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RESEARCH ARTICLE

Rough lemon (*Citrus jambhiri Lush.*), a potential rootstock with commercially high valued fruits: Characterization of rough lemon based on biochemical attributes

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Abstract

Rough lemon (*C. jambhiri* Lush.), widely employed as a rootstock in India as well as worldwide, is widely consumed in Assam. Its fruits are highly nutritious and in high local demand, often priced higher than Assam lemon during the harvest season. As a heterozygous species indigenous to the state, this crop displays extensive variation. These variations can be observed in the morphology of the plant and its fruits. However, limited research on its biochemical constituents has hindered efforts to promote this fruit for both processing and fresh consumption nationwide. This paper aims to provide a gist of the work conducted at Assam Agricultural University, based on biochemical characteristics and group them to assess their biochemical similarity. This study classifies the available germplasm of rough lemon collected across the state of Assam in three main groups based on biochemical constituents of fruit. This may be attributed to the genetic makeup of the crop or the influence of soil properties and nutrient availability in the study areas. Sensory evaluations were conducted to assess consumer preference for fresh consumption. Consequently, this research offers a distinct understanding of the specific type of rough lemon that can be chosen for fresh consumption as well as processing purposes.

Keywords: biochemical attributes; *C.jambhiri*; germplasm; genetic resource; quality; sensory

Introduction

Many citrus species are known for their antiseptic, antiviral, antioxidant, anti nitrosaminic; anticancer; anti-inflamatory, hypocholesterinemic; cardiotonic; sedative and stomachic activity and effect on capillaries (1 - 8). *C. jambhiri* is used as an antidiarrheal agent and is reported to improve digestion (9). These beneficial effects may be attributed to the components present in this citrus fruit and leaves like flavonoids, limonoids, coumarins and furanocoumarins. The rough lemon juice contains high citric acid and vitamin C (10-11). Various biologically important secondary metabolites reported in this genus include flavonoids, limonoids, coumarins, furanocoumarins, sterols, volatile oils, carotenoids and alkaloids (12-18). The peel of this citrus fruit is a potential source of essential oils (19) and yields oil in the range of 0.5-3 kg/t of fruit (20).

Rough lemon is found in diversified forms in different names in the region. There are two distinct types of rough lemons, acid and sweet types in terms of differences in their sweetness to acid blend (21). Research on rough lemon diversity in India is limited, primarily due to the dominance of *C. reticulata*, *C. sinensis* and *C. aurantifolia* with rough lemon mostly confined to homestead gardens in rural areas. So far, there is no well-defined variety of rough lemon in Assam that has been identified as a commercial type for fresh consumption. In Assam, rough lemon is more popular for table use than as a rootstock. Therefore, identifying superior varieties through biochemical characterization is necessary. Characterization of germplasm and presenting its diversity in their inherent biochemical constituents is essential. Biochemical characterization of germplasm is also essential for the crop's commercialization and for planning future improvement programs.

Materials and Methods

Five Rough lemon germplasm (T1-T5) were selected based on their morphological differences from six agro-climatic zones (L1-L6) in Assam, resulting in a total of thirty germplasm samples (AR01T1-AR06T5). The fruits were thoroughly washed and the juice was extracted. The Total Soluble Solids (TSS) of the fruit juice samples were determined using an Atago Digital Refractometer and the results were expressed in °Brix (22). The fruit juice pH was determined by a digital pH meter cyberscan 510 and average was recorded. The titratable acidity was estimated using the standard method from the Association of Official Analytical Chemists (23). Ascorbic acid content was determined using the 2,6-dichlorophenolindophenol dye method (24). The TSS-acid ratio was calculated by the following formula: TSS-acid ratio = TSS divided the acid content. Reducing sugar content was estimated by Dinitrosalicylic acid (DNS) method (25). TSS content was estimated using Anthrone method (26). Non-reducing sugar content was calculated using the formula: Non-reducing sugar = Total sugar - Reducing sugar.

The antioxidant activity of fruit juice samples was tested based on the radical scavenging effect on the DPPH free radical (27). Total flavonoid content in rough lemon juice was determined using aluminum chloride colorimetric assay (28). Limonin content in rough lemon juice and albedo was determined using a slightly modified version of the method (29). Total essential oil content in citrus peels were estimated by Hydrodistillation method and expressed as percentage on dry weight basis (30). Sensory evaluation methods were employed to assess human responses to fruit samples (31). The most widely used scale for measuring food acceptability is the 9-point hedonic scale which is employed in this study also.

Results and Discussion

The data presented in Table 1 revealed a significant difference in juice pH among the selected rough lemon accessions, with the highest pH (3.25) recorded in AR06T1 and the lowest pH (2.27) in AR04T1, resulting in an average value of 2.73. Juice pH showed a negative correlation with titratable acidity. The present finding is in conformity with previous studies (10, 11). The variation in juice pH might be due to variation in the acid content among different genotypes, which is affected by genetic makeup of individual plants and environmental conditions. The variation in juice pH across different locations was found to be statistically significant. The highest juice pH of 2.95 was recorded in location L6 (Table 2). The lowest pH (2.32) was recorded in location L4. A similar effect of location was previously reported in pummelo (32).

