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Genetic diversity studies of acid lime genotypes using morphological markers

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DOI: https://doi.org/10.22271/chemi.2020.v8.i3j.9302

Abstract
Genetic diversity was studied in 14 acid lime genotypes by using morphological markers with the help of Mahanalobis D^2 statistical analysis at All India Co-ordinated Research project on fruits Dr. P.D.K.V., Akola during 2018-19. The genotypes grouped into 3 clusters and maximum number of genotypes was included in cluster I and the cluster II and III having individual each genotype in cluster. The inter cluster distance in all the cases were higher than the intra cluster distance indicating wider genetic diversity among the genotypes of different groups. The highest intra and inter-cluster statistical distance was observed for the cluster I and lowest cluster distance recorded in between cluster II and III. Cluster I have the highest intra cluster distance (1120.04) which included 12 genotypes. The maximum average inter cluster distance was observed in between cluster II and III (4936.37). Lowest average inter cluster distance was between I and II (2346.10). The six axes accounted total variation among acid lime genotypes (92.9%) The characters spreading (E-W) (N-S) (m), ascorbic acid (mg/100ml), plant height (m), average weight of fruits, no. of fruits per plant, juice percentage etc. Showing maximum contribution towards genetic diversity. Magnitude of cluster means for different traits and performance of genotypes of cluster II and III may be considered as parents for hybridization program.

Keywords: Acid lime, genetic diversity, D^2 analysis, cluster distance, cluster means

Introduction
Acid lime (*Citrus aurantifolia*) is considered as one of the most important commercial fruit crops under the genus citrus. As a lime contain most important limiting vitamin like vitamin 'C' and mineral like calcium and iron. In India 899 thousand ha and 11515 MT respectively. In Maharashtra area under Citrus cultivation 135.66 Thousand ha and production 1176.77 MT most of area occupy by Amravati, Aurangabad, Jalna, Nagpur district of Maharashtra (Anon., 2018) [1].
Genetic diversity is an important factor in any crop improvement programme for obtaining high yielding variety. Multivariate analysis such as D^2 cluster analysis have been proved to be useful in selecting better genotypes for hybridization. Mahalanobis (1949) [4] D^2 analysis has been successfully used in measuring the diversity in several crops. An understanding of nature and magnitude of variability among the existing acid lime genotypes is a prerequisite for its improvement. The divergence analysis useful tool in quantifying the degree of divergence between biological population and it helps to the plant breeder in choosing the diverse parents for purposeful hybridization (Arunachalam, 1981; Samsuddin, 1985) [2, 7]. Since publish work on acid lime is scanty, the present study has been undertaken with 14 acid lime genotypes to understand the nature and magnitude of genetic divergence and the characters contributing genetic diversity by D^2 analysis.

Materials and methods
The present investigation was carried out during 2017-2018 and 2018-2019 at AICRP on (Fruits) Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola. Analytical work of the experiment was carried out at Analytical laboratory, Department of Horticulture, Dr. Panjabrao Deshmukh Krishi Vidyapeeth. The field experiment laid out in the Randomized Block Design (RBD) with 14 genotypes replicated 3 times with 2 plant per replication. Data were recorded of characters includes plant height (m),spreading (E-W,N-S) (m), no. of branches per plant, Stem girth (cm), canopy of plant (m³), leaf area (cm²), leaf lamina length (mm),

leaf lamina width (mm), petiole length (mm), petal length (mm), pedicel length (mm), no. of seed per fruit, no. of segment per fruit, peel thickness (mm), peel weight (g), Juice (%), fruit volume (cc), 100 seed weight (g), peel to fruit ratio, average rag weight percent to fruit weight (g), average weight of fruit(g), no. of fruits per plant, fruit yield per plant (kg), Total Soluble Solids (^{0}B), acidity (%), TSS acidity ratio, ascorbic acid (mg/100ml).

Genetic diversity was studied by following Mahalanobis (1949) [4] generalized distance (D^2) extended by Rao (1952) [6] All the statistical analysis was carried out using GENSTAT-5 computer software. Average intracluster distance was calculated by the following formula as suggested by Singh and Chaudhary (1997) [8].

