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Quantitative trait diversity in acid lime (*Citrus aurantifolia* Swing.) under West Bengal's conditions

R. Swamy Sekhar[1]* and S. Kundu[2]

*[1]Department of Fruit Science, College of Horticulture, Dr. Y.S.R. Horticultural University, Anantharajupeta-516105, Andhra Pradesh, India. [2]Department of Fruit Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur-741252, Nadia, West Bengal, India.*E-mail: swamysekhar88@gmail.com*

Abstract

The genetic diversity of acid lime was analyzed across one hundred genotypes in twelve West Bengal districts, using 22 quantitative characters for characterization. The data was statistically processed for descriptive, hierarchical cluster, discriminate, correlation and principal component analysis. Descriptive analysis revealed a prominent variation in all quantitative characters among different lime collections, with wide variations recorded in eleven quantitative characters (fruit weight, rind thickness, vesicle length, juice weight, juice volume, juice percentage, number of seeds per fruit, seed weight, seed length, seed width and non-reducing sugars). Ward's cluster analysis divided 100 lime genotypes into 5 clusters. Canonical discriminant function revealed that the major characters responsible for such clustering were fruit weight, vesicle length, seed length and seed width. PCA resulted in 9 components with a cumulative variance of 78.40 %. The biplot clarified the relation between genotypes and variables and the fruit characters distributed in the biplot contributed a considerable role to the differentiation of acid lime genotypes.

Key words: Clustering, dendrogram, principal component analysis, acid lime, quantitative characters

Introduction

Acid lime and lemon are India's third most important citrus fruits after mandarin and sweet orange. Globally, acid lime and lemon contribute about 12% of the total world citrus production, but in India, they contribute 26.5% of the Indian citrus area and 25.4% of Indian citrus production (Saxena, 2015). Acid lime is popular because of its regular fruiting habit and good shelf life. Fruits are good sources of vitamin C, minerals and salts. Acid lime and its juices probably have a greater variety of beverage, industrial and medicinal uses than any other fruits. Acid lime is consumed throughout the world in the form of refreshing drinks, beverages, pickles, squash and in cooking. Acid lime pickles are very popular not only in India but also in other parts of the world. It acts as an appetizer, stomachic, anticorbutic, and antihelmintic, and it checks biliousness. Besides, it prevents various diseases such as arthritis, piles, dysentery, colds, influenza, constipation and scurvy. So, it has tremendous potential for commercial exploitation. Despite the tremendous potential for commercial exploitation, acid lime is yet to be given prime importance in India. It is mostly grown in homesteads and kitchen gardens in India. Few varieties have been developed for acid lime but are not commercially accepted throughout India. The diverse eco-geographical distribution in India and spontaneous mutation and natural hybridization have given rise to a wide range of genetic diversity in citrus (Dubey *et al.*, 2016).

Most existing acid lime varieties have low yield, reduced juice content, and poor fruit quality, and are vulnerable to numerous pests and diseases, underscoring the need for a cultivar with improved fruit shape, thinner peel, fewer seeds, higher juice content, and enhanced flavor. Varieties should be available throughout the year with cluster bearing habit. Fruits must be of good quality concerning higher TSS, acidity and vitamin C. Fruits should have good storage life. The variety must be tolerant to pests (particularly leaf miner) and diseases (particularly citrus canker). The major motive of plant breeders lies in improving the qualitative and quantitative traits of the existing cultivars, which has been achieved via conventional breeding that involves whole genomes followed by the selection of the highest quality recombinants among several segregating individuals. Plenty of gene pool of acid lime are available in India, but it remains unexploited. Systemic collection, evaluation, and characterization are needed to shortlist them to identify active germplasm for developing new varieties by using the selection method.

