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Original Paper

Assessing the genetic diversity of twenty one *Colombo limon* L. genotypes through multivariate and covariance matrix analysis

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Genetic diversity of selected 21 colombo lemon (*Colombo limon* L.) genotypes were evaluated through multivariate analysis and also through covariance matrix to identify the promising parents for an auspicious crossing program for development of location specific high yielding genotypes. To view the endurance of significant heterogeneity, the genetic constitution of selected 21 genotypes were classified into four clusters (I, II, III & IV). Among them, the assemblage cluster II dominated the maximum number of genetic constitution, whereas assemblage cluster I possessed minimum number of genetic constitution. Surrounded by the genetic constitution, the first principal axis largely affords 47.1% of the variation. Inter genotypic gap was observed the highest (13.82) between the genetic constitution in both 'CL002' and 'CL017' genotypes, followed by genotypes 'CL002' and 'CL019'. The maximum intracluster distance was noticed (23.33) for cluster IV, whereas cluster III showed the lowest intracluster distance (10.62). In between assemblage clusters I and IV showed the highest interassemblage distance (165.64), while the lowest distance (44.84) was estimated between II and III assemblages. Considering the cluster mean bulky fruit remarked in cluster IV (256.00 g). Besides these the highest fruit yield plant^{-1} was also recorded in cluster IV (20.50 kg). In respect of both quantitative parameters, assemblage IV was quite diverse compare with all other assemblages. Contemplating the immensity of genetic distance and quantitative performance the genotypes 'CL002', 'CL005', 'CL006' and 'CL011' from cluster IV obviously pertinent for productive crossing program to promote high yielding colombo lemon genotypes.

Keywords: genetic diversity, colombo lemon, genotypes, multivariate analysis, clusters

1 Introduction

Citrus fruit production is widely popular agricultural activity in the world in respect of nutritional and economic aspect (Maya et al., 2012; Sharma et al., 2015). Among the tropical and subtropical fruits, citrus fruit occupies an eminent position next to mango and guava. In case of other citrus species, colombo lemon occupied a significant position compare with other export-oriented fruit crops in Bangladesh. Last three decade's expenditure expansion, production and per head expenditure for citrus fruit extensively increased (Spreen, 2010). Through natural and artificial crossing process most of the citrus fruits are develop from four original citrus fruits such as citron, pomelo, mandarin and papeda. Some of such species of Citrus include *Citrus aurantiifolia*, *Citrus crenatifolia*, *Citrus mangshanensis*, *Citrus medica*, *Citrus latipes*, *Citrus reticulate* and *Citrus trifoliate* (Mamede et al., 2020). Hybrid cultivar such as Kinnow, Sweet orange, Lemon etc. (Grosser et al., 2000; Abbate et al., 2012).

Colombo lemon consumed directly as fresh fruit or the extracted juice is used for pickles and beverage production (Kaysar et al., 2017). Popular lemon squash is also prepared with the combination of colombo lemon and other citrus fruits. Fresh lemon juice is being drunk as a source of cooling and stimulating liquid during summer and hot weather. In the case of climate change,

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new pest infestation, pathogen and international market competition, Bangladeshi citrus cultivation faces several impediments. To apprehension of genetic variation in citrus outlining and implementation of breeding, research is essential (Uzun et al., 2009). Moreover, germplasm collection, conservation and molecular studies should be carried out for future research (Maya et al., 2012). By following update characterization method genetic variability should be studied more precisely from the collected genetic materials (Rao and Hodgkin, 2002). For international citrus improvement program, it is prerequisite to accumulation, conservation and utilization of plant genetic materials around the world. In developing countries method of germplasm accumulation still not up to the mark though germplasm conservation activity for the development of cultivated plants has been well established (Hawkes 1981; Harlan, 1984; Roose et al., 2014).

