DOI : 10.5958/0974-0112.2022.00055.X

Indian J. Hortic. 79(4), December 2022: 394-401

Characterization of acid lime genotypes using SSR markers

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ABSTRACT

The determination of genetic variation at morphological and molecular level is of fundamental importance to the citrus breeders for the development of elite cultivars with desirable traits. This paper aimed to study the genetic diversity among seventy acid lime landraces using simple sequence repeat (SSR) markers. The results revealed a wide range of diversity in 70 genotypes in terms of fruit weight (30.50-56.26 g), juice (29.01-57.13%), acidity (6.18-8.35 %), and ascorbic acid (22.22-36.81 mg/100 ml juice). Twenty-five SSR markers were used to detect polymorphism among these genotypes. A total of 99 alleles were detected with an average of 4.71 alleles per locus. Polymorphism information content ranged between 0.41 (TAA15) to 0.93 (CT19) with an average of 0.73 and polymorphism ranged from 33.33 to 66.67 % with an average of 50.95 %. The markers were able to distributed the genotypes into two major clusters. The Jaccard's genetics similarity value varied from 0.08 to 0.60. Highest genetic similarity index was observed between JMU-Sum (58) and JMU-Sun (61) (0.60). However, least similarity index was observed between JMU-Jib (36) and JMU-Balli (30) (0.08). Out of all the selected genotypes, JMU-Nag (70) and JMU-Jib (36) were found most promising genotypes, and can be suggested for use in further breeding programmes.

Key words: *Citrus aurantifolia* Swingle, genetic diversity, SSR markers, polymorphism, primers.

INTRODUCTION

Acid lime (*Citrus aurantifolia* Swingle) accounts for 20 per cent of total citrus production (Ghosh *et al.,* 8) in India. In Jammu, lime accounts for an area of 4.97 thousand ha with the production of 12.74 thousand MT (Anon, 2). Though, a huge diversity of lime exists in Jammu region, however, to date, the genetic resources for acid lime have not been well characterized. The success of any crop improvement programme mostly depends on the nature and magnitude of genetic variability present in the crop, and extent to which the desirable characters are heritable.

To explore the possibility of new lime cultivar development, the genetic diversity analysis of available germplasm is an initial step for future variety management and development purposes. Morphological study is an essential component for the assessment of diversity and classification. Since morphological characters are influenced by environmental factors and they are only of limited use, alternate approaches, including application of appropriate molecular markers have now been increasingly adopted to address the problems in citrus taxonomy and genetics. Genetic diversity assessment in plants has now become far more simple, cost effective, reliable and reproducible; thanks to the introduction of PCR-based DNA marker techniques (RFLP and RAPD) that have been used to study the genetic diversity, taxonomy, cultivar identification (Novelli *et al*., 12) in various citrus species and simple sequence repeat (SSR) markers (Barkley *et al*., 4) to identify *Citrus* species with high accuracy. In general, SSR markers, due to their codominant nature and abundance in genome, are a good indicator for cultivar fingerprinting and hybrid prediction in orange cultivars (Shahnazari *et al.,* 14). The main purpose of this research is to study the qualitative characters, and to determine the genetic diversity of seventy acid lime landraces, and to select highly variable genotypes for breeding and variety development purposes, using SSR markers.

MATERIALS AND METHODS

The present research was carried out during the year 2017 to 2019. The survey was done from major lime growing districts *viz*., Jammu, Samba, Kathua, Udhampur and Reasi of Jammu region up to 755 m a msl (Table 1) to select promising accession among the diverse lime genotypes and assess variability in their physiological and morphological characteristics. Finally, plants of 70 seedling origin lime genotypes with divergent characters were selected at fruit maturity, and were subjected to analysis of acidity,

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Table 1. Accessions name, geographical location and locality of seventy acid lime genotypes taken into study.