Abhilash *et al.* (2017) assessed elite acid lime cultivars for quality metrics, highlighting the significance of choosing superior strains for enhanced productivity. In a similar vein, Amar Bahadur *et al.* (2018) evaluated the growth and yield of acid lime genotypes in Nepal, emphasizing the influence of environmental factors. Deshmukh *et al.* (2015) examined the performance of acid lime varieties in Akola, India, highlighting regional variability. Dinesh *et al.* (2018) investigated acid lime clones concerning growth, yield, and quality, emphasizing the necessity for evaluation across various locations. These studies highlight the importance of evaluating acid lime in regional contexts, consistent with the current study's emphasis in West Bengal.

In West Bengal, no standard and named varieties are available, although this state is endowed with an extremely diverse population of acid lime in its diverse agroecological conditions (Kundu *et al.*, 2010). It emphasizes the need for varietal improvement. Variation and selection are fundamental aspects

of any plant improvement programme. Fruit character is the main basis of germplasm selection and the study of the genetic diversity of fruits is of utmost importance to select the elite germplasm for breeding and varietal developmental programme. Acid lime demands the survey, identification of elite germplasm, and subsequent utilization through proper fruit characterization and comprehensive variability study. Limited research has been conducted on the diversity and characterization of acid lime in West Bengal. Considering the above points, this research program was initiated to assess variability and heterogeneity among different acid lime collections and identify superior commercial cultivation genotypes.

Materials and methods

Location of survey and selection of tree: Different areas of West Bengal were surveyed and first-hand information was collected from growers to identify their preferred genotypes. One hundred genotypes of acid lime were selected, covering twelve districts of West Bengal (North 24-Parganas, Nadia, Burdwan, Purulia, Hooghly, Bankura, South 24-Parganas, Birbhum, Howrah, Murshidabad, Purba Medinipur and Paschim Medinipur) during 2019-21. Different collections were named based on code used for different districts (first two letters) and the crop acid lime (last letter A). Thus, different genotypes were BNA (collected from Bankura), BRA (Bardhaman), BIA (Birbhum), HGA (Hooghly), HRA (Howrah), MUA (Murshidabad), NAA (Nadia), PNA (North 24 Parganas), PMA (Paschim Medinipur), PRA (Purba Medinipur), PUA (Purulia) and PSA (South 24 Parganas). Twenty-two quantitative characters were chosen from 'Citrus Descriptor' cited by Bioversity International (IPGRI, 1999) to characterize acid lime genotypes.

Sample Collection: Twenty fully matured and healthy fruits from each genotype were collected randomly from different directions of the canopy, quantitative observations including biochemical analysis were carried out at the Department of Fruit Science, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, West Bengal. Fruit and seed weight were measured by using electronic (digital) balance. Fruit length, diameter, rind thickness, vesicle length and seed size were measured by vernier caliper. The total soluble solids content of fruits was determined with the help of a digital refractometer, calibrated in ^{0}brix at 20 ^{0}C. Titratable acidity, total sugar, reducing sugar and non-reducing sugars were estimated by following the methods as described in A.O.A.C. (1984). Ascorbic acid was estimated using the method described by Ranganna (2000).

Statistical analysis: The data obtained was statistically processed for descriptive, hierarchical cluster, discriminant and principal component analysis. Descriptive statistics used the data to provide descriptions of the population. Ward's cluster analysis method was attempted to identify relatively homogeneous groups of varieties. Cluster members were further subjected to canonical discriminant analysis for multiple group problems to find out the characters responsible for such clustering. Principal component analysis was done to clarify the relation between genotypes and variables

Results

Descriptive analysis of one hundred acid lime genotypes (Table 1) indicated a higher co-efficient of variation (>20) for eleven quantitative characters *viz*., fruit weight, rind thickness, vesicle length, juice weight, juice volume, juice percentage, number of seeds per fruit, seed weight, seed length, seed width and non-reducing sugars, in which coefficient of variation was much higher (>50) in seed weight and number of seeds per fruit.