The highest TSS (8.87 °B) was recorded in accession AR06T4, while the lowest TSS (6.13 °B) was observed in AR02T1, with an average TSS of 7.54 °B. The highest TSS value (8.15 °B) was recorded in location L6, while the lowest (6.16 °B) was recorded in location L4. The variation in TSS content among the genotypes and different locations might be attributed to

Table 1. Biochemical quality parameters in juice of rough lemon accessions

Location/ District	Plant No.	Accession No.	Juice pH	TSS (°B)	Titratable acidity (%)	Reducing sugar (%)	Non reducing sugar (%)	Total sugar (%)	TSS acid ratio
Jorhat (L1)	1	AR01T1	2.72	7.37	5.21	1.08	1.50	2.58	1.41
	2	AR01T2	2.83	7.73	4.71	1.57	1.88	3.45	1.64
	3	AR01T3	2.75	7.13	6.01	1.14	1.58	2.72	1.19
	4	AR01T4	2.86	8.17	4.61	1.22	1.64	2.86	1.77
	5	AR01T5	2.82	7.70	5.21	1.13	1.51	2.64	1.48
Nagaon (L2)	6	AR02T1	2.67	6.13	6.10	0.77	1.05	1.82	1.00
	7	AR02T2	2.68	7.20	5.93	1.05	1.43	2.48	1.21
	8	AR02T3	2.62	8.10	5.83	1.45	1.79	3.24	1.39
	9	AR02T4	2.74	7.27	5.15	1.10	1.45	2.55	1.41
	10	AR02T5	2.63	7.17	5.62	1.03	1.37	2.40	1.27
Kokrajhar (L3)	11	AR03T1	2.77	8.27	4.04	1.62	1.90	3.52	2.04
	12	AR03T2	2.87	7.47	4.52	1.23	1.73	2.96	1.65
	13	AR03T3	2.92	8.50	5.51	1.48	1.78	3.26	1.54
	14	AR03T4	2.81	7.97	6.02	1.59	1.84	3.43	1.32
	15	AR03T5	3.09	8.13	5.21	1.25	1.72	2.97	1.56
Biswanath (L4)	16	AR04T1	2.27	6.73	5.86	0.98	1.17	2.15	1.15
	17	AR04T2	2.30	6.33	5.69	0.81	1.10	1.91	1.11
	18	AR04T3	2.32	6.63	5.44	0.93	1.17	2.10	1.22
	19	AR04T4	2.30	6.70	5.83	1.01	1.14	2.15	1.15
	20	AR04T5	2.41	6.63	6.06	0.95	1.11	2.06	1.09
Karbi Anglong (L5)	21	AR05T1	2.81	7.27	6.22	1.19	1.68	2.87	1.17
	22	AR05T2	2.78	8.27	6.42	1.15	1.65	2.80	1.29
	23	AR05T3	2.82	7.70	6.32	1.02	1.43	2.45	1.22
	24	AR05T4	2.77	7.47	5.22	1.47	1.79	3.26	1.43
	25	AR05T5	2.72	7.30	4.33	1.18	1.68	2.86	1.68
Cachar (L6)	26	AR06T1	3.25	8.53	4.22	1.57	1.86	3.43	2.02
	27	AR06T2	2.87	7.40	5.15	1.19	1.69	2.88	1.44
	28	AR06T3	2.78	8.17	4.20	1.64	1.86	3.50	1.95
	29	AR06T4	2.94	8.87	4.18	1.33	1.80	3.13	2.12
	30	AR06T5	2.91	7.77	5.04	1.47	1.79	3.26	1.54
Mean			**2.73**	**7.54**	**5.33**	**1.22**	**1.57**	**2.79**	**1.45**
S. Ed. (±)			**0.02**	**0.06**	**0.01**	**0.01**	**0.04**	**0.04**	**0.01**
$CD_{(0.05)}$			**0.03**	**0.12**	**0.02**	**0.03**	**0.08**	**0.07**	**0.02**
GCV(%)			**8.26**	**9.08**	**13.36**	**20.24**	**17.02**	**18.08**	**20.87**
PCV(%)			**8.29**	**9.13**	**13.36**	**20.29**	**17.29**	**18.15**	**20.90**
h^2(%)			**99.30**	**98.78**	**99.97**	**99.56**	**96.95**	**99.28**	**99.77**
GA(%)			**16.95**	**18.58**	**27.51**	**41.60**	**34.53**	**37.11**	**42.95**

*S.Ed-Standard error; Replication: 3; CD-Critical Difference; TSS: Total Soluble Solids; GCV: Genotypic Coefficient of Variation; PCV: Phenotypic Coefficient of Variation; h^2: heritability; GA: Genetic Advance

Table 2. Effect of locations on biochemical constituents of rough lemon juice

Locations	Juice pH	TSS (°B)	Titratable acidity (%)	TSS:acid ratio	Reducing sugar (%)	Non reducing sugar(%)	Total sugar (%)
L1	2.80	7.62	5.15	1.50	1.23	1.62	2.85
L2	2.67	7.17	5.73	1.26	1.08	1.42	2.50
L3	2.89	8.07	5.06	1.63	1.43	1.79	3.23
L4	2.32	6.61	5.78	1.14	0.94	1.14	2.08
L5	2.78	7.60	5.70	1.36	1.20	1.64	2.85
L6	2.95	8.15	4.55	1.81	1.44	1.80	3.24
Mean	**2.73**	**7.54**	**5.33**	**1.45**	**1.22**	**1.57**	**2.79**
S.Ed. (±)	**0.006**	**0.03**	**0.005**	**0.006**	**0.01**	**0.01**	**0.02**
CD (0.05)	**0.013**	**0.07**	**0.010**	**0.013**	**0.01**	**0.03**	**0.03**

sugar and acid content of the rough lemon accessions which is influenced by genetic makeup as well as environmental factors. A negative correlation was found between TSS and titratable acidity.