S. No.	Accessions name	Geographical location of selected plant			Locality
		Latitude	Longitude	Elevation	
1.	JMU-Log (1), JMU-Log (2), JMU-Log (3), JMU-Log (4), JMU-Log (5), JMU-Log (6), JMU-Log (7), JMU-Log (8), JMU-Log (9)	32°26.155‟N-32°26.689‟N	075°03.313‟E-075°29.866‟E	451-484m	Logate
2.	JMU-Bar (10), JMU-Bar (11), JMU-Bar (12), JMU-Bar (13)	32°26.466‟N-32°25.907‟N	075°30.082‟E-075°28.727‟E	421-475m	Barwal
3.	JMU-Kat (14), JMU-Kat (15), JMU-Kat (16), JMU-Kat (17)	32°39.509‟N-32°39.754‟N	075°02.533‟E-075°02.674‟E	397-412m	Katwalta
4.	JMU-Uttar (18), JMU-Uttar (19), JMU-Uttar (20), JMU-Uttar (21)	32°39.154‟N-32°38.988‟N	075°03.569‟E-075°03.670‟E	389-405m	Uttarbehani
5.	JMU-Gura (22), JMU-Gura (23), JMU-Gura (24), JMU-Gura (25)	32°35.841‟N-32°35.853‟N	075°02.261‟E-075°02.267‟E	405-407m	Gurhasalathia
6.	JMU-Taror (26)	32°36.238‟N	074°56.934‟E	315m	Tarore
7.	JMU-Balli (27), JMU-Balli (28), JMU-Balli (29), JMU-Balli (30)	32°52.504‟N-32°52.522‟N	075°07.833‟E-075°07.851‟E	628-633m	Balli
8.	JMU-Neeli (31), JMU-Neeli (32), JMU-Neeli (33)	32°52.480‟N-32°52.513‟N	075°07.862‟E-075°07.879‟E	625-634m	Neeli nalla
9.	JMU-Jib (34), JMU-Jib (35), JMU-Jib (36), JMU-Jib (37), JMU-Jib (38)	32°55.093‟N -32°55.195‟N	075°03.248‟E-075°03.313‟E	703-710m	Jib thathi
10.	JMU-Tikri (39), JMU-Tikri (40)	32°57.137‟N-32°57.139‟N	074°58.734‟E-074°58.742‟E	757-759m	Tikri
11.	JMU-Pana(41), JMU-Pana(42), JMU-Pana(43), JMU-Pana (44)	33°03.958‟N-33°03.973‟N	074°48.237‟E-074°48.240‟E	400-401m	Panasa
12.	JMU-Chet(45), JMU-Chet (46), JMU-Chet (47), JMU-Chet (48)	33°02.457‟N-33°04.125‟N	074°35.071‟E-074°45.973‟E	429-716m	Cheater
13.	JMU-Duggi (49)	33°03.998‟N	074°36.943‟E	599m	Duggi
14.	JMU-Lait (50), JMU-Lait (51)	33°03.940‟N-33°04.037‟N	074°36.992‟E-074°36.923‟E	597-608m	Later
15.	JMU-Godd (52), JMU-Godd (53), JMU-Godd (54), JMU-Godd (55), JMU-Godd (56)	33°03.106‟N-33°03.158‟N	074°37.311‟E-074°37.535‟E	615-633m	Godder
16.	JMU-Sum (57), JMU-Sum (58), JMU-Sum (59), JMU-Sum (60)	32°57.430‟N-32°57.672‟N	074°40.462‟E-074°40.495‟E	534-590	Sumah
17.	JMU-Sun (61), JMU-Sun (62), JMU-Sun (63), Sun (64)	32°57.629‟N-32°57.676‟N	074°40.468‟E-074°40.517‟E	586-601m	Sungal
18.	JMU-Nag (65), JMU-Nag (66), JMU-Nag (67), JMU-Nag (68), JMU-Nag (69), JMU-Nag (70)	32°48.235‟N-32°48.284‟N	074°54.684‟E-074°54.806‟E	365-390	Nagrota

ascorbic acid (A.O.A.C. 3), juice per cent by using standard protocols at Division of Fruit Science, and molecular study was done in the School of Biotechnology and Molecular Laboratory, SKUAST-Jammu. Characterization of fruit and seed was based on IPGRI descriptors (IPGRI, 9). The data recorded during the investigation was statistically analysed with the help of INDOSTAT statistical package.

For molecular characterization total genomic DNA was isolated using the modified CTAB (Cetyl trimethyl ammonium bromide) method (Doyle and Doyle, 7). A set of 25 arbitrary highly polymorphic SSR primers were selected from literature published previously (Cristofani *et al.* 5; Shahzadi *et al.,* 15, Shrestha *et al.,* 17) for characterization of lime germplasm (Table 2). These primers were selected based on polymorphic information content (PIC) values. PCR amplification was performed in a 10 µl total reaction volume containing $MgCl_2$ (1.2 µl), dNTPs mix (1.00 µl), PCR buffer (1.00 µl), Forward

Table 2. List of selected SSR primers along with their primer sequence and melting temperature.