Table 1. Variability study of different quantitative characters of acid lime

Characters	Minimum	Maximum	Mean	SD	CV (%)
Fruit weight (g)	20.75	100.00	50.85	18.96	37.29
Fruit diameter (mm)	27.76	57.40	41.29	6.42	15.56
Fruit length (mm)	31.22	91.94	52.36	9.90	18.92
Oil glands (/cm^2)	32.00	78.00	53.13	9.06	17.06
Rind thickness (mm)	0.91	2.86	1.84	0.48	25.81
Number of segments	8.00	14.33	10.42	1.03	9.89
Vesicle length (mm)	6.39	21.50	9.90	2.17	21.91
Juice weight (g)	12.00	35.50	21.44	4.63	21.57
Juice volume (ml)	12.00	35.00	21.15	4.59	21.69
Juice percentage	22.56	78.79	46.02	14.16	30.78
Number of seeds	0.00	40.00	15.22	8.78	57.69
Seed weight (g)	0.00	0.16	0.07	0.03	50.00
Seed length (mm)	0.00	11.29	7.43	2.86	38.53
Seed width (mm)	0.00	8.20	3.89	1.63	41.91
Acidity (%)	3.81	8.64	5.24	0.78	14.83
pH	1.42	2.60	1.86	0.24	12.94
TSS (^{o}brix)	5.60	9.80	7.17	0.85	11.79
TSS: Acid	0.92	1.90	1.39	0.22	16.11
Ascorbic acid (mg/100 mL juice)	28.30	54.60	37.56	5.48	14.59
Reducing sugars (%)	1.05	1.85	1.21	0.12	9.97
Total Sugars (%)	1.62	2.85	2.11	0.30	14.44
Non reducing sugars (%)	0.32	1.66	0.86	0.28	32.98

Table 2. Clusters of acid lime genotypes based on quantitative characters using Ward's clustering method

Cluster number	Cluster member
1	BNA 2, BIA 4, PMA 6, BRA 8, PUA 1, BRA 7, PSA 5, MUA 7, NAA 16, HGA 1, BRA 10, PSA 3, HGA 9, NAA 18, PSA 6, HGA 8, NAA 19, PNA 3, HGA 3, BNA 4, PRA 2, BRA 9, PRA 3, PSA 7, BRA 11, BRA 6, PSA 1, HGA 4, PNA 4, PSA 4, PMA 5, PNA 9, HRA 4, HGA 12, NAA 1, NAA 24, HRA 2.
2	NAA 8, BIA 2, NAA 13, NAA 14, NAA 6, BRA 1, PMA 3, MUA 8, NAA 5, NAA 3, MUA 1, NAA 15, BRA 2.
3	NAA 2, BNA 1, BRA 5, PUA 2, BRA 12, PNA 11, PMA 1, PUA 4, BRA 3, NAA 17, PNA 6, HRA 1, NAA 11, BIA 1, PNA 8, NAA 10, NAA 7, PMA 4, HGA 2, MUA 5, HGA 10, NAA 9, MUA 2, MUA 3, MUA 6, NAA 12, MUA 4, NAA 4.
4	NAA 21, NAA 20, NAA 25, HGA 11, HGA 5, BIA 3, BNA 3, PNA 5, HGA 7, PNA 2, PNA 7, BRA 4, PNA 1, PUA 3.
5	PRA 1, NAA 22, NAA 23, HGA 6, PNA 10, HRA 3, PMA 2, PSA 2

The variation of 11 quantitative characters (Table 1) with higher co-efficient of variation was wide in fruit weight (20.75-100 g), rind thickness (0.91-2.86 mm), vesicle length (6.39-21.50 mm), juice weight (12.00-35.50 g), juice volume (12.00-35.00 ml), juice percentage (22.56-78.79), number of seeds per fruit (0-40), seed weight (0-0.16 g), seed length (0.00-11.29 mm), seed width (0.00-8.20 mm) and non-reducing sugars (0.32-1.66 %). The wide variation in fruit weight was due to variations in fruit length (31.22-91.94 mm), juice percentage (22.56-78.79%) and fruit diameter (27.76-57.40 mm). Present study revealed wide range of variation in TSS (5.6-9.80^{o}brix), acidity (3.81-8.64%), TSS/

