching) and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithms were used. Moreover, the correlation coefficient (r), based on Mantel Z-statistics, was done. It showed that Jaccard coefficient had the highest correlation coefficient (0.92). Similarity on dendrogram ranged from 0.46 to 0.96, and two Minaab genotypes have shown the higher similarity. In dendrogram (Fig. 1), samples were classified into four main groups, at 0.56 percent of similarity. It is clear from Fig 1 that dendrogram was based on Jaccard coefficient and UPGMA algorithm method.

At 0.46 level of similarity, the first group was separated from other samples. This included just sweet lime (S55). Second group was generated at 48.8%. This group contained three Darab genotypes (D8, D9 and D11). The third main group was found at 53.3% of similarity. All standard samples that used in the present study (Mexican lime, Persian lime, Rough lemon and Lisbon lemon) were placed within this group. The fourth group was the largest cluster covering 27 genotypes. This group was divided into two sub-clusters (4A and 4B). Sub-cluster 4A covered most of Darab and Manojan genotypes plus three genotypes of Minaab. Three Minaab genotypes (M1-1, M1-5 and M5-1) plus A8 (genotype of Manojan) showed high levels of similarity. Sub-cluster 4B covered all samples of Minaab in addition to two genotypes from Darab (D1) and Manojan (A7). These two last genotypes (D1 and A7), especially D1, were different from other members of the sub-cluster.

Discussion

Molecular markers are powerful and suitable tools for estimating genetic diversity, determining different parentages, and revealing phylogenetic relationships among various citrus genotypes (Sharma et al., 2015). To conserve and utilize genetic resources, assessment of genetic diversity is essential. Evaluation of genetic diversity was used for selection and monitoring of genetic resources, and helped to predict the possible capability of the genotypes in breeding (Chakravarthi and Naravaneni, 2006). One of the PCR-based markers is amplified fragment length polymorphism (AFLP), which rapidly generates hundreds of highly replicable bands from DNA of any organism (Mueller and Wolfenbarger, 1999). The time and cost efficiency, replicability and resolution of AFLPs are superior or equal to those of other markers (Mueller and Wolfenbarger, 1999). Four primer combinations used for assessment of genetic diversity among 30 genotypes of acid lime. Six standard cultivars produced 126 bands, of which 69.84 present showed polymorphic pattern. Total polymorphic information content was calculated (0.48). This amount of PIC showed efficiency of primers in distinguishing genetic differences between samples (Roldain-Ruiz, 2000). In the previous studies (Pang et al. 2007), it was found that the measured polymorphic percentage was lower than the percentage of phylogenetic relationships within *Citrus* and its related genera; but, in comparing to one species of *Citrus*, this amount of polymorphic was proportionally high. All samples in the genetic analysis of pummelo cultivars [*Citrus maxima* (Burm.) Merrill] were belonged to one species (Nartvaranant and Nartvaranan, 2011). In another investigation by Pang et al. (2007), six AFLP combination primers were used to understand phylogenetic relationships within *Citrus* and its related genera (95.32%). It was found that the applied primer combinations were suitable and efficient in investigating genetic diversity in the given samples, according to polymorphic percentage and polymorphic information content.

The results of cophenetic test (Mantel, 1967) revealed that Jaccard coefficient method and UPGMA algorithm were the best tools for designing dendrogram. Calculated cophenetic coefficient has shown that % 92 of data in the similarity matrix is presented in dendrogram. In the similarity matrix of Jaccard coefficient, calculated similarities were in range of 0.24 to 0.96. Shrestha et al. (2011) and Munankarmi et al. (2014) reported same range of similarity in limes. Al-Sadi et al. (2012) found different results. They identified two reasons for low genetic diversity in Oman. First, all cultivated acid limes have been introduced into Oman from a common source. The second reason was the low level of genetic diversities of acid lime in Oman.