S. No.	Name	Forward sequence	Reverse sequence	Tm Calculated (°C)
1	TAA45	GCACCTTTTATACCTGACTCGG	TTCAGCATTTGAGTTGGTTACG	63.6
2	TAA41	AGGTCTACATTGGCATTGTC	ACATGCAGTGCTATAATGAAGT	58.4
3	TAA15	GAAAGGGTTACTTGACCAGGC	CTTCCCAGCTGCACAAGC	64.2
4	TAA3	AGAGAAGAAACATTTGCGGAGC	GAGATGGGACTTGGTTCACACG	66.7
5	CAT01	GCTTTCGATCCCTCCACATA	GATCCCTACAATCCTTGGTCC	63.6
6	CAC15	TAAATCTCCACTCTGCAAAAGC	GATAGGAAGCGTCGTAGACCC	62.9
7	TAA27	GGATGAAAAATGCTCAAAATG	TAGTACCCACAGGGAAGAGAGC	62.2
8	CT19	CGCCAAGCTTACCACTCACTAC	GCCACGATTTGTAGGGGATAG	64.5
9	TC26	CTTCCTCTTGCGGAGTGTTC	GAGGGAAAGCCCTAATCTCA	62.9
10	AG14	AAAGGGAAAGCCCTAATCTCA	CTTCCTCTTGCGGAGTGTTC	63.3
11	GT03	GCCTTCTTGATTTACCGGAC	TGCTCCGAACTTCATCATTG	62.9
12	BQ623065	GGTGTTGTTCTCGCAACAGA	CGGCAGCCTATTGCTACTTC	63.9
13	BQ624307	TTCAAGCCAAAGCAAGAGGT	ACCCAAATGCTCAAAACACC	63.8
14	BQ624796	ACGATGACCAAGAATCCAGC	AAGATCCCACAAGCCATCAC	63.9
15	C24033	GCAGCAATTCTGAAGGAAGG	ACGGCCTCAATGGAACCTAT	64.1
16	C24317	ACTGCTGTTCACCCTGTTCC	GAGAGCTTTCGAGCCTTTGA	63.9
17	CTG1006372	TCAGCACTGAATCCAATCCA	GTGAGAGCTTGAGGCTGACC	64.3
18	TAA33	GGTACTGATAGTACTGCGGCG	GCTAATCGCTACGTCTTCGC	63.6
19	CCSM 3	GCAATGCACCTTGTCATTAG	CATCACAGGCACTTATGCAG	61.3
20	CCSM 9	GACTGGATTAGAGTTCTCTG	ATGGATGTGTTATCTCACTC	53.2
21	CCSM 17	ACATGGACAGGACAACTAAG	GTTATGATACGTCTGTGTCC	55.4
22	CCSM 40	ACAAGAGTCGCAACAATC	GACAACAGTGGCAATACC	56.1
23	CCSM 59	GCAGATATGATGATGATG	ACAACTTCACAATGTTGCAC	54.9
24	CCSM 64	CGCCATTATGGATGATTG	GGTGATTAGATGTGTGAGGA	58.6
25	CCSM 70	GCAAGGAGTTAGTAATGTGG	CTCGTGTGCAAGTTGCAT	58.7

Primer (0.8 µl), Reverse Primer (0.8 µl), template DNA (1.00 µl), Taq DNA polymerase (0.2 µl) and sterile water (4 µl). PCR master mix was thoroughly mixed and subjected to polymerase chain reaction (PCR). The thermal cycling was programmed as initial denaturation cycle at 94°C for of 5 min, followed by a loop of 35 cycles each consisting of denaturation (at 94°C for 1 min), annealing (at 48-62°C for 45s), elongation (at 72°C for 2 min) and the final extension was performed (at 72°C for 4 min).

The PCR products were subjected to electrophoresis on a 3% agarose gel and the gel was stained with 5mg/ml ethidium bromide solution. The amplified fragments were visualized under UV light and photographed using gel documentation system (Minilumi by DNR bio-imaging system, Israel). The size of the PCR products was determined by using 100 bp DNA ladder. The bi-nominal data matrix was recorded and total number of bands obtained, alleles per locus, percentage polymorphism and PIC were estimated. Per cent polymorphism was calculated by dividing the number of polymorphic bands by the total number of scored bands. The variation in the number of alleles across multiple loci within a population were expressed as the average number of alleles per locus.