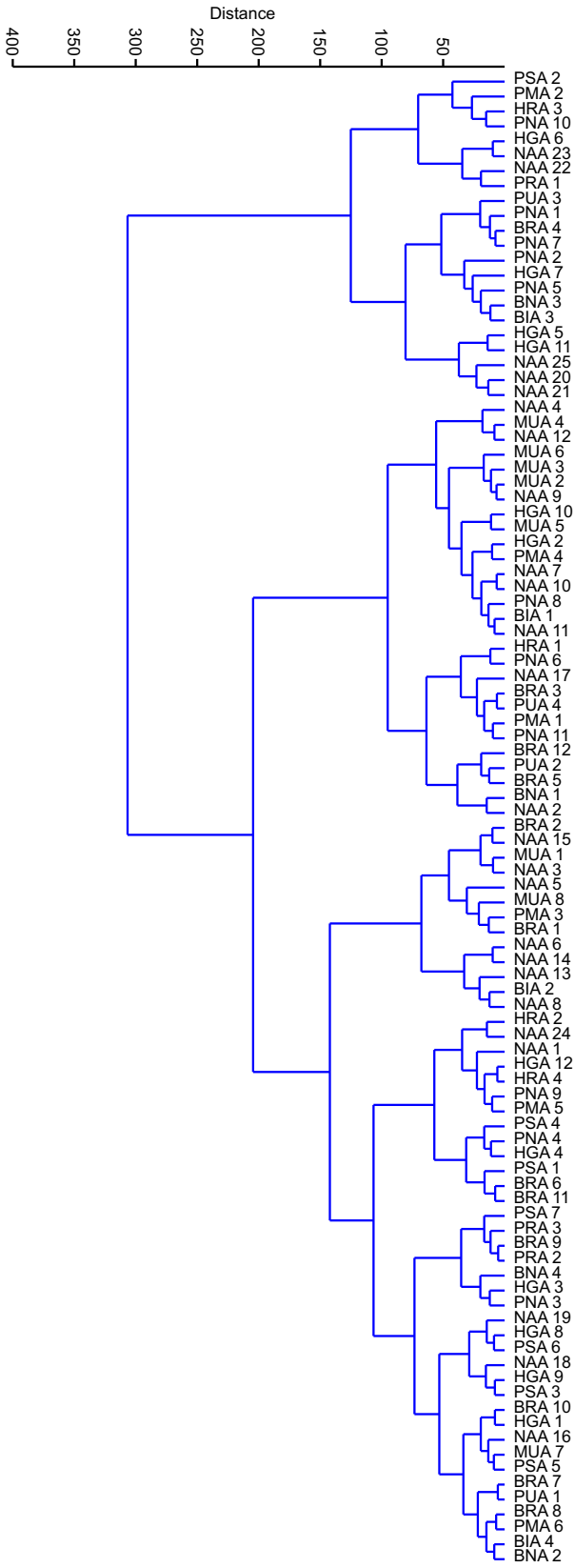

Fig. 1. Dendrogram (Ward's method) of different acid lime genotypes using quantitative characters

acid ratio (0.92-1.90), pH (1.42-2.60), total sugars (1.62-2.85%), reducing sugars (1.05-1.85%), non-reducing sugars (0.32-1.66%) and ascorbic acid (28.30-54.60 mg per100 mL juice).

Ward's cluster analysis divided 100 acid lime collections into 15 clusters with allowed distance 108 considering 22 quantitative characters (Table 2 and Fig. 1). The results showed that all the clusters were distant each other and cluster 1 was the largest one consisting of 37 acid lime genotypes followed by cluster 3 with 28 genotypes, cluster 4 with 14 genotypes, cluster 2 with 13 genotypes and cluster 5 with 8 genotypes. The canonical discriminant function revealed the major characters responsible for such clustering were fruit weight, vesicle length, seed length and seed width (Table 3). Four components were derived with eigenvalue more than 1 with a cumulative variance of 100%. Among 4 components, component 1 alone showed 88.1 variance and was highly loaded with seed length. Component 2, 3, and 4 were loaded with vesicle length, fruit weight and seed width, respectively.