The data presented in Table 1 and Table 2 revealed that titratable acidity varied significantly among different accessions and locations. The highest titratable acidity (5.78 %) was recorded in location L4, while the lowest titratable acidity (4.55 %) was recorded in location L6. The variation in titratable acidity might be due to genetic variation among the accessions, maturity stage of harvested fruits, soil nutrient status and environmental conditions prevailing in different locations.

The data presented in Table 1 revealed significant variation in TSS acid ratio among the accessions with the highest TSS-acid ratio of 2.12 recorded in AR06T4 while the lowest ratio of 1.00 recorded in AR02T1 with an average ratio of 1.45. A significant variation in TSS-acid ratio was observed among different locations. The variation in TSS-acid ratio might be due to genetic differences among the accessions, soil fertility status and environmental conditions.

The significantly high percentage (1.64 %) of reducing sugar was recorded in AR06T3 which was statistically *at par* with AR06T1 (1.57 %), AR01T2 (1.57 %), AR03T1 (1.62 %) and AR03T4 (1.59 %). The lowest reducing sugar per cent was recorded to be 0.77 % in AR02T1 with a mean reducing sugar per cent of 1.22 %. Effect of different locations on reducing sugar per cent was found to be statistically significant (Table 2). Perusal of the data revealed that the highest non-reducing sugar content of 1.90 % was recorded in AR03T1, which was comparable to AR06T1, AR06T3, AR06T4, AR06T5, AR01T2, AR02T3, AR03T2, AR03T3 and AR03T4, while the lowest (1.05 %) was recorded in AR02T1, with an average of 1.57 % across the accessions. This variation might be due to genotypic differences, as well as varying soil and climatic conditions. Regarding location effect, the highest non-reducing sugar content (1.80 %) was recorded in location L6, which was comparable to location L3 (1.79 %) and the lowest (1.14 %) was recorded in location L4.

Significant differences were observed in total sugar content, with an average total sugar percentage of 2.79 % among the accessions. The effect of different locations on total sugar content was significant, with the highest percentage (3.24 %) recorded in location L6, comparable to location L3 (3.23 %) and the lowest (2.08 %) recorded in location L4. The observed variation could be due to the heterozygous nature of the genotypes, as well as varying soil and climatic conditions.

The ascorbic acid content in rough lemon fruits varied significantly as presented in Table 3, among accessions, with a notable influence of location. This observation aligns with early studies (33, 10). The variation in ascorbic acid content is likely attributed to genetic factors, fruit positioning, soil nutrient levels and environmental differences (34). Similarly, total flavonoid content in the juice varies significantly among both accessions and locations. These variation patterns are consistent with genetic control, as observed in local pummelo and mandarin accessions in China (35, 36). The variation observed in our study can be attributed to genetic factors, soil nutrients and environmental conditions across the locations.

Significant variation was observed among the selected accessions for total antioxidant activity among accessions (presented in Table 3) with a mean total antioxidant activity of 85.18 %. The data on total antioxidant activity is presented in Table 4. revealed that there was significant variation also among different locations.

The variation in total antioxidant activity could be attributed to differences in the selected genotypes as well as variations in climatic conditions. The ascorbic acid contributed between 60 % and 100 % of the antioxidant potential in citrus fruits, whereas phenolic compounds played a major role in the antioxidant activity of non-citrus fruits (37). In the present study, the ascorbic acid content of the juice exhibited a strong positive correlation with total antioxidant activity. Similar high positive correlation between vitamin C and total antioxidant activity in *Citrus junos* fruits (38). These findings suggest that ascorbic acid is likely one of the key contributors to the antioxidant activity of rough lemon juice.

The results of HPLC analysis of limonin extract of juice and albedo for limonin content as presented in Table 3, revealed significant variations among rough lemon accessions and among the locations. The variation in limonin content among the accessions might be due to differences in genetic makeup and soil nutrient status. Variety is an important factor in determining the limonin content of citrus fruits (39). The soil nutrient status *i.e.* low nitrogen and potassium content in soil might increase limonin content of citrus juice (40). In the present study limonin was analyzed in the juice and scrapped albedo portion, which might contain higher content of limonin as compared to juice alone.

Significant variation was observed among the selected accessions for essential oil content of peels of rough lemon. While there was no significant variation among different locations.