Polymorphic information content (PIC): The PIC value ranges from '0' (monomorphic) to '1' (highly discriminative with many alleles in equal frequencies). The markers with more alleles have more polymorphism information content. Average PIC indicates the ability of utilized markers to differentiate genotypes. It was calculated according to following formula.

$$PIC\ Value = 2fi\ (1\text{-}fi)$$

Where, fi = frequency of bands present and
1-fi = frequency of bands absent

Bi-nomial data matrix of all the genotypes generated from SSR primers were subjected to the UPGMA (Unweighted pair group method with arithmetic averages) analysis and a dendrogram was constructed using NTSYSpc software package version 2.11a (Rohlf, 13). Genotypes were divided in various clusters and sub-clusters based on genetic diversity among them and linkage distance was calculated.

RESULTS AND DISCUSSION

In the current study the genotypic differences were observed in respect of fruiting season, duration of fruiting season, fruit shapes, and seed colour. Data in Table 3 depict that among the studied acid lime genotypes, fruiting season was found early in 41.43 per cent and midseason in 58.57 per cent genotypes. The spheroid fruit shape was found in 52.86 per cent followed by ellipsoid fruit shape (47.14 per cent). Majority of the genotypes had convex type (74.29 per cent) shape of fruit base followed by truncate (25.71 per cent) (Plate 1). The average number of seeds per fruit varied from 5-9 in 41.43 per cent genotypes and 10-19 in (58.57 per cent) of acid lime genotypes. Clavate seed shape was found in 55.72 per cent genotypes, ovoid in 18.57 per cent and remaining had semi-deltoid (25.71 per cent). The seed colour was white in 18.57 per cent, cream in 44.29 per cent genotypes and green seed colour was observed in 37.14 per cent of acid lime genotypes. JMU-Log (1) and JMU-Godd (56) were observed to be the earliest to mature by 8th August, whereas genotype JMU-Jib (34), JMU-Pana (42), JMU-Pana (43) and JMU-Nag (67) were found to be the last to start fruit maturity with 22nd August. JMU-Log (1), JMU-Log (2), JMU-Godd (56) and JMU-Godd (57) were earliest to end its fruiting season by 15th August, and JMU-Jib(38), JMU-Tikri (39), JMU-Tikri (40), JMU-Pana (44), JMU-Pana(45), JMU-Pana(46), JMU-Pana (47) and JMU-Pana (48) were last to end its fruiting season on 28th August. Fruit shapes assessed in the present study were also supported by the findings of Yadlod *et al.* (19) who described different fruit shapes (oval, ovate, round, ellipsoid, spheroid and spherical) in *Kagzi* limes which were under genetic control.

Table 3. Frequency of fruit characterization of seventy acid lime genotypes.

Fruit characteristics	Category	Number of genotypes	Frequency (%)
Fruiting season	Early	29	41.43
	Midseason	41	58.57
Fruit Shape	Spheroid	37	52.86
	Ellipsoid	33	47.14
Shape of fruit base	Convex	52	74.29
	Truncate	18	25.71
Shape of fruit apex	Mammiform	70	100
	Clavate	39	55.72
	Ovoid	13	18.57
	Semi-deltoid	18	25.71
Seeds per fruit	5-9	29	41.43
	10-19	41	58.57
Seed colour	White	13	18.57
	Cream	31	44.29
	Yellowish	0.00	0.00
	Green	26	37.14

In all the 70 acid lime genotypes studied, high range of variations were recorded for fruit weight (30.50-56.26g), juice (29.01-57.13%), acidity (6.18-8.35%), and ascorbic acid (22.22-36.81 mg / 100 ml juice) (Table 4). Maximum (56.26 g) and minimum fruit weight were recorded in genotype JMU-Nag (70) And JMU-Log (4) genotypes, respectively. Since characters related to fruit size and fruit morphology are the main traits that account towards phenotypic diversity in citrus and citrus related species (Khan *et al.,* 10). The fruits of JMU-Nag (70) proved most acidic (8.35 per cent). Similarly, Abhilash *et al.* (1) recorded the highest titratable acid content in KLS-23 (8.85 %) strains of *Kagzi* lime. Significant variation was observed in ascorbic acid content of fruit in the different genotypes of acid lime. The highest content of ascorbic acid (36.81 mg/100ml juice) content was found in JMU-Nag (70) and lowest in genotype JMU-Log (4) (22.22 mg/100 ml juice). The fruits of JMU-Nag (70) had the highest juice content (57.13 per cent) followed by JMU-Pana (41) (56.70 per cent),

Table 4. Variability present in physico-chemical characters of seventy acid lime genotypes.