Table 3. Canonical discriminant function coefficient based on quantitative characters of acid lime

Variable coefficients	Function			
	1	2	3	4
Fruit weight	-0.015	-0.018	0.067	0.005
Vesicle length	-0.237	0.715	-0.008	-0.067
Seed length	1.049	0.016	0.228	-0.458
Seed width	0.800	0.180	-0.116	0.914
(Constant)	-7.821	-6.970	-4.593	-0.258
Eigenvalue	20.667[a]	1.741[a]	.761[a]	.297[a]
% of Variance	88.1	7.4	3.2	1.3
Cumulative %	88.1	95.5	98.7	100.0
Canonical Correlation	0.977	0.797	0.657	0.479

Unstandardized coefficients

The principal component analysis resulted in 9 components using 22 quantitative characters with a cumulative variance of 78.40 percent concerning eigenvalue more than 1 (Table 4). Loading of 22 quantitative

Table 4. Component matrix resulted by PCA for quantitative characters of acid lime

Variables	Components matrix								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Fruit weight	0.84	0.20	0.25	0.22	-0.05	0.09	-0.17	-0.01	-0.14
Fruit diameter	0.75	-0.01	0.11	-0.08	-0.06	-0.37	-0.14	0.08	-0.14
Fruit length	0.59	-0.03	0.12	0.43	-0.19	0.10	0.39	-0.04	-0.24
Oil glands/cm^2	0.42	-0.29	0.12	-0.10	-0.21	0.35	-0.21	0.26	0.33
Rind thickness	0.22	-0.13	-0.10	0.20	-0.05	-0.03	0.63	0.30	0.22
Number of segments	0.38	-0.05	0.16	-0.32	0.16	-0.41	-0.35	0.19	0.17
Vesicle length	0.51	0.03	0.13	-0.16	0.32	0.10	-0.07	-0.31	0.19
Juice weight	0.65	0.25	-0.50	-0.26	0.23	0.23	0.07	0.17	0.03
Juice volume	0.63	0.24	-0.51	-0.28	0.27	0.20	0.10	0.18	0.01
Juice percentage	-0.56	-0.01	-0.58	-0.36	0.22	0.08	0.25	0.10	0.16
No. of Seed	0.12	0.60	-0.29	0.28	-0.13	0.17	0.01	-0.13	-0.25
Seed weight	-0.09	0.74	-0.02	0.12	-0.17	-0.26	-0.06	0.12	0.31
Seed length	-0.17	0.74	-0.04	0.14	-0.29	0.03	0.08	-0.10	0.01
Seed width	-0.03	0.67	-0.13	-0.14	-0.05	-0.46	-0.14	-0.07	0.17
Acidity	-0.10	0.10	0.13	-0.40	-0.72	0.39	-0.11	0.17	0.02
pH	0.00	-0.03	0.44	0.24	-0.01	-0.15	0.19	0.11	0.53
TSS	-0.31	0.20	0.08	0.22	0.08	0.56	-0.48	0.34	0.01
TSS: Acid	-0.21	0.10	-0.03	0.54	0.72	0.09	-0.26	0.03	0.01
Ascorbic acid	-0.14	0.13	0.12	0.30	0.10	-0.18	0.10	0.71	-0.18
Reducing sugars	-0.23	-0.01	0.13	-0.41	0.04	-0.32	-0.01	0.28	-0.48
Total sugars	-0.10	0.38	0.65	-0.39	0.34	0.19	0.25	0.03	-0.16
Non reducing sugars	-0.05	0.40	0.64	-0.31	0.29	0.27	0.27	-0.07	0.06
Eigenvalue	3.65	2.54	2.18	1.88	1.78	1.60	1.35	1.16	1.10
Variability (%)	16.61	11.54	9.93	8.56	8.11	7.26	6.16	5.25	5.00
Cumulative %	16.61	28.14	38.07	46.63	54.74	62.00	68.16	73.41	78.41

