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RESEARCH ARTICLE

Studies on Diversity of Acid Lime (*Citrus aurantifolia* Swing.) based on Important Fruit Characters under West Bengal Conditions

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The study on diversity of acid lime was undertaken using one hundred genotypes collected from twelve districts of West Bengal in the duration of 2015-17. Genotypes were selected for studies on the basis of *in situ* observation as well as information provided by the farmers. Based on consumer's preference, six important quantitative fruit characters (fruit weight, rind thickness, juice percentage, number of seed, TSS: acid, ascorbic acid) were recorded. Descriptive analysis revealed a wide range of variation particularly in fruit weight, rind thickness, juice percentage and number of seed. The genotypes were grouped into eighteen clusters by Hierarchical cluster analysis. The major characters responsible for such grouping by canonical discriminant function were fruit weight, number of seed and ascorbic acid. PCA of six fruit characters of acid lime showed three components with cumulative variance of 67.6% According to biplot analysis, genotypes HRA 3, PMA 2, NAA 21 may be exploited for higher fruit weight; genotypes NAA 2, HGA 2, MUA 3, PMA 4 for higher juice content and genotypes NAA 24, PMA 6, NAA 5, NAA 1, BRA 5 for excellent biochemical quality.

Key Words: Biplot, Cluster analysis, Descriptive analysis, Discriminant analysis, PCA

Introduction

Acid lime, an important commercial species of citrus, is considered to be indigenous to India and is extensively cultivated in many states under tropical and subtropical climatic conditions. India is the largest producer of acid lime in the world (IIFPT, 2021). Lime commonly called lebu or nimbu are very popular for its refreshing juice. Demand of fresh fruit is always high all the year round, particularly during the summer months when the price of a fruit goes up. The fruits having bigger in size with more juice and less seed content are always in market demand. In spite of tremendous potentiality for commercial exploitation, acid lime is yet to be given due importance in India. It is mostly grown in homestead and kitchen gardens in India. Few varieties have been developed for acid lime but they are not well accepted throughout India. The diverse eco-geographical distribution in India and the occurrence of spontaneous mutation and natural hybridization have given rise to a wide range of variability in citrus (Dubey *et al*., 2016).

In West Bengal, no named varieties are available. Varieties are classified only 'Pati' (round) and 'Kagzi' (oval) based on fruit shape although this state is endowed with extremely diverse population of lime in diverse agro-ecological zones (Kundu *et al*., 2010). It emphasizes the needs for varietal improvement. Acid lime should have got the importance which definitely demands the survey, identification of elite germplasm and its subsequent utilization through proper fruit characterization and comprehensive variability study. The quality of acid lime fruits primarily depends on fruit weight, rind thickness, juice percentage, number of seeds, TSS/Acid ratio and ascorbic acid which may vary according to the climate, temperature, soil fertility and genotypes. However study of fruit characters at different agro ecological regions of West Bengal is meager in the past and very limited literatures are available in this aspect. Therefore, the present study was undertaken to determine the variation of fruit characters of acid limes grown in different districts of West Bengal and select elite genotypes for commercial cultivation.

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Materials and Methods

One hundred genotypes of acid lime covering twelve districts of West Bengal were selected by thorough survey and first hand information from growers during 2015-17. The different collections were named based on code of different districts and the first letter 'A' of acid lime. Thus different genotypes were named as 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). Based on consumer's preference, six important quantitative characters (fruit weight, rind thickness, juice percentage, number of seed, TSS: acid, ascorbic acid) were chosen from 'Citrus Descriptor' of Bioversity International (IPGRI, 1999) and studied for characterization of selected lime genotypes. Twenty fully matured and healthy fruits from each genotype were collected randomly from different directions of the canopy and brought to laboratory of Fruit Science of Bidhan Chandra Krishi Viswavidyalaya for recording six core quantitative observations including analytical works. All the genotypes were seedling in origin and naturally each genotype consists of single plant. However survey was conducted during 2015-16 for recording observations of each genotype during two successive years 2016 and 2017 to minimize the effect of environment. Electronic (digital) balance was used for recording fruit weight. Fruit rind thickness was measured by slide calipers. Total soluble solids content of fruits was determined with the help of a digital refractometer and calibrated in ºbrix at 20 ºC. Titratable acidity is estimated by treating against standard alkali (N/10 NaOH) solution using phenolphthalein as an indicator and ascorbic acid was estimated by the method as described by Ranganna (2000).

The data obtained was statistically processed for descriptive analysis, hierarchical cluster analysis, discriminant analysis, principal component and biplot analysis. Descriptive statistics used the data to provide descriptions of the population. Hierarchical cluster analysis following single linkage (nearest neighborhood) method, where distance matrix was Euclidian, 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 characters responsible for such clustering. Principal component Analysis and Biplot analysis were done to clarify the relation between genotypes and variables. Data was analyzed by using SAS 9.3 software.

Results and Discussion

Descriptive analysis revealed a wide range in fruit weight (20.75 – 100.00 g), rind thickness (0.91 – 2.86 mm), juice percentage (22.56– 78.79), seed number (0.00 – 40.00), TSS/acid ratio (0.92–1.90) and ascorbic acid (28.30 – 54.60 mg/100 ml juice) among one hundred lime collections (Table 1). Prominent variation of physical characters of lime fruits was obtained earlier by Kundu *et al*. (2010) in West Bengal, Shambhulingappa *et al*. (2015) and Abhilash *et al*. (2017) in Karnataka. Among 6 quantitative characters, coefficient of variation in the present study was much higher (≥25) in fruit weight (37.29), rind thickness (25.81), juice percentage (30.78) and seed number (57.69). Higher coefficient of variation obtained in these four characters could be interpreted with a high degree of heterozygosity and therefore genetic variation with regard to these traits might be high. Hence these traits can be utilised for selection in plant breeding. Lesser coefficient of variation (<15) in fruit characters was found earlier by Shambhulingappa *et al*. (2015) and Yadlod *et al*. (2018) whereas Dubey *et al*. (2016) obtained much wider coefficient of variation (9.52-227.12) in lime.