Characters	Mean ± SE	Range	Coefficient of variation (%)	CD at 5%
Fruit weight (g)	45.62±1.06	30.50-56.26	4.04	3.93
Juice (%)	44.75±1.13	29.01-57.13	4.36	3.17
Acidity (%)	6.95±0.19	6.18-8.35	4.63	0.53
Ascorbic acid (mg / 100 ml juice)	28.99±0.85	22.22-36.81	5.13	2.39

JMU-Jib (36) (55.78 per cent) and JMU-Nag (65) (54.61 per cent) with no significant difference, while it was lowest JMU-Log (6) (29.01 per cent). Juice is an important parameter possessing high value in processing, which is related to various attributes including fruit size (Dabbarma and Hazarika, 6).

The results obtained from SSR marker analysis allowed to characterize and determine the genetic diversity, existed in 70 acid lime genotypes. The banding pattern and polymorphism detected by SSR primers GAT03 are shown in Plate 1. Out of 25 SSR markers 22 markers exhibited polymorphisms and

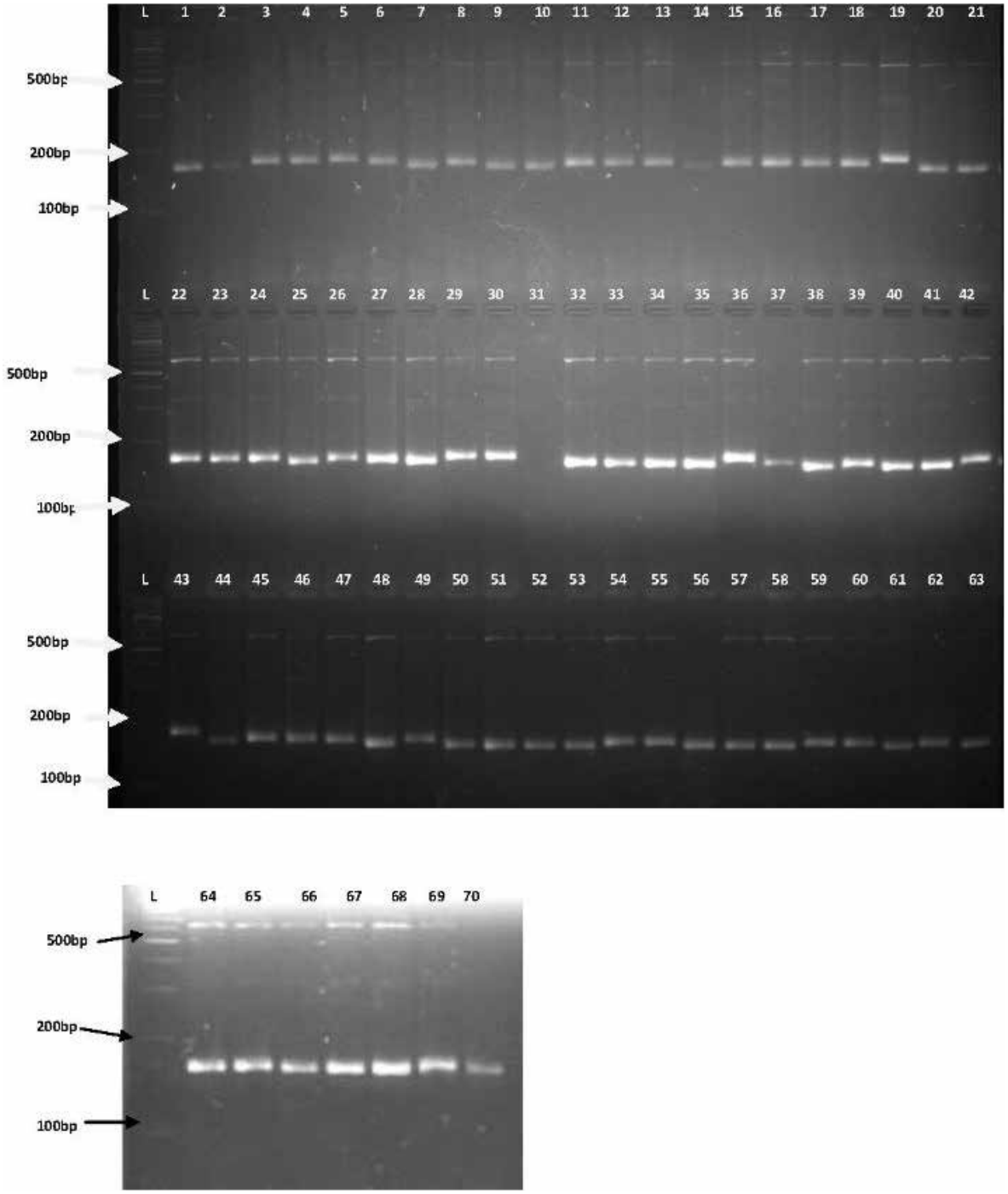

Plate 1. Genotyping of 70 acid lime genotypes using GAT03 primer (L stands for ladder 100bp, and black arrows showing Polymorphic bands).

total 99 alleles were observed by 21 polymorphic SSR loci. Amplified DNA fragments ranged from 50-292 base pairs and the number of alleles ranged from 3 to 6 with an average of 4.71 alleles per locus. The highest number of alleles were observed in TAA41, CAC 15, CT19, CCSM40, CCSM64 and CCSM 70 loci and lowest alleles were amplified in TAA15, C24317, CCSM3, CCSM9 and CCSM59 loci. Primers showing more alleles per locus were found efficient in studying diversity at particular locus. Sharafi *et al.* (16) also identified 49 polymorphic alleles while evaluating genetic variation in acid lime accessions. Barkley *et al.* (4) stated that the number of alleles gives an indication about the level of genetic diversity in species or varieties alongwith phenotypic evaluation.