Genetic data based on cluster analysis revealed that S55 was the most distinct sample. According to morphological characters and previous genetics investigations (Al-Anbari et al., 2014), this kind of grouping was predictable. Shahsavar et al. (2007), when studied Fars province lime genotypes, used genotype D8 (Cucumber lime). They reported that this genotype disported from other samples. Thus, it was found that the generation of second main group, containing three

Table 1. Acid lime samples used in AFLP analysis.

Plant code	Scientific name	Location	Plant code	Scientific name	Location
D1	*Citrus* sp.	Darab	M1-5	*Citrus* sp.	Minab
D2	*Citrus* sp.	Darab	M1-7	*Citrus* sp.	Minab
D3	Citrus sp.	Darab	M1-10	Citrus sp.	Minab
D4	*Citrus* sp.	Darab	MI1-13	*Citrus* sp.	Minab
D5	*Citrus* sp.	Darab	M1-14	*Citrus* sp.	Minab
D6	*Citrus* sp.	Darab	M2-2	*Citrus* sp.	Minab
D7	*Citrus* sp.	Darab	M2-8	*Citrus* sp.	Minab
D8	*Citrus* sp.	Darab	M2-10	*Citrus* sp.	Minab
D9	*Citrus* sp.	Darab	M4-2	*Citrus* sp.	Minab
A5	*Citrus* sp.	Manojan	M4-5	*Citrus* sp.	Minab
A6	*Citrus* sp.	Manojan	M5-2	*Citrus* sp.	Minab
A7	*Citrus* sp.	Manojan	M1-5	*Citrus* sp.	Minab
A8	*Citrus* sp.	Manojan	S37	*Citrus aurantifolia*	Ramsar
A9	*Citrus* sp.	Manojan	S46	*Citrus* × *limon*	Ramsar
A10	*Citrus* sp.	Manojan	S49	*Citrus jambhiri*	Ramsar
M1-1	*Citrus* sp.	Minab	S51	*Citrus* × *latifolia*	Ramsar
M1-3	*Citrus* sp.	Minab	S54	*C. medica*	Ramsar
M1-4	*Citrus* sp.	Minab	S55	*Citrus limetta*	Ramsar

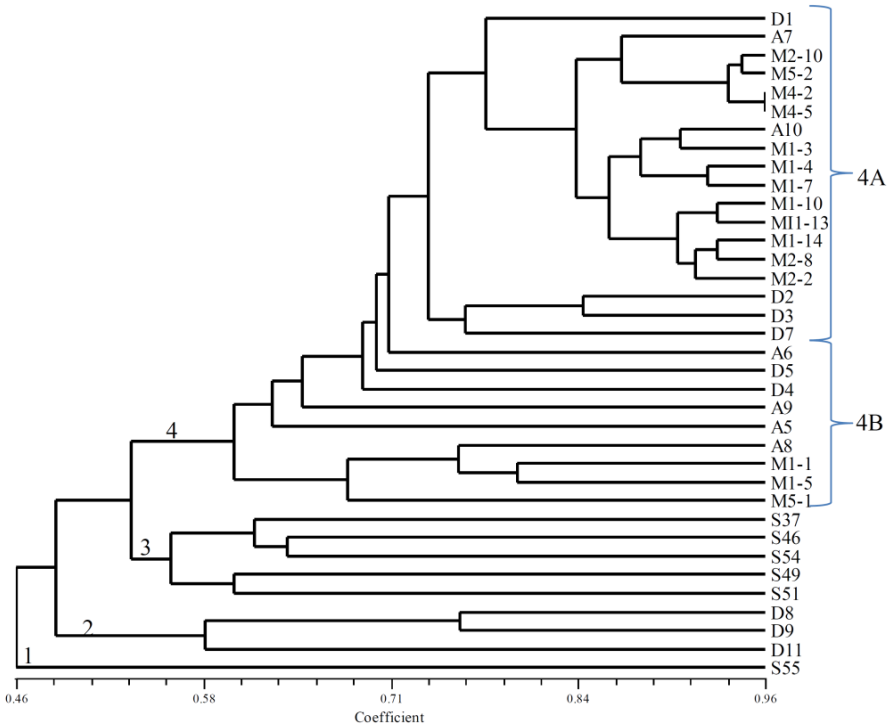

Fig 1. Dendrogram of 36 acid lime genotypes using AFLP markers based on UPGMA.