Hierarchical cluster analysis following single linkage divided 100 acid lime collections into 18 clusters with allowed distance 1.701 considering six core quantitative characters. All the clusters were distant each other and cluster 1 was the largest one consisting of 78 lime

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

Characters	Minimum	Maximum	Mean	Std. Deviation	CV (%)
Fruit weight (g)	20.75	100.00	50.85	18.96	37.29
Rind thickness (mm)	0.91	2.86	1.84	0.48	25.81
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
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

genotypes followed by cluster 10 with three genotypes (Table 2). Canonical discriminant function revealed the major characters responsible for such clustering were fruit weight, seed number and ascorbic acid (Table 3). Cluster analysis is able to identify physico-chemical variability among different clusters. The variation among clusters might be due to heterozygosity, seedling population and nucellar embryony. From earlier studies it was noted five main clusters from 19 genotypes of lime and lemon by Zandkarimi *et al*. (2011), 5 clusters by Shrestha *et al*. (2012) and 4 clusters by Kumar *et al*. (2013) from lime diversity. The more number of clusters in the present study might be due to more collection of lime genotypes from different agro-climatic zones.

Principal component analysis of six core quantitative characters of acid lime resulted three components with cumulative variance of 67.583 per cent with reference to eigen value more than 1 (Table 4). The eigen value was high in F1 (1.758) and low in F3 (1.047). The component matrix F1 alone contributed 29.315 per cent of total variance with highly positive loading of juice percentage (0.917) and negatively loading of fruit weight (-0.933). The component F2 explained 20.813 per cent of total variance having positively loaded higher scored variables of TSS: Acid (0.721) and ascorbic acid (0.679). The component F3 explained 17.454 per cent of total variance. The positively loaded characters in this component were rind thickness (0.793) whereas number of seed was negetatively loaded (-0.442). So, the cumulative variance for quantitative traits revealed great variability with high genetic diversity and the traits had significant contribution in lime diversity. The present results are more or less similar with the earlier findings of Shrestha *et al.* (2012) who obtained 71.3 % accumulative variance using seven quantitative characters. In contrast, Zandkarimi *et al*. (2011) obtained higher accumulated variance of 85.99% using 31 characters and Dubey *et al*. (2016) obtained cumalative variation of 99% using 11 physico-chemical characters.

Biplot analysis of 100 lime genotypes indicated that six core quantitative fruit characters distributed in loading plot contributed a considerable role to the

Table 2. Clusters of acid lime genotypes based on six core quantitative characters using single linkage clustering method on squared Euclidean distance matrix

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

Table 3. Canonical Discriminant function Coefficient based on six core quantitative characters ofacid lime

Variable coefficients	Function		
	1	2	3
Fruit weight	0.072	0.009	0.031
Number of Seed	-0.137	0.017	0.081
Ascorbic acid	0.016	0.244	-0.034
Eigen value	2.542a	1.225a	.187a
% of Variance	64.3	31.0	4.7
Cumulative %	64.3	95.3	100.0
Canonical Correlation	0.847	0.742	0.397
(Constant)	-2.216	-9.837	-1.537
Unstandardized coefficients			

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

Variables	Component matrix		
	F1	F2	F3
Fruit weight	**-0.933**	0.021	0.028
Rind thickness	-0.064	-0.266	**0.793**
Juice percentage	**0.917**	-0.063	-0.016
Number of Seed	-0.138	0.437	**-0.442**
TSS: Acid	0.144	**0.721**	0.136
Ascorbic acid	0.025	**0.679**	0.448
Eigen value	1.758	1.248	1.047
Variability %	29.315	20.813	17.454
Cumulative %	29.315	50.129	67.583

differentiation of acid lime genotypes. Genotypes from the 1st quadrant (NAA 24, PMA 6, NAA 5, NAA 1, BRA 5, PUA 1, BIA 1, BIA 2, HRA 1, BNA 1 etc.) had higher mean values of ascorbic acid and TSS: acid ratio Genotypes distributed in the 2nd quadrant of scoring plot had higher number of seed (HGA 7, BIA 3, PNA 5, HGA 8, PSA 1, PNA 4 etc.) and fruit weight (HRA 3, PMA 2, NAA 21 etc.) Similarly, in the 3rd quadrant of biplot, genotypes (HGA 9, HRA 2, PNA 9, NAA 25, BRA 6, HGA 1, PRA 2, NAA 18 etc.) had higher rind thickness and higher in juice percentage (NAA 2, HGA 2, MUA 3, PMA 4, NAA 7, MUA 2, PNA 8 etc.) .

Conclusion

From the present study, it could be concluded that there is a profound phenotype diversity among acid lime collections. Genotypes HRA 3, PMA 2, NAA 21 may be exploited for higher fruit weight, genotypes NAA 2, HGA 2, MUA 3, PMA 4 for higher juice content and genotypes NAA 24, PMA 6, NAA 5, NAA 1, BRA 5 for excellent biochemical quality. Few may be utilized as important breeding material for development of improved varieties.

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