Clustering of rough lemon accessions based on quantitative biochemical characters

Cluster analysis was done based on twelve quantitative

Table 3. Biochemical constituents in rough lemon accessions

Location/ District	Plant No.	Accession No.	Total flavonoid content (mg/100 mL)	Ascorbic acid (mg/100 mL)	Total antioxidant activity (%)	Limonin in albedo and juice (mg/100 mL)	Essential oil (%)
Jorhat (L1)	1	AR01T1	26.12	59.41	91.23	23.62	4.72
	2	AR01T2	32.72	53.24	75.65	10.42	3.39
	3	AR01T3	29.95	66.98	94.90	13.43	4.50
	4	AR01T4	21.57	52.02	68.11	15.73	4.33
	5	AR01T5	34.72	58.27	87.03	18.55	3.89
Nagaon (L2)	6	AR02T1	20.25	72.14	97.64	21.51	4.06
	7	AR02T2	28.72	66.18	91.42	14.69	3.39
	8	AR02T3	46.58	65.61	91.45	23.21	4.39
	9	AR02T4	36.34	61.45	78.52	20.79	4.72
	10	AR02T5	20.77	64.40	84.48	16.71	4.56
Kokrajhar (L3)	11	AR03T1	30.82	63.03	88.15	10.52	4.22
	12	AR03T2	25.22	55.06	76.95	23.23	3.78
	13	AR03T3	25.12	43.41	72.88	15.21	4.56
	14	AR03T4	27.49	65.50	89.52	24.07	4.06
	15	AR03T5	31.89	58.40	87.56	22.31	4.78
Biswanath (L4)	16	AR04T1	36.02	74.01	91.86	20.47	4.06
	17	AR04T2	27.74	65.17	88.50	23.08	4.22
	18	AR04T3	26.40	63.99	88.21	22.65	4.72
	19	AR04T4	32.68	69.12	88.88	17.42	5.06
	20	AR04T5	37.85	76.19	94.66	19.82	3.39
Karbi Anglong (L5)	21	AR05T1	32.89	62.15	88.86	10.43	4.28
	22	AR05T2	36.03	64.14	85.55	13.40	4.39
	23	AR05T3	33.14	63.17	85.05	12.91	3.72
	24	AR05T4	29.72	62.01	85.96	16.78	4.06
	25	AR05T5	24.86	59.17	91.57	15.23	4.56
Cachar (L6)	26	AR06T1	26.32	56.54	80.71	17.75	4.61
	27	AR06T2	21.04	51.46	71.47	21.40	4.17
	28	AR06T3	19.33	53.51	71.49	17.23	4.67
	29	AR06T4	37.80	60.50	84.05	14.25	3.72
	30	AR06T5	21.47	57.60	82.97	20.65	4.06
Mean			**29.39**	**61.46**	**85.18**	**17.92**	**4.23**
S.Ed. (±)			**0.38**	**6.60**	**1.10**	**0.04**	**0.10**
CD ($_{0.05}$)			**0.76**	**13.20**	**2.20**	**0.08**	**0.19**
GCV (%)			**21.91**	**8.38**	**8.87**	**23.76**	**10.27**
PCV (%)			**21.96**	**15.59**	**9.01**	**23.76**	**10.63**
h^2 (%)			**99.49**	**28.89**	**96.92**	**99.99**	**93.30**
GA (%)			**45.01**	**9.28**	**17.99**	**48.94**	**20.44**

*S.Ed-Standard error; Replication: 3; CD-Critical Difference; TSS: Total Soluble Solids; GCV: Genotypic Coefficient of Variation; PCV: Phenotypic Coefficient of Variation; h^2: heritability; GA: Genetic Advance

Table 4. Effect of locations on biochemical constituents of rough lemon juice

Locations	Total flavonoid content (mg/100 mL)	Ascorbic acid (mg/100 mL)	Total antioxidant activity (%)	Limonin (mg/100 mL)	Total essential oil (%)
L1	29.02	57.98	83.38	16.35	4.17
L2	30.53	65.95	88.70	19.38	4.22
L3	28.11	57.08	83.01	19.07	4.28
L4	32.14	69.21	90.42	20.69	4.29
L5	31.33	62.13	87.40	13.75	4.20
L6	25.19	55.92	78.14	18.26	4.24
Mean	**29.39**	**61.38**	**85.18**	**17.92**	**4.23**
S.Ed. (±)	**0.18**	**0.94**	**0.49**	**0.02**	**-**
CD ($_{0.05}$)	**0.38**	**2.05**	**1.06**	**0.05**	**NS**

Non significant: NS

biochemical characters using SPSS software. Data revealing squared Euclidean distance matrix of proximity of thirty rough lemon accessions based on quantitative biochemical characters are presented in Fig. 1. It interprets that AR04T3 and AR04T2, both of Biswanath district, are the nearest relatives while AR03T1 of Kokrajhar and AR02T1 of Nagaon district are the farthest relatives among all the thirty accessions based on quantitative biochemical characters. Further, dendrogram created by hierarchical clustering using wards linkage represented the clustering of accessions into different groups based on their biochemical quality characters (Fig. 2). Accessions belonging to the same group are considered to have similar biochemical characters while accessions of varied characters were grouped in different groups accordingly (Table 5). Thirty accessions were clustered into two main groups. First group included seven accessions namely, AR02T1and AR02T5 of Nagaon and AR04T1, AR04T2, AR04T3, AR04T4 and AR04T5 of Biswanath, belonging to two agroclimatic zones. It can be interpreted that all the five accessions of Biswanath district are clustered in same group indicating strong environmental influence and genetic similarity among the accessions in terms of biochemical characters. However, similar results were not found with respect to accessions of Nagaon district of the same group. The second group comprises of 23 accessions which are divided further into two subgroups namely, 'Group II a' and 'Group II b'. First subgroup consists of 7 accessions namely, AR01T2 and AR01T4