Darab genotypes (D8, D9 and D11), is correct. Presence of four standard cultivars (Mexican lime, Persian lime, Rough lemon and Lisbon lemon) in the given group could be predicted in Iran, because limes are propagated by seeds. Persian lime was not the common cultivar in the given places, and gene flow was not occurred from them to other germplasms. High similarity levels were reported for citron and lemon (Al-Anbari et al., 2014, Shahsavar et al., 2007). Same classification was found in cluster analyses of four standard samples (Mexican lime, Persian lime, Rough lemon and Lisbon lemon). However, it seems that placing Mexican lime in this group was wrong. It should be considered that many different factors such as frequent occurrence of hybridization, apomixes, polyploidy and bud mutations could legitimatize this kind of classification (Kumar et al., 2010).

Twenty seven genotypes were placed in the fourth main group divided into two sub-clusters. One (sub-cluster 4A) showed high level of differences among members. Differences in this sub-cluster were high because in these regions (Darab and Manojan), other species of *Citrus* (such as Mandarin, sweet orange and grape fruit) are cultivated. In the second sub-cluster (4B) that covered most of Minaab genotypes and two genotypes from Darab (D1) and Manojan (A7), similarity has been in a very high level, so, it could be comprehended that these genotypes may have the same parents, and because of propagation by seed through years,

Table 2. AFLP primer combinations used in genetic diversity experiments with 36 citrus samples and their statistics Information.

Combination code	Primer Combination	Total No. of fragments	No. of polymorphic fragment	Polymorphic percent	Polymorphic Information Content (PIC)
C1	E_{CGC}-M_{AGA}	32	24	75	0.48
C2	E_{CCA}-M_{AGA}	26	17	65.38	0.40
C3	E_{CCA}-M_{AGT}	37	27	72.97	0.48
C4	E_{CGC}-M_{AAG}	31	20	64.52	0.50
Total	-	126	88	69.84	0.48

they differ from each other. Therefore, for many years, Mexican lime, as the most important citrus product, was cultivated in these regions. So, there is the possibility that it was the parent of these genotypes, especially when it was found that the similarity of those genotypes and Mexican lime is about 0.57. Obviously, more research is needed to substantiate this assertion.

Generally, several factors involved in the estimation of genetic relationships between samples, including number of markers used in the study, distribution of markers throughout genome, and mechanism of evaluating samples (Powell et al., 1996). About the number of markers, it is believed that utilizing a high number of markers is more reliable. However, Ellis et al., (1997) reported that 80% of genomic relationship was covered with six primer combinations. In the present study, four AFLP primer combinations were applied. According to previous surveys, four primer combinations utilized in this investigation are suitable. Campus et al., (2005) used two AFLP primer combinations in Mandarin, and Al-Sadi et al., (2012) utilized four combinations reliably. Results of present investigation suggested a high level of genetic diversity between Iranian acid lime genotypes. The main reason of this amount of diversity is the method of propagation. In contrast, Al-Sadi et al., (2012) found low level of genetic diversity in acid lime in Oman.

The results of this investigation are expected to be used by researchers to design breeding program for special aim such as resistance to disease and tolerance to abiotic stress like drought and salinity by estimating suitable crossing.