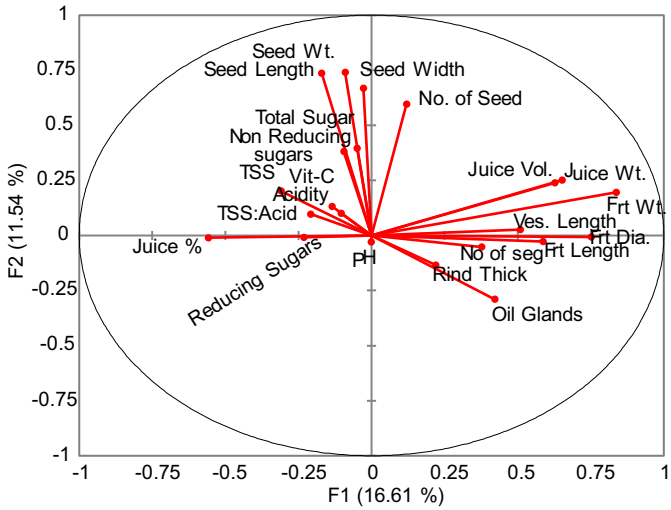

Fig. 2. Loading biplot of PCA (F1 Vs F2) for quantitative characters of acid lime

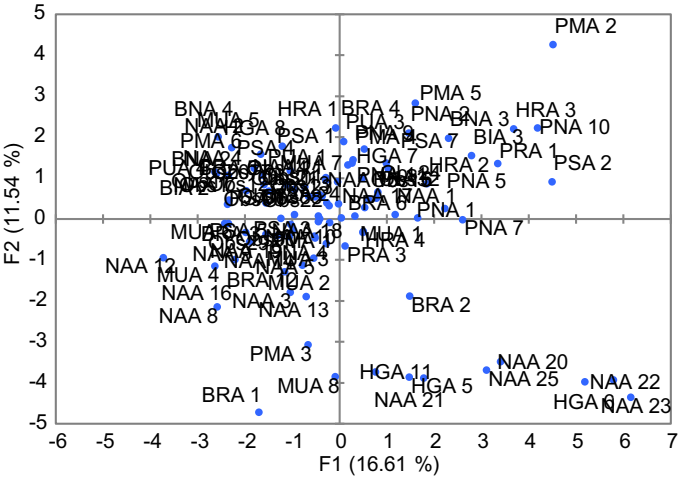

Fig. 3. Scoring biplot of PCA (F1 Vs F2) for acid lime genotypes based on quantitative characters

and scoring of 100 acid lime genotypes distributed among four quadrants of biplot (Figs. 2 and 3) indicated that genotypes remained in the 1st quadrant of the scoring plot (PMA 2, PMA 5, PSA 2, HRA 3, PNA 10, PRA 1, BIA 1, PNA 2, PUA 3 *etc.*) had higher mean values of 5 characters that were loaded in 1st quadrant of loading plot (number of seeds, juice volume, juice weight, fruit weight and vesicle length). Again, genotypes confined in the 2nd quadrant of the scoring plot (HRA 1, BNA 4, HGA 8, PMA 6, PSA 1, PUA 4, MUA 5, PUA 1 *etc.*) had higher values of 9 characters (seed width, seed weight, seed length, total sugar, non reducing sugars, TSS, ascorbic acid, acidity and TSS: acid ratio). Similarly in the 3rd quadrant of the biplot, genotypes (MUA 8, PMA 3, BRA 1, NAA 8, NAA 1, MUA 4, NAA 13, MUA 2 *etc.*) were higher in the content of pH, reducing sugars and juice percentage and in the 4th quadrant of biplot, genotypes (BRA 2, PNA 7, PRA 3, NAA 20, NAA 23, NAA 25, HGA 11, HRA 4 *etc.*) had higher values of rind thickness, oil glands, number of segments, fruit length and fruit diameter.