Case	Squared Euclidean Distance																													
	AR01T1	AR01T2	AR01T3	AR01T4	AR01T5	AR02T1	AR02T2	AR02T3	AR02T4	AR02T5	AR03T1	AR03T2	AR03T3	AR03T4	AR03T5	AR04T1	AR04T2	AR04T3	AR04T4	AR04T5	AR05T1	AR05T2	AR05T3	AR05T4	AR05T5	AR06T1	AR06T2	AR06T3	AR06T4	AR06T5
AR01T1	0.00																													
AR01T2	2.78	0.00																												
AR01T3	0.84	2.13	0.00																											
AR01T4	1.43	1.08	1.95	0.00																										
AR01T5	0.54	1.31	0.68	1.00	0.00																									
AR02T1	1.49	5.52	1.57	4.12	1.99	0.00																								
AR02T2	1.25	1.93	0.53	2.06	0.53	1.21	0.00																							
AR02T3	1.20	2.30	1.34	2.52	0.95	3.68	1.87	0.00																						
AR02T4	0.38	2.19	0.91	1.07	0.41	2.03	1.27	1.07	0.00																					
AR02T5	0.48	2.50	0.46	1.26	0.68	1.04	0.69	2.01	0.56	0.00																				
AR03T1	2.58	0.77	2.19	1.38	1.70	6.00	2.74	2.23	2.37	2.70	0.00																			
AR03T2	0.87	1.27	1.88	0.62	0.60	3.22	1.61	1.65	0.88	1.35	1.75	0.00																		
AR03T3	1.70	1.03	1.83	0.49	1.26	4.92	2.37	1.98	1.44	1.69	1.50	1.14	0.00																	
AR03T4	1.07	1.95	1.32	1.98	1.04	3.27	1.61	0.63	1.42	1.63	2.15	1.08	1.45	0.00																
AR03T5	0.46	2.01	1.12	1.09	0.59	3.10	1.81	0.81	0.57	1.17	1.71	0.76	0.96	0.75	0.00															
AR04T1	1.17	3.98	1.07	3.28	1.20	0.92	0.87	1.92	1.17	0.98	4.36	2.43	3.94	2.43	2.32	0.00														
AR04T2	1.03	4.70	1.57	3.21	1.47	0.43	1.23	2.80	1.19	0.81	5.26	2.43	4.04	2.92	2.60	0.48	0.00													
AR04T3	0.57	4.22	1.25	2.52	1.24	0.73	1.38	2.31	0.76	0.50	4.31	2.03	3.20	2.44	1.88	0.65	0.16	0.00												
AR04T4	1.01	4.23	0.89	2.89	1.47	1.08	1.42	2.24	0.88	0.58	4.16	2.87	3.31	2.77	2.15	0.73	0.56	0.31	0.00											
AR04T5	1.88	4.23	1.48	4.07	1.43	0.79	0.71	2.45	1.81	1.47	5.01	2.99	4.74	2.82	3.11	0.47	0.68	1.14	1.19	0.00										
AR05T1	1.40	1.49	0.18	1.77	0.77	2.41	0.62	1.48	1.16	0.81	2.00	1.97	1.42	1.47	1.35	1.60	2.29	1.98	1.49	1.98	0.00									
AR05T2	1.22	1.71	0.38	1.57	0.64	2.65	0.81	1.00	0.91	0.86	2.05	1.83	1.21	1.21	0.99	1.53	2.30	1.92	1.47	2.01	0.24	0.00								
AR05T3	1.39	1.82	0.50	1.75	0.52	1.72	0.22	1.75	1.07	0.71	2.73	1.79	1.82	1.73	1.59	1.16	1.61	1.64	1.41	1.14	0.37	0.35	0.00							
AR05T4	0.91	0.71	0.70	0.97	0.45	3.03	0.96	0.83	0.86	1.00	0.92	0.65	0.84	0.50	0.64	2.01	2.53	2.03	2.11	2.45	0.65	0.73	1.02	0.00						
AR05T5	0.67	1.43	0.84	0.86	0.64	2.74	1.34	1.73	0.84	0.79	0.91	0.87	1.29	1.57	0.77	2.01	2.25	1.58	1.76	2.81	1.10	1.29	1.51	0.54	0.00					
AR06T1	1.96	1.34	2.57	0.85	1.62	5.86	3.28	2.19	1.89	2.58	0.73	1.08	0.89	1.70	0.82	4.85	5.20	4.12	4.42	5.67	2.50	2.23	3.02	1.06	1.09	0.00				
AR06T2	0.80	1.47	1.57	0.42	0.73	2.91	1.56	1.90	0.72	0.90	2.23	0.25	0.69	1.16	0.78	2.39	2.18	1.73	2.38	3.10	1.56	1.46	1.48	0.73	0.99	1.34	0.00			
AR06T3	2.08	1.26	2.81	0.54	1.94	5.97	3.44	2.63	1.94	2.35	0.91	1.03	0.70	1.89	1.36	4.82	4.88	3.77	4.15	5.85	2.68	2.52	3.28	1.11	1.14	0.43	1.04	0.00		
AR06T4	2.31	0.81	2.36	1.13	1.09	5.52	2.31	1.89	1.92	2.66	0.50	1.22	1.52	2.10	1.38	3.76	4.68	4.03	4.16	4.21	2.13	1.80	2.15	1.06	1.16	0.78	1.87	1.33	0.00	
AR06T5	0.83	1.04	1.32	0.69	0.67	3.31	1.48	1.30	1.05	1.18	1.23	0.30	0.68	0.47	0.52	2.68	2.83	2.24	2.76	3.21	1.37	1.30	1.59	0.25	0.68	0.72	0.37	0.72	1.22	0.00

This is a dissimilarity matrix

Fig. 1. Squared Euclidean distance matrix of proximity of 30 rough lemon accessions based on quantitative biochemical character.