Average intra-cluster distance $D^2 = \sum_{i=1} D_i^2 / n_i$

Where,

$\sum D_i^2$ = The sum of the distance between all possible combinations ($n_i n_j$) of genotypes involved in a cluster.
n_i= Number of entries in cluster i
n_j= Number of entries in cluster j

Result and discussion

In this study the fourteen-acid lime were grouped into three cluster based on D^2 (Table 1). The distribution pattern indicates that the maximum number (12 each) genotypes were included in cluster I and followed by II and III. The minimum number of genotypes were included in cluster II and III.

Table 1: Grouping of acid lime genotypes into different clusters

Cluster Number	Number of genotypes	Genotypes
I	12	PDKV Lime, PDKV Bahar, PDKV Trupti, Kagzi lime local, Akola lime-1, Sriganganagar local, Akola lime-4, Akola lime-5, Sai Sarbati, Tenali, Mangalipattu, Pramalini.
II	1	PDKV Chakradhar
III	1	Vikram

The maximum intra and inter cluster distance are presented in Table 2. The inter cluster distance were higher than the average intra cluster distance, which indicated the wide genetic diversity among the acid lime genotypes of different groups than those of same cluster (Rahman and munsur, 2009) [5]. Average intra and inter cluster statstical distances for 27 characters calculated by 'Tocher's method' have been presented in Table 20. The variation for the intra cluster distance ranged from zero (Cluster I and II) to 1120.04 (Cluster I) The cluster I having the highest intra cluster distance (1120.04) which included 12 genotypes. Cluster II and III were with zero intra cluster distance and included one genotype each. The maximum average inter cluster distance was observed between Cluster II and III (D=4936.37) followed by cluster I and III (D=4936.37), lowest average inter cluster distance was found between cluster I and II (D= 2346.10). Hybridization among the genotypes drawn from widely divergent cluster with high yield potential would likely to manifest maximum heterotic combinations as well as new recombination with desired traits. Similar finding was observed in pummelo genotypes (Gaikwad, 2013) [3].

Table 2: Average intra and inter cluster distance

	Cluster I	Cluster II	Cluster III
Cluster I	1120.04	2346.10	4779.68
Cluster II		0.00	4936.37
Cluster III			0.00

Based on the cluster means value of 27 different character shown in (Table 3). Difference in cluster means existed for almost all the characters studied. The highest cluster mean value for plant height (4.80 m), no. of fruits per plant (1724), fruit yield per kg (89.86 kg), juice per cent (51.32 %) recorded in cluster I. In cluster II stem girth (60.25 cm), no. of fruits per plant (2132.33), fruit yield per kg (71.20), Total Soluble Solids (8.23 ^{0}B), juice per cent (59.40). In cluster III canopy of plant (81.53 m), no. of fruits per plant (2137), fruit yield per kg (89.94), Total Soluble Solids (8.35 ^{0}B), Total Soluble Solid and acidity ratio (1.16). These characters show the highest source of variation to estimate the diversity in acid lime genotypes.

Table 3: Cluster means of different characters

S. No.	Characters	Cluster		
		I	II	III
1	Plant height(m)	4.80	4.45	4.45
2	Spreading(E-W and N-S) (m)	4.93	4.47	5.72
3	No. of branches per plant	7.42	5.67	6.67
4	Stem girth(cm)	52.87	60.25	52.51
5	Canopy of plant (m^3)	55.86	47.12	81.53
6	Leaf area (cm^2)	16.88	12.89	10.87
7	Leaf lamina length(mm)	52.17	47.67	45.33
8	Leaf lamina width (mm)	39.14	34.33	34.67
9	Petiole length (mm)	10.28	7.00	10.00
10	Petal Length (mm)	2.80	1.70	1.73
11	Pedicel length (mm)	2.12	1.70	2.03
12	No. of seed per fruit	10.73	1.33	11.33
13	No. of segment per fruit	11.00	9.67	11.00
14	Peel thickness (mm)	1.54	1	1.70
15	Peel weight (g)	8.21	3.69	8.57
16	Juice (%)	51.32	59.40	49.18
17	Fruit Volume (cc)	40.20	33.22	41.69
18	100 seed weight (g)	7.09	3.33	7.41