Discussion

Shambhulingappa *et al.* (2015) and Yadlod *et al.* (2018) found a lesser coefficient of variation (<15) in fruit characters where, as Dubey *et al.* (2016) obtained a much wider coefficient of variation (9.52-227.12) in acid lime. Wide variations recorded in eleven quantitative characters could be interpreted with a high degree of heterozygosity; therefore, genetic variation regarding these traits was very high. Hence these traits can provide scope for selection in plant breeding.

Prominent variation of physical characteristics of acid lime fruits was obtained earlier by Kundu *et al.* (2008) in West Bengal, Shambhulingappa *et al.* (2015) in the Bijapur district of Karnataka and Abhilash *et al.* (2017) in Vijayapura district. The range of variation in fruit length and size obtained by Shambhulingappa *et al.* (2015) and Yadlod*et al.* (2018) was less than that obtained in the present studies. The mean fruit weight of acid lime (50.85 g) was also found to be higher in the present studies than 34.40 g obtained by Shambhulingappa *et al.* (2015) and 39.3 g by Shreshta *et al.* (2012 b). But, Ghosh *et al.* (2012) obtained much higher fruit weight (167 g) and fruit size (7.1 x 6.2 cm) in Pati Hybrid at the laterite zone of West Bengal. However, Akhtar *et al.* (2013) obtained a similar range of variation in fruit weight and size compared to present studies.

The variation of juice percentage (Table 1) of present studies showed a significant variation (22.56-78.79%) than obtained by Athani *et al.* (2009), Shambhulingappa *et al.* (2015) and Abhilash *et al.* (2018). The wider variation of juice percentage might be due to more collections in the present studies. Interestingly, the mean juice percentage (46.02%) of acid lime was more or less similar to that of earlier findings. The variation of rind thickness (0.91-2.86 mm), number of seeds per fruit (0.00-40.00), and seed weight (0.00-0.16 g) in the present study was higher in comparison to earlier findings of Athani *et al.* (2009) and Shambhulingappa *et al.* (2015). In contrast, Akhter *et al.* (2013) obtained much higher seed weight (0.22-4.33 g) and rind thickness (0.21-0.62 cm). However, rind thickness (0.91-2.13 mm) and number of seeds per fruit (6.63-36.03) obtained by Prasad (1989) were at par with the present findings.

Wide variations in the chemical composition of fruits were also noticed by so many earlier (Shambhulingappa *et al.,* 2015; Abhilash *et al.*, 2017). The wide variation in biochemical composition of fruits in the present study offers a wide scope for breeding to develop desirable hybrids. The cluster variation might be due to heterozygosity, seedling population and nucellar embryology. In West Bengal, acid limes are planted mainly as a seedling population with other citrus species, which might result from cross-pollination; thus, wide genetic variation exists in different agroclimatic cultivation zones. Shrestha *et al.* (2012 a) found 5 clusters from 62 acid limes in Nepal, whereas Kumar *et al.* (2013) noted 4 clusters from 6 acid lime varieties at Periyakulam, Tamilnadu.

Shrestha *et al.* (2012 a) obtained 3 components with 71.3 percent accumulative variance using 7 quantitative characters, whereas Dubey *et al.* (2016) obtained 4 components with 99% variation using 11 physico-chemical characters. In the present studies, out of 22 quantitative characters, the contribution of fruit weight, fruit diameter, fruit length, oil glands, number of segments, vesicle length, juice weight, juice volume and juice percentage as observed in the first two components of PCA leads to the conclusion that these characters contributed more to the total variation observed in 100 genotypes. Therefore, the natural gene pool of acid lime from different agro-climatic zones of West Bengal was diverse, suggesting its high genetic potential, which could be used to find valuable well-adapted genotypes of intended traits.

The study revealed that there is a profound diversity among acid lime collections, and few genotypes may be exploited for various attributes based on consumer acceptance. Few of the genotypes *viz*., PUA 1, MUA 3, PNA 7, PSA 2, PNA 10, HRA 3, BIA 3, MUA 7 may be exploited as breeding material for the development of improved varieties.

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