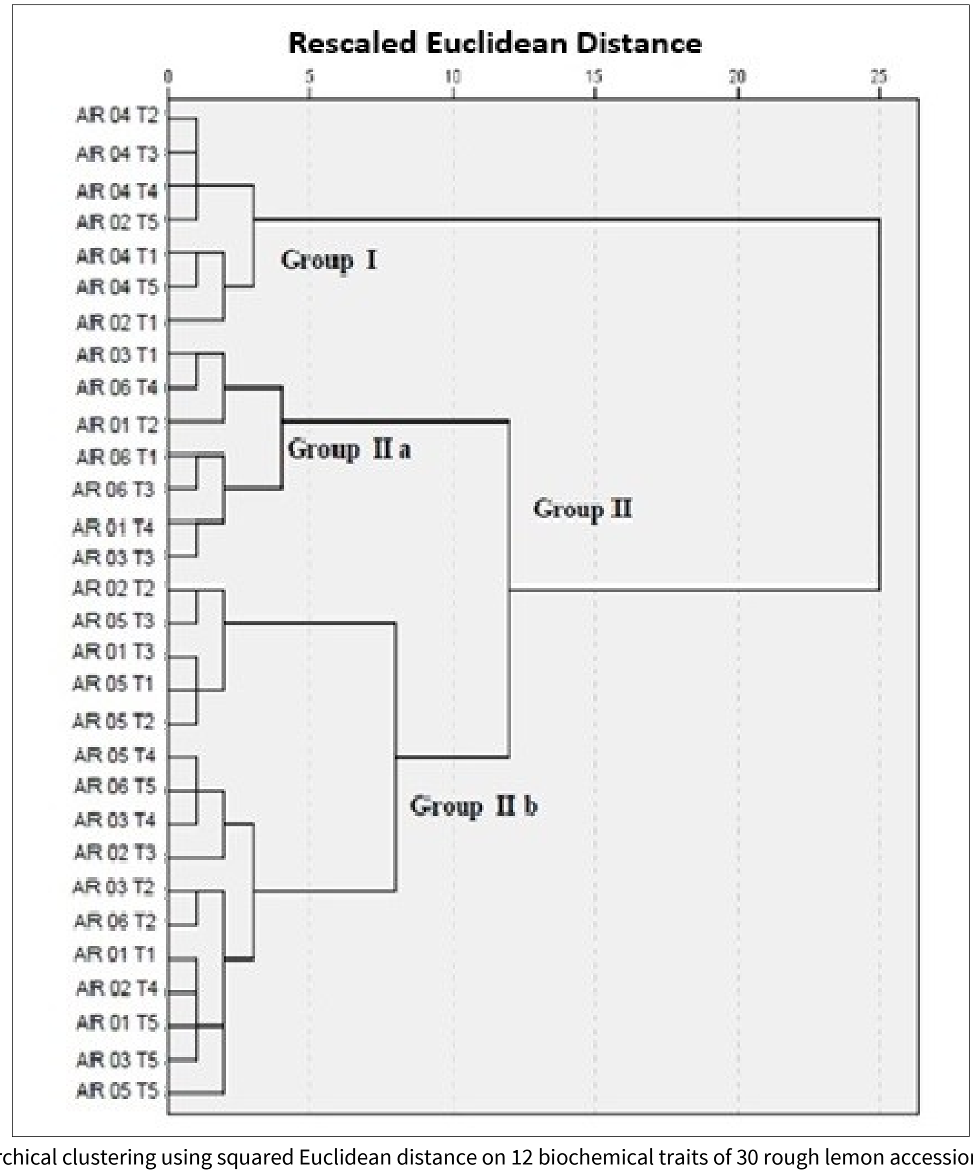

Fig. 2. Ward's hierarchical clustering using squared Euclidean distance on 12 biochemical traits of 30 rough lemon accessions.

Table 5. Grouping of rough lemon accessions using cluster analysis based on biochemical characters

	Accessions in each cluster
Group I	AR02T1, AR02T5, AR04T1, AR04T2, AR04T3, AR04T4, AR04T5
Group II a	AR01T2, AR01T4, AR03T1, AR03T3, AR06T1, AR06T3, AR06T4
Group II b	AR01T1, AR01T3, AR01T5, AR02T2, AR02T3, AR02T4, AR03T2, AR03T4, AR03T5, AR05T1, AR05T2, AR05T3, AR05T4, AR05T5, AR06T2, AR06T5

of Jorhat, AR03T1 and AR03T3 of Kokrajhar and AR06T1, AR06T3 and AR06T4 of Cachar. Rest of 16 accessions constituted the second subgroup 'Group II b'. This cluster included accessions namely, AR01T1, AR01T3 and AR01T5 of Jorhat, AR02T2, AR02T3 and AR02T4 of Nagaon, AR03T2, AR03T4 and AR03T5 of Kokrajhar, AR05T1, AR05T2, AR05T3, AR05T4 and AR05T5 of Karbi-Anglong and AR06T2 and AR06T5 of Cachar. It is noticeable that 5 accessions from Karbi Anglong district had been clustered in the same group indicating similar biochemical characters among the accessions which might be due to close genetic relationship and similar growing conditions.