Though Colombo lemon has been cultivated for many years at Narsingdi district of Bangladesh, only one variety which is suggestive achievement has been made in varietal improvement (Hoque et al., 2017; Kaysar et al., 2017). In homestead and kitchen garden this species could also be grown very easily. To find out disease resistant/tolerant colombo lemon variety is essential because it shows susceptibility to citrus canker. Characterization and identification of genotype are based on phenotypic inspection (Zamani et al., 2013). However, for primary examination qualitative characteristics are also considered an important phenomenon. In compliance with Iezzoni and Pritts (1991), multivariate analysis is a useful tool for morphological characterization of enormous data set (Das and Gupta, 1984). Genetic variation existed in any population contribute a dominant role, in case of successful crossing among the accessions (Nwosisi et al., 2019). This genetic variation among the accession can be grouped and identified by following principal component analysis (PCA) and cluster analysis (CA) techniques (Odony et al., 2011). Availability of genetic materials and vast genetic variation is mandatory for varietal development of any crop. For this selection, assembling and determining citrus species of superior quality and high yield is a prerequisite. Regarding continuous embryo transformation and wide reproductive congruity with homogeneous species, enormous genetic variation has been subsisting among all the citrus species which are cultivated (Breto et al., 2001). Nonetheless, this heterogeneity, very few research was focused to identify highly productive and good quality colombo lemon germplasm. At Narsingdi region, ample genetic variability of colombo lemon gives a scope to identify enticing types clone which is resistant to disease as well as high nutritive value and good handling qualities. The core objective of the research work was to singularize and determine the variability of quantitative characters within 21 colombo lemon which was collected from the different agro-climatic zones of Narsingdi. Moreover, identification of the most important variables for discrimination among the accession in another vital task. As the origin and distribution of citrus genera are diverse, it was hypothesized that among the fruits indicative genetic heterogeneity will be found. Contemplating the mentioned importance and abundance of citrus fruits in Narsingdi region, the present investigation was conducted to study the genetic diversity of colombo lemon.

2 Materials and methods

2.1 Survey of locations and plant selection

Surveys were made during 2018–2019 in six different upazillas under the Narsingdi district of Bangladesh. It was the activity of *in situ* evaluation as to where the plant has been grown. A centre of diversity was studied by consulting and surveying the densely cultivated area and also the related Geographical Indicator (GI) was identified through discussion with skilled fruit scientists and Department of Agricultural Extension (DAE) officials at district and upazilla level. The plants of Narsingdi location were selected from farmer's field in Shibpur upazilla, Belabo, Monohordi, Polash, Narsingdi Sadar and Raipura upazilla of Narsingdi district. The topography of the inspected area ranged between 90° 39′ 8″ E and 90° 50′ 28″ E longitudes, 23° 56′ 18″ N and 24° 4′ 33″ N latitude, and 11 m to 13 m altitude. Considering the growth and yield of different locations total of 21 superior selected trees of colombo lemon were identified. For future cataloguing selected each tree was marked with a unique number. Different growth and yield contributing characteristics, such as the age of the tree, fruiting periodicity, bud initiation time, ripening period and fruit yield were computed consulting with respective grower through personal communication.

2.2 Collection of samples

Five mature fruits samples were collected from selected trees by following fruit maturity index and gathered to the laboratory of Regional Horticulture Research Station (RHRS) of Bangladesh Agricultural Research Institute (BARI) at Narsingdi. The samples were aggregated to determine quantitative characteristics viz., fruit weight of the individual sample, fruit size, rind weight, rind thickness, pulp weight, no. of seeds/fruit, per cent TSS. With the help of electronic balance fruit weight was estimated, while fruit size and rind thickness were recorded by using digital slide calipers. Moreover, digital

