

Characterization of the Genetic Diversity of Acid Lime (*Citrus aurantifolia* (Christm.) Swingle) Cultivars of Eastern Nepal Using Inter-Simple Sequence Repeat Markers

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Table S1. ISSR primer details, Total Number of amplified Bands (TNB), Number of Polymorphic Bands (NPB), Percent Polymorphism (PP), amplicon size range, Polymorphic Information Content (PIC), Band Informativeness (I_B) and Resolving power (R_P) computed for different ISSR primers used to generate ISSR profiles in 60 *Citrus aurantifolia* (Acid lime) accessions.

S.N.	Primer Code	Primer Sequence (5' – 3')	Primer Length (bp)	TNB	NPB	PP	Amplicon size range (bp)	PI C	I _B	R _P
1	C1	TCTCTCTCTCTC TCTCTCCC	20	7	5	71.4 3	550-1800	0.8 5	1.7 7	12.4 0
2	C2	AGCAGCAGCA GCGT	14	10	10	100	500-3200	0.8 3	0.8 3	8.33
3	C4	CTCCTCCTCGC	11	14	14	100	300-2200	0.8 2	0.4 2	5.87
4	C5	CACCACCACGC	11	12	11	91.6 7	600-2500	0.8 3	0.6 0	7.23
5	C7	HVHGAGAGAG AGAGAGAT	18	17	15	88.2 3	250-2000	0.8 9	0.8 7	14.8 7
6	C8	TCCTCCTCCTCC TCCRY	17	9	7	77.7 8	520-2800	0.8 7	1.4 7	13.2 3
7	C9	BDBTCCTCCTCC TCCTCC	18	9	6	66.6 7	520-2000	0.8 6	1.5 0	13.5 6
8	C10	HVHTCCTCCTC CTCCTCC	18	11	8	72.7 3	500-2000	0.7 9	0.7 8	8.63
9	UBC 807	AGAGAGAGAG AGAGAGT	17	9	9	100	450-1300	0.7 4	0.5 1	4.63
10	UBC 810	GAGAGAGAGA GAGAGAT	17	16	16	100	390-1980	0.8 5	1.0 0	12.0 0

11	UBC 812	GAGAGAGAGA GAGAGAA	17	12	9	75.0 0	450-1500	0.9 1	1.0 3	16.6 0
12	UBC 825	ACACACACAC ACACACT	17	9	5	55.5 6	500-2000	0.8 7	1.5 1	13.6 3
13	UBC 834	AGAGAGAGAG AGAGAGYT	18	7	5	71.4 3	310-1550	0.8 1	1.2 8	8.96
14	UBC 835	AGAGAGAGAG AGAGAGYC	18	10	10	100	400-2900	0.8 6	1.2 9	12.9 6
15	UBC 836	AGAGAGAGAG AGAGAGYA	18	10	10	100	320-1450	0.8 7	1.1 3	11.3 0
16	UBC 841	GAGAGAGAGA GAGAGAYC	18	10	10	100	320-1700	0.8 7	1.0 8	10.8 3
17	UBC 842	GAGAGAGAGA GAGAGAYG	18	12	12	100	320-1650	0.8 9	1.0 4	12.5 3
18	UBC 857	ACACACACAC ACACACYG	18	18	17	94.4 4	300-3000	0.9 3	1.2 8	23.1 6
19	UBC 873	GACAGACAGA CAGACA	16	14	13	92.8 6	470-3000	0.8 9	0.9 6	13.4 3
20	UBC 888	BDBCACACACA CAGACA	17	9	7	77.7 8	480-1450	0.8 7	1.4 7	13.2 3
21	UBC 889	DBDACACACAC ACACACA	18	9	5	55.5 6	480-1400	0.8 7	1.7 0	15.3 3
Total				234	204	Average		0.8 5	1.1 2	12.0 3

Average Polymorphic Bands per primer = 9.72

Average Polymorphism = 87.18

H, non-G; Y, Pyrimidine; B, non-A; D, non-C; V, non-T

Table S2. Analysis of Molecular Variance (AMOVA) for 60 Acid lime accessions from three agro-ecological regions.

Source of variation	df	SS	MS	Estimated variance	Total variance	P-value	ϕ_{PT}
Among zones	2	185.227	92.613	3.539	14%	< 0.001	0.139
Within zones	57	1248.298	21.900	21.900	86%		
Total	59	1433.525		25.439	100%		

Df: Degree of freedom; SS: Sum of square; MS: Mean sum of square; ϕ_{PT} : Indicator of the genetic differentiation

Table S3. Correlation Coefficient values generated from Mantel test of original similarity matrices.

	Simple Matching (SM)	Jaccard (J)	Dice (D)
Simple Matching (SM)	*****	0.98143	0.98318
Jaccard (J)		*****	0.99710
Dice (D)			*****

Table S4. Consensus Fork Index (CIc) values generated for the UPGMA based phenograms using different similarity coefficients.

	Simple Matching (SM)	Jaccard (J)	Dice (D)
Simple Matching (SM)	*****	0.74138	0.74138
Jaccard (J)		*****	1.00000
Dice (D)			*****

Table S5. Cophenetic correlation coefficients value (r) obtained for different similarity matrices *viz.* Simple Matching, Jaccard's and Dice.

Clustering module of similarity	Simple Matching	Jaccard	Dice
UPGMA	0.88396	0.89800	0.90356

Table S6. Percentage genetic similarities observed within and between three agro-ecological zones for various Acid lime samples based on Dice similarity matrix generated from ISSR profile.

Zone/Zone	High-hill	Average	Mid-hill	Average	Terai	Average
High-hill	75-95	86.02	68-95	83.6	57-95	82.68
Mid-hill			70-94	82.94	57-94	79.98
Terai					57-94	79.33

Zone: Agro-ecological zone

Table S7. Genetic variation observed for *C. aurantifolia* samples representing different agro-ecological zones as revealed by POPGENE ver. 1.32.

Population of <i>Citrus aurantifolia</i>	Sample Size	Number of polymorphic bands	PPB (%)	H	I
High-hill	20	129.00	55.130	0.173	0.262
Mid-hill	21	156.00	66.670	0.202	0.308
Terai	19	163.00	69.660	0.215	0.325
Average		149.33	63.820	0.197	0.300
Species level (Multipopulation)	60	204	87.180	0.223	0.348

PPB = Percent Polymorphic Bands/loci; H = Nei's gene diversity; I = Shannon's information index

Table S8. Acid lime accessions belonging to two major and three minor clusters of UPGMA phenogram.

S.N.	Clusters	Accessions
1	I	LT-1, LT-2, LT-3, LT-12, LT-8, LT-10, LT-14, LT-15, LT-11, LT-19, LT-5, LT-7, LT-6 , LT-17, LT-18, LT-20, LT-21, LT-22, LD-25, LD-27, LD-30, LD-29, LT-23, LD-24, LT-13, LD-26, LT-4, LD-31, LT-16, LD-28
2	II	LD-32, LD-33, LS-36, LS-37, LS-39, LS-42, LD-45, LS-38, LS-40, LM-43, LM-44, LS-41, LD-45, LD-48, LD-50, LD-49, LM-51, LM-54, LS-34, LS-35, LM-52, LM-55, LKv-60, LKm-61, LKr-62
3	III	LT-9
4	IV	LD-59
5	V	LS-56, LS-57, LD-58