19	Peel to fruit ratio	0.19	0.12	0.18
20	Average rag weight to percent fruit weight (g)	0.48	0.11	0.14
21	Average weight of fruit(g)	46.30	34.98	43.89
22	No. of Fruits per plant	1724	2132.33	2137
23	Fruit yield per plant (kg)	89.86	71.20	89.94
24	Total Soluble Solids (^{0}B)	6.80	8.23	8.35
25	Acidity (%)	7.30	7.06	7.18
26	TSS Acidity ratio	0.94	1.10	1.16
27	Ascorbic acid (mg/100ml)	30.20	28.61	28.12

Grouping was also independently by using principle component of analysis (PCA) to verify grouping obtained through D^2 statistics in six dimensional Vectors (Table 4). Contribution of the characters towards genetic divergence of the genotypes results of PCA (Principle Component Analysis) revealed that in vector I the important character responsible for genetic diversity in the major axis of differentiation were spreading (E-W) (N-S) (0.31), ascorbic acid (0.28), average weight of fruit (0.28), fruit yield per plant (0.28), acidity (0.27), Total soluble solids and acidity ratio (0.27), plant height (0.26) and total soluble solids(0.26) were maximum source of variation. In vector II stem girth (0.34), spreading (E-W) (N-S) (0.31) and average rag weight percent to fruit weight (0.19) were maximum source of variation. In vector III average weight of fruits (0.60), canopy of plant (0.50), pedicel length (0.42), no of fruits per plant (0.40) and peel thickness (0.22) which having maximum source of variation in genetic variability. In vector IV peel weight (0.35), juice percentage (0.35), ascorbic acid (0.21) characters contribute maximum variation.

In vector V pedicel length (0.43), canopy of plant (0.41), petiole length (0.40), no. of branches per plant (0.34) and stem girth (0.30).

In vector VI characters peel to fruit ratio (0.39), Total Soluble Solids (0.35), Total Soluble Solids and acidity ratio (0.19), spreading (0.14) and plant height (0.13) were important source of variation. The first six canonical roots have accounted for the first six canonical roots has accounted for 92.9% of the observed variability. First canonical roots ($\lambda 1$) has accounted 42.03% of the observed variability in the material while, comparatively less variability accounted by the rest of 5 canonical roots ($\lambda 2$ % = 25.16%, $\lambda 3$ % = 9.60%, $\lambda 4$ % = 6.82%, $\lambda 5$ % = 5.21%, $\lambda 6$ % = 4.08%). It indicated the completion of major portion of differentiation in first six phases. Contribution of various characters towards genetic diversity influence by the quantitative parameter. Fruit yield per kg accounted 42.86 % of the observed contribution in variability, followed by canopy of plant account 36.46 % of the observed total contribution in variability, No. of fruits per plant account 16.48 % contribution on total variation and stem girth which account 2.2 % contribute in total variation in selected acid lime genotypes. And other characters which account positive but less contribution in genetic variability of acid lime genotypes.