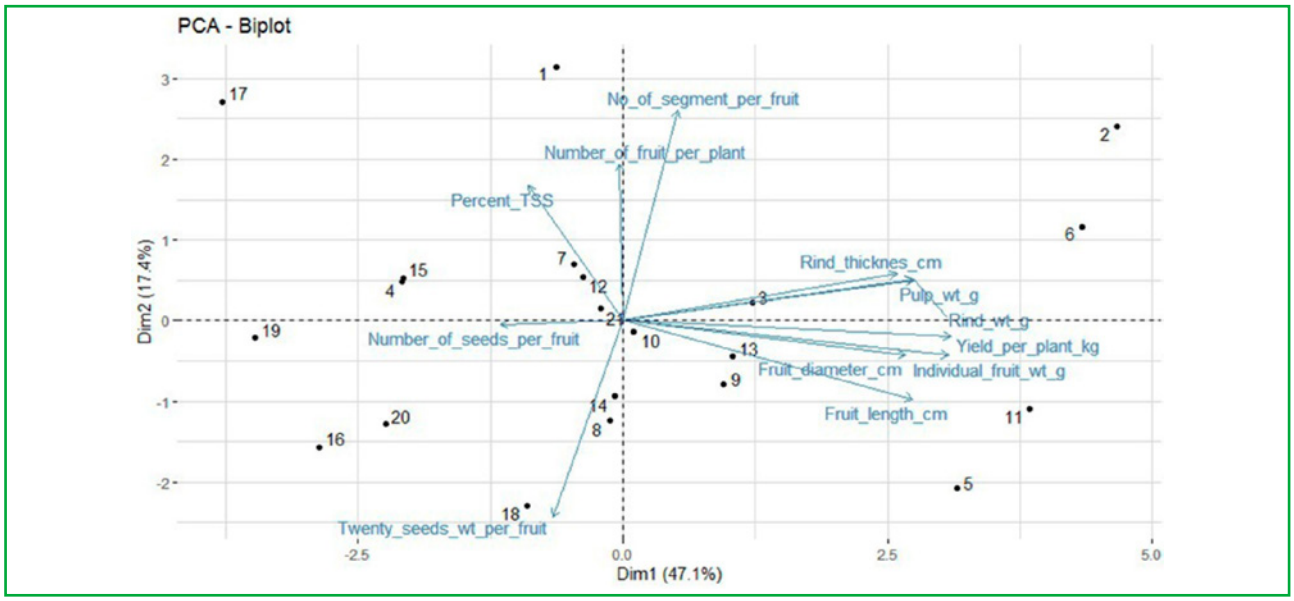

Figure 1 Biplot diagram of 21 colombo lemon genotypes based on their principal component scores

refractometer was utilized to estimate total soluble solids (% TSS Brix).

2.2 Data analysis

Through multivariate analysis techniques viz. Principal component analysis (PCA), Principal coordinate analysis (PCO), Canonical variate analysis (CVA) and Cluster analysis (CLSA) using R 3.6.3 software each character's mean datum was computed.

3 Results and discussion

3.1 Principal component analysis (PCA)

Principal components were computed from the genotype records which were attained from the first component and succeeding components with latent roots. Latent vectors of the first two principal components were discussed about the augmentation of the different morphological traits towards divergence. Eigenvalues of each principal component axes were generated by principal component analysis. In the case of the first axis, the variation among the genotypes combined computing for fruit length is 47.10 while Eigenvalues of two of these unities accounted for 64.45%. (Table 1; Figure 1). Among 12 characters which represented 21 colombo lemon genotypes accounted for 75.86% of total variation and this was accounted by the first three principal axes. From the principal component analysis of 22 lentil genotypes, Alam et al. (2011) found 78.13% total variation of the first three Eigenvalues for three principal coordination axis. For coconut, the least possible acceptable value of cumulative Eigenvalue of the principal component is 75% (Emannuel, 2002).

3.2 Construction of the biplot diagram

The significance of the augmentation of the correlative variable to the principal component is determined by the cosine of the angle between a vector and an axis. Individual fruit weight and yield/plant are the extensive contributors to the dim1. On the other hand number of seeds/fruit is the most important contributor to the dim2. Correlation between the correlative variables marked by the cosine of the angle and in between pair of vectors (Figure 1). The point which shows similar direction are highly correlated variable whereas variables are nearly perpendicular to each other which do not correlate. Individual fruit weight and yield/plant are highly correlated. The number of seeds/fruit is almost uncorrelated with the other variables.

3.3 Cluster analysis (CLA)

For non-hierarchical clustering, the 21 genotypes were arranged into four different clusters with the application of the covariance matrix. In accordance with the result of the principal component analysis of the genotypes, the clustering pattern was fixed. To align the genotypes into more or fewer uniform groups cluster analysis is used. The distribution pattern marked the highest number of genotypes (8) constituted in cluster II followed by cluster III (7). Cluster IV included less number of genotypes (4) compared with the mentioned two clusters. In cluster I, the least number of genotypes was found. Usually, heterogeneity is correlated with topographic heterogeneity but genetic heterogeneity is not directly linked with topographic dissemination (Luan et al., 2008 and Bauer et al., 2010). Considering the study the clustering arrangement of the genotypes explain that

Table 1 Eigenvalues and percentage of variation for corresponding 12 component characters in colombo lemon genotypes

Principle of component axis	Principal component characters	Eigenvalues	Percentage of total values accounted	Cumulative percentage
I	fruit length (cm)	5.652	47.10	47.10
II	fruit diameter (cm)	2.082	17.35	64.45
III	individual fruit weight (g)	1.369	11.41	75.86
IV	rind weight (g)	1.062	8.85	84.71
V	no. of fruit/plant	0.671	5.59	90.31
VI	rind thickness (cm)	0.371	3.09	93.41
VII	pulp weight (g)	0.301	2.51	95.93
VIII	no. of segment/fruit	0.197	1.64	97.57
IX	no. of seeds/fruit	0.169	1.41	98.98
X	20 seed weight/fruit (g)	0.063	0.53	99.52
XI	% TSS	0.055	0.46	99.98
XII	yield/plant (kg)	0.001	0.01	100.00