Materials and methods

Plant materials and DNA extraction

Fresh young leaf samples were taken from 30 acid lime genotypes collected from various locations of the south and central regions of Iran. Six species, Mexican lime (C. auranifolia), Persian lime (*Citrus* × *latifolia*), Sweet lime (*C. limetta*), Citron (*C. medica*), Lisbon lemon (*C. limon*) and Rough lemon (*Citrus jambhiri*), from Citrus Research Institute of Iran in Ramsar city (north of Iran), were used as standard samples. The leaves stored at -80 ºC until being used for DNA extraction (Table 1). Total DNA was extracted according to protocol introduced by the Diversity Arrays Technology Pty Ltd (DArT P/L) company (Diversityarrays, 2007).

AFLP analyses

AFLP analysis was conducted as described by Vos et al., (1995) with some modifications. Genomic DNA was digested at 37ºC for 180 min using *Eco*RI and *Mse*I enzymes (2 µl of 10X Tango buffer, 2.5 U *Eco*RI; 2.5 U *Mse*I, ~300 ng of genomic DNA, and dH2O up to a volume of 20 µl). A 5 µl ligation mixture (0.5 µl of 10X Tango buffer, 2.5 pmol *Eco*RI adaptor (5´-CTCGTAGACTGCGTACC/ AATTGGTACGCAGTCTAC-3´), 25 pmol *Mse*I adaptor (5´-GACGATGAGTCCTGAG/TACTCAGGACTCAT-3´), 1 U T4 DNA ligase and 50 mM of ATP-Sodium salt and dH_2O (up to a volume of 5 µl) was added to the digested DNA and incubated for 60 min at 37 ºC and then by 120 min at 20ºC. DNA template was prepared by diluting ligation product with 10X dH_2O and 3µl of the resulting digestion-ligation mixture. DNA template (6µl) was used for PCR pre-amplification by adding 0.5mM of each primer, 2.5µl of 10X Taq buffer (containing $MgCl_2$), 200µM of each four dNTPs, and 1 unit of Taq DNA polymerase, in a final volume of 25µl. PCR conditions consisted of 94°C for 3 min; 35 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 1 min; and one final cycle of 72°C for 5 min. The pre-selective amplification product was diluted by adding 50 µl of dH_2O to the 5 µl of it. These mixtures were used as DNA template in a selective amplification. This amplification was performed in DNA template (diluted pre-selective amplification) 6 µl, 0.5 mM of each primers, 2.5µl of 10X Taq buffer (containing $MgCl_2$), 200 µM of each four dNTPs, and 1 units of Taq DNA polymerase, in a final volume of 25µl. PCR conditions consisted of 94°C for 3 min; 10 cycles of 94°C for 30s, 60°C for 30s, and 72°C for 1 min (the annealing temperature was reduced every cycle by 1°C to 56°C); 26cycles of 94°C for 30s, 56°C for 30s, and 72°C for 1 min; and one final cycle of 72°C for 5 min. PCR products were run on a 6%-denaturing polyacrylamide gel and visualized by silver staining. AFLP fingerprinting was first performed on five acid lime random sub-samples using nine primer pair combinations. Out of these, four selective primer pair combinations, which produced the highest number of polymorphic fragments, were chosen for analysis of the entire samples (Table 1).

Data analysis

AFLP data were scored as present (1) or absent (0) for every amplified locus within the size range of 50–1000 base pairs (bp). Genetic similarity based on AFLP data was calculated by making a pairwise comparison between all samples according to the Jaccard coefficient using the Simqual module of NTSYS-pc software version 2.01 (Rohlf 1998). Similarity matrix was compared using the Mantel matrix-correspondence test using MxComp module in NTSYS-pc ver 2.1 (Rohlf 2000). Then, the UPGMA cluster analysis was performed. The level of Polymorphic Information Content was calculated as PICi = 2fi(1 - fi), where PICi is the PIC of marker i, fi the frequency of ith marker fragment when present and 1 - fi is the frequency of *ith* marker when absent (Roldain-Ruiz, 2000).

References

Al-Anbari A, Kanawapee N, Al-Kazragi TA, Al-Jewari H, Al.Mashhadani A, Barusrux S, Pornpongrungrueng P,

Table 3. Jaccard similarity coefficients matrix among 36 acid lime samples.