PIC value provides an estimate of the discrimination power of a marker by taking into account not only number of alleles at locus, but also the relative frequencies of those alleles in the genotypes. PIC content values ranged from 0.41 to 0.93 with an average of 0.73. Most of the primers showed high PIC values proving to be more efficient in characterization of genetic diversity of acid lime genotypes. Teklewood and Becker (18) reported that the marker with a high PIC value can better differentiate genetic accessions. Highly informative markers SSR markers such as AG14, CT19 and GT03 have also been reported by Barkley *et al.* (4) in citrus. In the study, percentage of polymorphism ranged from 33.33 per cent (TAA41, C24317, CCSM3 and CCSM59) to 66.67 per cent (CT19, CCSM9, CCSM40 and CCSM70) with an average of 50.95 per cent (Table 5). Similar findings were also reported by Naz *et al.* (11).

The UPGMA dendrogram depicting the genetic relationships among the 70 acid lime genotypes and classified into two major clusters, Cluster I, Cluster II with sub- clusters (Fig. 1). Cluster I comprised of only one genotype *viz.,* genotype -36 (JMU-Jib 36) and Cluster II, the largest cluster, consists of 69 acid lime genotypes. Cluster II was divided into two sub clusters II A and II B. Sub cluster II A comprised of sixty-four acid lime genotypes which was further

Table 5. Details of selected SSR primers.

S. No.	Name	Amplicon Size (bp)	Allele per locus	Polymorphism percent (%)	PIC (Polymorphism Information Content) value
1	TAA45	80-150	5	60.00	0.65
2	TAA41	50-170	6	50.00	0.83
3	TAA15	160-200	3	33.33	0.41
4	TAA3	130-170	5	40.00	0.74
5	CAT01	50-180	5	60.00	0.72
6	CAC15	180-225	6	50.00	0.90
7	TAA27	80-125	5	60.00	0.85
8	CT19	50-155	6	66.67	0.93
9	AG14	125-140	5	40.00	0.58
10	GT03	120-180	5	60.00	0.89
11	BQ623065	269-284	4	50.00	0.77
12	BQ624307	133-145	4	50.00	0.63
13	BQ624796	240-253	5	60.00	0.76
14	C24317	131-146	3	33.33	0.52
15	CTG1006372	269-303	5	40.00	0.79
16	CCSM 3	144-292	3	33.33	0.72
17	CCSM 9	144-292	3	66.67	0.73
18	CCSM 40	144-292	6	66.67	0.85
19	CCSM 59	144-292	3	33.33	0.65
20	CCSM 64	144-292	6	50.00	0.79
21	CCSM 70	144-292	6	66.67	0.81
Range			3-6 (Av. 4.71)	50.95	0.41 -0.93 (Av. 0.73)