Genetic variability, heritability and genetic advance in biochemical constituents and quality traits

An analysis of genetic variability parameters among the evaluated biochemical and quality traits revealed a wide range of variation, indicating substantial potential for genetic improvement through selection. High Genotypic Coefficient of Variation (GCV) and Phenotypic Coefficient of Variation (PCV) values were observed for several traits, notably reducing sugar (20.24 % and 20.29 %), total flavonoid content (21.91 % and 21.96 %), limonin in albedo and juice (23.76 % for both) and TSS (20.87 % and 20.90 %). The minimal differences between GCV and PCV for these traits suggest that the expression of these traits is predominantly governed by genetic factors with minimal environmental influence. Heritability in the broad sense (h^2) was remarkably high for most traits, with values exceeding 99 % for titratable acidity, total flavonoid content, total sugar and limonin content, indicating strong genetic control. Genetic Advance as a percentage of the mean (GA %) was also high for these traits, particularly for limonin (48.94 %), reducing sugar (41.60 %), total sugar (37.11 %) and total flavonoids (45.01 %), which further supports the predominance of additive gene action and suggests that these characters can be effectively improved through direct selection. On the other hand, traits such as ascorbic acid content exhibited lower heritability (28.89 %) along with a comparatively higher difference between GCV and PCV (7.21 %), indicating a greater influence of environmental factors and less scope for genetic improvement through selection alone. However, despite this, traits like essential oil content (GCV: 10.27 %, h^2: 93.3 %, GA: 20.44 %) and non-reducing sugar (GCV: 17.02 %, h^2: 96.95 %, GA: 34.53 %) still demonstrated considerable genetic influence and moderate improvement potential. Overall, the traits with high heritability, high GCV and high GA, namely reducing sugar, TSS, total flavonoid content, limonin content and total sugar emerges as promising targets for selection in citrus breeding programs aimed at enhancing fruit quality and biochemical traits.

Correlation studies: Correlation of fruits per tree and biochemical parameters with soil quality parameters

Correlation among the biochemical parameters and soil quality parameters from various accessions are presented in Fig. 3. Data revealed that yield (fruits per tree) exhibits significant positive correlation with organic carbon and available nitrogen in soil. TSS, total sugar, reducing and non-reducing sugar and TSS-acid ratio has positive correlation with available potassium in soil. However, ascorbic acid and limonin content was found to correlate negatively with available potassium. The interpretation of strong correlations among soil and quality as well as yield are portrayed in Table 6.

Sensory evaluation

Mean data of ten evaluators with respect to peel and pulp colour, smell, taste and aftertaste are presented in Table 7. The highest score (9.00) for peel colour in AR03T5 and AR06T1. Accessions AR01T2, AR01T5, AR03T2, AR02T2, AR02T3 and AR03T5 recorded the highest score (9.00) for pulp colour. In context of taste, AR03T1, AR06T1 and AR06T4 recorded the highest score (9.00). In citrus fruits, a bitter aftertaste is felt, higher the bitterness, the lower is the scores recorded in this context. Among 30 accessions, AR05T2 recorded least bitterness and the best score (8.50) for aftertaste. In terms of smell, fourteen accessions namely AR01T1, AR01T3, AR01T4, AR02T1, AR02T4, AR03T1, AR03T3, AR03T5, AR04T1, AR04T3,

	Soil pH	Organic Carbon	Available N	Available P	Available K	Juice pH	TSS	Titratable acidity	Reducing Sugar	Non Reducing Sugar	Total Sugar	TSS acid Ratio	Total Flavanoid Content	Ascorbic acid	Anti Oxidant activity	Limonin	Essential oil	Yield
Soil pH	1																	
Organic Carbon	-.031	1																
Available N	-.189	.851**	1															
Available P	.744**	-.061	-.178	1														
Available K	.288	.108	.102	.203	1													
Juice pH	.022	-.072	.013	.024	.587**	1												
TSS	.080	-.270	-.146	-.072	.726**	.758**	1											
Titratable acidity	-.175	.321	.240	.000	-.236	-.439*	-.485**	1										
Reducing Sugar	.091	-.161	.027	-.135	.556**	.657**	.785**	-.544**	1									
Non Reducing Sugar	.004	-.228	-.073	-.129	.591**	.727**	.837**	-.540**	.876**	1								
Total Sugar	.049	-.200	-.023	-.136	.592**	.715**	.838**	-.560**	.969**	.968**	1							
TSS acid Ratio	.167	-.338	-.246	-.044	.499**	.635**	.786**	-.912**	.721**	.736**	.752**	1						
Total Flavanoid Content	-.025	.049	.084	.148	.231	-.195	.113	.287	-.067	.001	-.035	-.134	1					
Ascorbic acid	-.058	.173	.038	.052	-.449*	-.652**	-.615**	.566**	-.588**	-.617**	-.622**	-.617**	.406*	1				
Antioxidant activity	-.250	.129	.057	-.030	-.268	-.449*	-.507**	.513**	-.463**	-.469**	-.481**	-.549**	.365*	.833**	1			
Limonin	-.062	.100	.077	.018	-.426*	-.233	-.318	.096	-.258	-.297	-.287	-.256	-.107	.152	.132	1		
Essential oil	-.147	.055	.056	-.105	.090	-.031	.032	-.087	.022	-.030	-.004	.055	-.200	-.155	-.069	.202	1	
Yield	-.023	.826**	.685**	.138	.173	.096	-.245	.340	-.156	-.140	-.153	-.337	.029	.229	.269	.143	.058	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Fig. 3. Correlation among soil parameters, yield (fruits per tree) and fruit quality parameters.