	D1	D2	D3	D4	D5	D7	D8	D9	D11	A5	A6	A7	A8	A9	A10	M1-1	M1-3	M1-4	M1-5	M1-7
D1	1.00																			
D2	0.81	1.00																		
D3	0.72	0.84	1.00																	
D4	0.69	0.73	0.70	1.00																
D5	0.70	0.74	0.71	0.59	1.00															
D7	0.67	0.78	0.75	0.68	0.63	1.00														
D8	0.53	0.58	0.53	0.47	0.44	0.59	1.00													
D9	0.61	0.61	0.59	0.49	0.49	0.58	0.76	1.00												
D11	0.49	0.51	0.47	0.44	0.39	0.54	0.57	0.60	1.00											
A5	0.66	0.73	0.67	0.56	0.67	0.60	0.54	0.67	0.45	1.00										
A6	0.67	0.62	0.68	0.56	0.68	0.60	0.38	0.47	0.42	0.61	1.00									
A7	0.77	0.69	0.67	0.64	0.70	0.64	0.53	0.55	0.49	0.60	0.72	1.00								
A8	0.56	0.56	0.57	0.52	0.50	0.53	0.52	0.43	0.37	0.46	0.52	0.65	1.00							
A9	0.60	0.63	0.67	0.59	0.57	0.58	0.45	0.42	0.37	0.51	0.56	0.66	0.73	1.00						
A10	0.82	0.85	0.81	0.74	0.72	0.79	0.55	0.57	0.51	0.68	0.74	0.82	0.71	0.75	1.00					
M1-1	0.58	0.55	0.50	0.52	0.47	0.44	0.55	0.45	0.39	0.43	0.42	0.58	0.74	0.64	0.60	1.00				
M1-3	0.75	0.76	0.73	0.73	0.65	0.70	0.47	0.50	0.44	0.60	0.67	0.75	0.72	0.76	0.91	0.67	1.00			
M1-4	0.79	0.88	0.78	0.77	0.69	0.75	0.57	0.60	0.53	0.70	0.66	0.73	0.60	0.65	0.89	0.62	0.87	1.00		
M1-5	0.64	0.64	0.59	0.60	0.55	0.53	0.59	0.49	0.43	0.52	0.52	0.64	0.77	0.61	0.72	0.80	0.70	0.75	1.00	
M1-7	0.74	0.81	0.79	0.77	0.70	0.76	0.53	0.55	0.49	0.66	0.72	0.80	0.62	0.66	0.89	0.58	0.87	0.92	0.70	1.00
M1-10	0.75	0.76	0.73	0.73	0.71	0.71	0.49	0.52	0.48	0.61	0.73	0.80	0.63	0.67	0.89	0.59	0.88	0.86	0.74	0.93
MI1-13	0.74	0.75	0.70	0.70	0.73	0.67	0.48	0.51	0.45	0.63	0.70	0.77	0.60	0.67	0.86	0.59	0.84	0.86	0.73	0.89
M1-14	0.70	0.71	0.69	0.69	0.67	0.66	0.45	0.48	0.42	0.62	0.74	0.76	0.59	0.66	0.84	0.55	0.83	0.81	0.69	0.88
M2-2	0.75	0.73	0.71	0.68	0.71	0.66	0.50	0.52	0.47	0.65	0.76	0.78	0.58	0.62	0.83	0.60	0.82	0.83	0.71	0.87
M2-8	0.76	0.77	0.75	0.69	0.72	0.72	0.50	0.53	0.47	0.68	0.79	0.82	0.64	0.68	0.91	0.55	0.83	0.81	0.69	0.88
M2-10	0.82	0.74	0.72	0.69	0.72	0.72	0.53	0.55	0.49	0.60	0.71	0.85	0.61	0.66	0.88	0.60	0.80	0.78	0.66	0.85
M4-2	0.80	0.75	0.72	0.67	0.70	0.70	0.53	0.55	0.49	0.60	0.75	0.86	0.62	0.66	0.89	0.61	0.81	0.79	0.64	0.86
M4-5	0.80	0.75	0.73	0.67	0.70	0.70	0.54	0.56	0.50	0.61	0.75	0.86	0.63	0.67	0.89	0.59	0.81	0.79	0.67	0.86
M5-1	0.67	0.56	0.52	0.51	0.56	0.48	0.63	0.53	0.46	0.50	0.53	0.67	0.63	0.50	0.64	0.65	0.57	0.60	0.76	0.62
M5-2	0.86	0.76	0.73	0.70	0.73	0.74	0.54	0.57	0.51	0.61	0.75	0.90	0.63	0.67	0.89	0.59	0.81	0.80	0.65	0.86
S37	0.62	0.62	0.62	0.60	0.56	0.59	0.36	0.41	0.38	0.58	0.60	0.59	0.44	0.53	0.66	0.39	0.62	0.63	0.45	0.67
S46	0.53	0.55	0.56	0.52	0.55	0.56	0.40	0.41	0.36	0.57	0.50	0.56	0.47	0.49	0.63	0.39	0.59	0.60	0.47	0.64
S49	0.57	0.57	0.58	0.54	0.47	0.63	0.33	0.36	0.42	0.51	0.61	0.55	0.41	0.48	0.64	0.36	0.60	0.61	0.42	0.62
S51	0.48	0.56	0.54	0.45	0.45	0.59	0.35	0.38	0.38	0.46	0.45	0.48	0.36	0.39	0.58	0.29	0.53	0.54	0.37	0.56
S54	0.48	0.47	0.50	0.45	0.42	0.46	0.29	0.32	0.34	0.46	0.49	0.48	0.43	0.40	0.54	0.35	0.53	0.51	0.41	0.55
S55	0.49	0.48	0.47	0.40	0.44	0.41	0.24	0.27	0.30	0.38	0.51	0.49	0.43	0.56	0.53	0.39	0.54	0.48	0.38	0.49