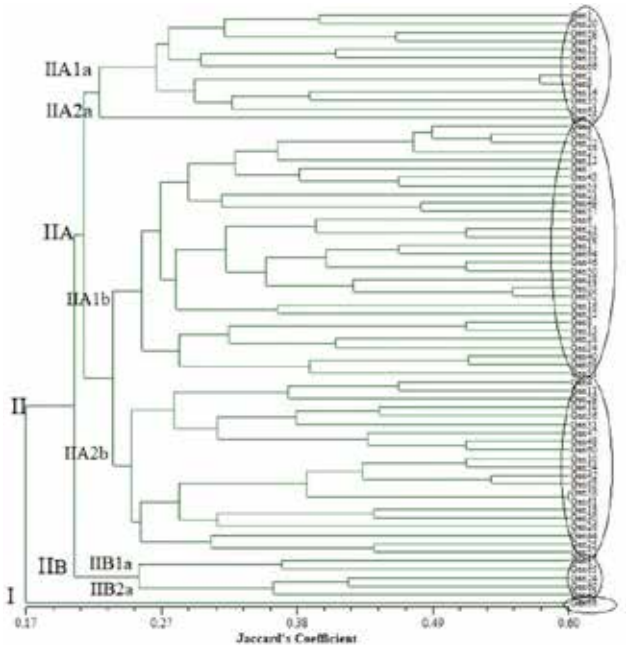

Fig. 1. UPGMA dendrogram showing genetic relationship among 70 acid lime genotypes based on SSR data.

divided into four sub-sub clusters such as II A1a, II A2a and II A1b, II A2b. Sub cluster II B further divided into two sub-sub clusters such as II B1a and II B2a (Table 5). Based on Jaccard's coefficient similarity level, the similarity varied from 0.08 to 0.60. Highest genetic similarity index was observed in JMU-Sum (58) and JMU-Sun (61) followed by JMU-Log-2 and JMU-Log-9, and JMU-Godd (55) and JMU-Sun (62). These genotypes were found to be closely related which showed the highest genetic similarity index. However, least similarity index was observed in genotype JMU-Jib(36) followed by JMU-Balli (30) (0.08), JMU-Log(7) and JMU-Gura (23) (0.08), JMU-Jib(36) and JMU-Log(1) (0.08). On the basis of cluster dendrogram results and similarity index, genotype JMU-Jib (36) is more diverse to others. This inference was correspondingly congruent with the results (five cluster groups) while doing cluster analysis with SSR markers in lime germplasm in terai to high hills of Nepal (Shrestha *et al.,* 17). On the basis of Jaccard's similarity coefficient, least similarity index was observed in genotypes JMU-Jib(36), JMU-Nag(70) and JMU-Pana(41) which were found to be the most promising genotypes. Selections showing highest dissimilarity coefficient value between them, suggested that a rich genetic variation exists between them and they can be used as prospective parents in further breeding programmes to get segregates. Similar results were shown by Shrestha *et al.* (17) and Sharafi *et al.* (16) in their studies where the similarity values were ranged from (0.73-0.75) and (0.19-0.25), respectively in acid lime accessions. Dendrogram from SSR markers placed acid lime genotypes in diversified groups, thereby, indicating that these markers vary in their efficiency in extracting similarities and differences among genotypes.

Therefore, based on study conducted on qualitative characters and diversity analysis, it can be concluded that the genotypes JMU-Nag (70) proved to be the superior genotypes with excellent quality acid lime production which can be further used in breeding programmes to get segregates.

AUTHORS' CONTRIBUTION

Conceptualization of research (SK, AS, PB, RS); Designing of the experiments (AS, MS, RS); Contribution of experimental materials (SK, AS, RS); Execution of field/lab experiments and data collection (SK, AS); Specialized person for diseased problems (VG), Analysis of data and interpretation (AS, MS, RS, GKR); Preparation of the manuscript (SK, AS).

DECLARATION

The authors declare no conflict of interest.

REFERENCES

1. Abhilash, K., Kerutagi, M.G., Nagesh, N., Satish, D. and Manjula, K. 2017. Evaluation of the elite strains of acid lime (*Citrus aurantifolia* Swingle) for the quality parameters. *Int. J. Chemical Studies*, **5**: 112-17.