Table 4: Canonical root analysis of different characters

S. No	Characters	Vectors					
		I	II	III	IV	V	VI
1	Plant height (m)	0.266	-0.002	0.033	-0.176	-0.064	0.130
2	Spreading(E-W and N-S) (m)	0.314	0.310	0.071	-0.258	0.047	0.146
3	No. of Branches per plant	-0.202	-0.176	-0.116	0.161	0.347	-0.038
4	Stem Girth (cm)	0.058	0.340	0.046	0.043	0.309	-0.285
5	Canopy of plant (m^3)	0.161	-0.032	0.506	-0.408	0.412	-0.471
6	Leaf area (cm^2)	-0.283	-0.104	0.054	-0.036	-0.089	0.090
7	Leaf lamina length (mm)	0.123	-0.156	-0.408	-0.304	-0.094	0.097
8	Leaf lamina width (mm)	0.064	-0.263	0.323	-0.178	-0.248	0.073
9	Petiole length (mm)	0.015	-0.227	-0.341	-0.211	0.406	-0.266
10	Petal length (mm)	0.148	-0.251	-0.136	0.174	0.220	0.347
11	Pedicel length (mm)	-0.038	-0.143	0.428	-0.271	0.431	0.073
12	No. of seed per fruit	0.168	-0.293	-0.011	-0.085	-0.037	-0.125
13	No. of segment per fruit	-0.004	-0.316	0.195	0.161	-0.222	-0.366
14	Peel thickness (mm)	0.086	-0.225	0.220	-0.399	-0.139	-0.038
15	Peel weight (g)	0.214	-0.154	0.153	0.356	0.003	-0.238
16	Juice (%)	-0.198	-0.175	0.006	0.356	-0.097	0.254
17	Fruit Volume (cc)	-0.220	-0.196	0.210	-0.136	0.186	-0.046
18	100 seed weight (g)	0.165	-0.299	0.104	-0.022	0.198	-0.023
19	Peel to fruit ratio	-0.229	-0.177	0.133	0.025	0.062	0.398
20	Average rag weight to percent fruit weight (g)	-0.248	0.192	0.114	-0.082	0.146	0.121
21	Average weight of fruit (g)	0.281	0.061	0.601	-0.361	0.143	0.083
22	No. of Fruits per plant	0.208	-0.011	0.405	-0.068	-0.219	0.176
23	Fruit yield per plant (kg)	0.287	0.116	0.017	-0.106	0.057	-0.068
24	Total Soluble Solids (^{0}B)	0.261	0.014	0.060	0.054	0.209	0.359
25	Acidity (%)	0.272	-0.096	-0.063	0.134	0.080	0.041
26	TSS Acidity ratio	0.272	0.065	-0.124	0.204	0.068	0.196
27	Ascorbic acid (mg/100ml)	0.285	0.014	0.051	0.214	0.117	-0.053

The principle component of analysis revealed that the vector (I to VI) were show high positive value for spreading (E-W) (N-S) (m), average weight of fruits(g), no. of fruits per plant (kg), fruit yield per plant, ascorbic acid (mg/100 ml), acidity

(%), TSS Acidity ratio. In Vector II spreading (E-W) (N-S) (m) and stem girth. Such positive results indicated that maximum contribution towards divergence. Negative value in all six vectors indicated that the lowest contribution towards genetic divergence. The greater divergence in the present material is due to positive value characters which will offer a good scope for improvement of yield through rational selection of parents for producing heterotic acid lime hybrid.

Conclusion
Crosses involving parents belonging to most diverse genotypes are expected to exhibit maximum heterosis and create wide variability in genetic diversity. Considering magnitude of genetic distance, contribution of different traits toward the total divergence, magnitude of cluster means for different traits and performance the genotypes of cluster II and III may be considered as parents for future hybridization program.

References
1. Anonymous. Indian Horticulture Database, National Horticulture Board, 2018. www.nhb.gov.in
2. Arunachalam VA. Genetic distance in plant breeding. Indian Journal of Genetics. 1981; 4:226-236.
3. Gaikwad KA. Survey and characterization of pummelo genotypes (*Citrus grandis* L. Osbeck) grown in coastal region of Maharashtra. The Ecocsan, Special Issue: 2013; 8:353-362.
4. Mahalanobis PC. Historical note on the D^2Statistics. Sankhya. 1949; 19:237-239.
5. Rahman MM, Munsur MAZ. Genetic divergence analysis of lime. J Bangladesh Agril. Univ. 2009; 7(1):33-37.
6. Rao CR. Advanced Statstical Methods in Biometrical Research. John Wiley and Sons. Inc. New York. 1952, 390.
7. Samsuddin AKM. Genetic diversity in relation to heterosis and combining analysis in spring wheat. Theo. Appl. Genet. 1985; 70:306-308.
8. Singh PK, Chaudhary RD. Biometrical methods in quantitative genetic analysis, Kalyani publishers, New Delhi, 1997, 178-185.