Table 3 Continued. Jaccard similarity coefficients matrix among 36 acid lime samples.

	M1-10	MI1-13	M1-14	M2-2	M2-8	M2-10	M4-2	M4-5	M5-1	M5-2	S37	S46	S49	S51	S54	S55
M1-10	1.00															
MI1-13	0.93	1.00														
M1-14	0.92	0.91	1.00													
M2-2	0.90	0.90	0.92	1.00												
M2-8	0.92	0.88	0.93	0.92	1.00											
M2-10	0.88	0.85	0.84	0.85	0.90	1.00										
M4-2	0.86	0.83	0.82	0.84	0.88	0.95	1.00									
M4-5	0.90	0.86	0.85	0.87	0.91	0.95	0.96	1.00								
M5-1	0.66	0.65	0.61	0.66	0.67	0.72	0.67	0.71	1.00							
M5-2	0.87	0.84	0.82	0.84	0.88	0.95	0.93	0.93	0.74	1.00						
S37	0.63	0.62	0.64	0.61	0.66	0.64	0.62	0.62	0.45	0.65	1.00					
S46	0.60	0.62	0.61	0.58	0.61	0.61	0.58	0.59	0.51	0.62	0.62	1.00				
S49	0.58	0.55	0.59	0.59	0.65	0.62	0.60	0.58	0.41	0.63	0.61	0.54	1.00			
S51	0.52	0.49	0.53	0.51	0.56	0.51	0.51	0.52	0.34	0.52	0.57	0.53	0.60	1.00		
S54	0.52	0.48	0.52	0.50	0.55	0.52	0.50	0.51	0.42	0.54	0.61	0.64	0.54	0.58	1.00	
S55	0.53	0.52	0.52	0.49	0.52	0.52	0.54	0.52	0.33	0.51	0.55	0.41	0.43	0.40	0.50	1.00

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