2. Anonymous. 2018. *District-Wise/Kind-Wise estimated Area under Major Horticulture Crops J&K*. Department of Horticulture, Jammu and Kashmir.

3. AOAC. 1980. *Offical Methods of Analysis*, Association of Offical Analytical Chemists, 13th edition. Washington. DC. Pp. 1018.

4. Barkley, N.A., Roose, M.L., Krueger, R.R. and Federici CT. 2006. Assessing genetic diversity and population structure in a citrus germplasm collection utilizing simple sequence repeat markers (SSRs). *Theor. Appl. Genet.* **112:** 1519-31.

5. Cristofani, M., Figueiredo, J.O., Penteado, M., Targon, N. and Machado M.A. 2003. Differentiation of lemon varieties by microsatellites. *Laranja. Cord.* **241:** 165-75.

6. Dabbarma, N. and Hazarika, B.N. 2016. Effect of plant growth regulators and chemicals on yield and quality of acid lime (*Citrus aurantifolia* Swingle) under foot hill condition of Arunachal Pradesh. *Int. J. Agri. Env. & Biotech.* **9**: 231-236.

7. Doyle, J.J. and Doyle, J.L. 1990. Isolation of Plant DNA from fresh tissue. *Focus.* **12:** 13-15.

8. Ghosh, D., Bhose, S., Manimekalai, R. and Gowda, S. 2013. Molecular detection of Candidatus Phytoplasma spp. causing witches' broom disease of acid lime (*Citrus aurantifolia*) in India. *J. Plant. Biochem. Biotech.* **22:** 343-47.

9. IPGRI. 1999. *Descriptors of Citrus*. International Plant Genetic Resource Institute. Rome, Italy.

10. Khan, M.M., Mumtaz, S., Ahmad, S., Abbas, M. and Khan, I. A. 2008. Some studies on the morphology of Kinnow mandarin and Feutrell's early. *Pak. J. Agri. Sci.* **45**: 424-31.

11. Naz, S., Shahzadi, K., Rashid, S., Saleem, F., Zafarulla. and Ahmad S. 2014. Molecular characterization and phylogenetic relationship of different citrus varieties of Pakistan. *J. Animal Plant Sci.* **24:** 315-20.

12. Novelli, V.M., Machado, M.A. and Lopes, C.R. 2000. Isoenzymatic polymorphism in *citrus spp*. and *Poncirus trifoliata* (L.) Raf. (Rutaceae). *Genet. Mol. Biol.* **23:** 1163-68.

13. Rohlf, F.J. 2000. NTSYSpc, numerical taxonomy and multivariate analysis system, version 2.11a. New York, Excel Software, pp, 44.

14. Shahnazari, N., Noormohammadi, Z., Sheidai, M. and Koohdar, F. 2022. A new insight on genetic diversity of sweet oranges: CAPs-SSR and SSR markers. *J. Genet Eng. Biotechnol.* **20**: 105.

15. Shahzadi, K., Naz, S. and Riaz, S. 2014. Assessing genetic diversity of Pakistani citrus varieties using microsatellite markers. *J. Animal Plant Sci.* **24:** 1752-57.

16. Sharafi, A.A., Abkenar, A.A., Sharafi, A. and Masaeli, M. 2016. Genetic variation assessment of acid lime accessions collected from south of Iran using SSR and ISSR molecular markers. *Physiol. Mol. Biol. Plants.* **22:** 87-95.

17. Shrestha, R.L., Dhakal, D.D., Gautum, D.M., Paudyal, K.P. and Shrestha, S. 2012. Genetic diversity assessment of acid lime (*Citrus aurantifolia* Swingle) landraces in Nepal, using SSR markers. *American J. Plant Sci.* **3:** 1674-81.

18. Teklewood, A. and Becker, H. 2006. Geographic *pattern of genetic* diversity among 43 Ethiopian mustard (*Brassica carinata A. Braun) accessio*n **a**s revealed by RAPD analysis. *Genet. Resour. Crop Evol.* **53**: 1173–85

19. Yadlod, S.S., Bhalerao, R.V. and Pingle, S.N. 2018. Variability Studies of Strains of Kagzi Lime (*Citrus aurantifolia* Swingle) in Latur District of Maharashtra. *Agri. Sci. Dig.- Research J.* **38:** 48-51.

Received : February, 2022; Revised : November, 2022; Accepted : November, 2022