Table 6. Interpretation of strongly significant influences of soil parameters on fruit quality and yield

Soil parameter	Quality/Yield parameter	r value	Significance level	Interpretation
Organic carbon	Yield	0.826	p < 0.01	High organic matter improves yield substantially
Available N	Yield	0.685	p < 0.01	Strong influence of nitrogen on yield
Available K	TSS	0.726	p < 0.01	
Available K	Reducing sugar	0.556	p < 0.01	Potassium boosts sugar accumulation
Available K	Non reducing sugar	0.591	p < 0.01	
Available K	TSS/Acid ratio	0.499	p < 0.01	Enhances sweetness-to-acidity balance
Available K	Juice pH	0.587	p < 0.01	Positively related with the juice pH

Table 7. Sensory evaluation of rough lemon accessions

Plant No.	Accession No.	Peel colour	Pulp colour	Taste	Aftertaste	Smell	Overall acceptability
1	AR01T1	5.00	7.33	5.67	4.50	9.00	6.30
2	AR01T2	6.33	9.00	6.33	7.00	7.33	7.20
3	AR01T3	6.33	8.67	4.67	7.00	9.00	7.13
4	AR01T4	7.33	7.00	7.50	7.50	9.00	7.67
5	AR01T5	7.00	9.00	5.00	6.33	8.67	7.20
6	AR02T1	7.33	8.33	4.67	4.00	9.00	6.67
7	AR02T2	8.67	9.00	4.00	7.50	6.00	7.03
8	AR02T3	5.67	9.00	5.50	5.00	8.33	6.70
9	AR02T4	6.67	8.50	5.00	4.50	9.00	6.73
10	AR02T5	4.67	7.00	4.33	5.00	8.67	5.93
11	AR03T1	6.67	8.33	9.00	7.00	9.00	8.00
12	AR03T2	7.00	9.00	7.00	5.67	8.00	7.33
13	AR03T3	8.33	7.33	5.67	5.67	9.00	7.20
14	AR03T4	5.33	7.67	5.67	5.00	8.50	6.43
15	AR03T5	9.00	9.00	5.00	4.33	9.00	7.27
16	AR04T1	7.00	8.00	4.00	4.00	9.00	6.40
17	AR04T2	4.67	7.50	4.50	5.00	8.67	6.07
18	AR04T3	5.00	6.00	5.00	4.67	9.00	5.93
19	AR04T4	6.00	7.00	4.00	5.00	9.00	6.20
20	AR04T5	7.33	7.50	4.00	6.00	6.00	6.17
21	AR05T1	6.33	8.00	4.33	7.33	8.67	6.93
22	AR05T2	8.00	6.50	5.00	8.50	9.00	7.40
23	AR05T3	5.33	7.00	4.83	7.00	8.00	6.43
24	AR05T4	7.00	7.67	5.00	5.67	8.50	6.77
25	AR05T5	8.33	6.00	6.67	5.00	9.00	7.00
26	AR06T1	9.00	8.00	9.00	5.50	8.67	8.03
27	AR06T2	7.33	5.50	5.67	4.00	9.00	6.30
28	AR06T3	8.00	7.00	8.33	5.00	8.33	7.33
29	AR06T4	7.67	7.33	9.00	8.33	8.00	8.07
30	AR06T5	7.33	7.00	5.67	4.33	8.50	6.57
Mean:		6.86	7.67	5.67	5.71	8.49	6.88
Standard deviation of samples		1.25	0.99	1.54	1.33	0.79	0.61

AR04T4, AR05T2, AR05T5, AR06T2 recorded the highest score (9.00). Overall acceptability of AR06T4 of Cachar District (Barak valley zone) recorded the best score of 8.07.

Conclusion

Thus, from the above observations on quality and sensory characters, it can be concluded that the environment of Cachar district suits well for the cultivation of rough lemon, basically used for table purposes. However, correlation of the quality and yield characters with the soil parameters generates light on nutrient management of rough lemon for quality improvement which otherwise grows as a neglected crop in homestead gardens. The characterization of rough lemon types collected across the state may help in crop improvement research in rough lemon especially those which focuses on the use of fruits for table as well as pharmaceutical purposes.

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Authors' contributions

UK, PD, MDP have made substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data. BKB, BG and AK have been involved in statistical analysis of data, presentation and drafting the manuscript or revising it critically for important intellectual content and PP have been involved in all the above activities included in collection of germplasm, laboratory analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

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