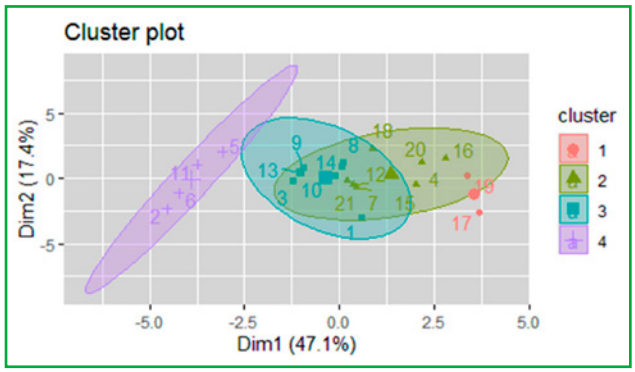

Figure 2 Cluster plot represents the distribution of 21 colombo lemon genotypes. In the figure similar symbol against number like circle, triangle, the square plus sign indicates the same cluster. In the case of cluster 1 parabolic diagram absent because of few cluster members

the genotypes didn't constitute a single cluster which is originating from the same location. It means genetic diversity and geographic diversity is not always correlated. Because of regular reciprocation of germplasm among the scientist and producer, it is happening. Thus, it could be terminated that in case of crossing and cultivar selection genetic heterogeneity should be considered and insist on more than topographic heterogeneity. Different clusters compositions with their interrelated genotypes in each cluster were presented in Table 2 and Figure 2.

3.4 Different characters cluster mean values of Colombo lemon

In Table 3 mean value of different cluster which are based on twelve characters are presented. In case of fruit length the highest mean value was noticed in cluster IV (11.70 cm) followed by cluster III (10.70 cm). The shortest fruit length (9.14 cm) was originated by the genotypes of cluster I. The genotypes under the cluster IV originated the highest (6.94 cm) fruit diameter followed by cluster III (6.49 cm). Cluster I delineated the lowest mean values (6.26 cm) of this trait. Regarding individual fruit weight, the highest result was observed in cluster IV (256.00 g) while the least weighted fruit was found in cluster I (114.00 g). The highest rind weight was found in cluster IV (129.00 g) while rind weight found lowest in cluster I (66.80 g). The number of fruit per plant got maximum (80.50) in cluster I followed by cluster II (80.30) and

Table 2 Distribution of 21 genotypes in different clusters

Cluster	Genotypes	Number of genotypes
I	CL 017, CL 019	2
II	CL 004, CL 007, CL 012, CL 015, CL 016, CL 018, CL 020, CL 021	8
III	CL 001, CL 003, CL 008, CL 009, CL 010, CL 013, CL 014	7
IV	CL 002, CL 005, CL 006, CL 011	4
Total genotypes		21

Table 3 Addressing different characters of colombo lemon genotypes cluster mean values

Characters	I	II	III	IV
Fruit length (cm)	9.14	10.30	10.70	11.70
Fruit diameter (cm)	6.26	6.30	6.49	6.94
Individual fruit weight (g)	114.00	179.00	211.00	256.00
Rind weight (g)	66.80	76.50	82.20	129.00
No. of fruit/plant	80.50	80.30	79.80	79.90
Rind thickness (cm)	0.585	0.679	0.681	0.855
Pulp weight (g)	106.00	102.00	128.00	153.00
No. of segment/fruit	11.80	11.40	11.50	11.70
No. of seeds/fruit	8.34	10.60	7.56	7.12
20 seed weight/fruit (g)	2.82	2.93	2.78	2.79
% TSS	7.40	6.56	6.57	6.53
Yield/plant (kg)	9.71	14.40	16.80	20.50

minimum in cluster III (79.80). Rind thickness obtained largest in cluster IV (0.855 cm) and smallest in cluster I (0.585 cm). Cluster IV showed the highest pulp weight (153.00 g) and cluster II showed the lowest pulp weight (102.00 g). In the case of the number of segment fruit^{-1}, the cluster I showed maximum mean value (11.80) and cluster II showed minimum mean value (11.40). The number of seeds per fruit showed maximal (10.60) in cluster II whereas in cluster IV it was delineated least in number (7.12). In cluster II twenty seed gravity showed the highest result (2.93 g) on the other hand lowest gravity (2.78) was shown in cluster III. Cluster I showed the highest% TSS (7.40) and the lowest% TSS (6.53) was found in cluster IV. The highest yield per plant was obtained from cluster IV (20.50) whereas the lowest one was from cluster I (9.71).

3.5 Principal coordinate analysis (PCO)

For adjutant support of principal component analysis principal coordinate analysis was complied. In between the genotypes, 'CL 002' and 'CL 017' maximum inter genotypic gap (13.82) was noticed which was followed by 'CL 002' and 'CL 019' (13.33), CL 006 and CL 017 (13.05). The minimum gap was noticed between the genotypes 'CL 008' and 'CL 014' (2.50) followed by 'CL 018' and 'CL 021' (2.81), 'CL 015' and 'CL 020' (3.17) (Table 4; Figure 3). According to Singh and Chaudhary (1985), the intracluster distance was determined from inter genotypic distance. Genotypes number in the cluster and significance of the

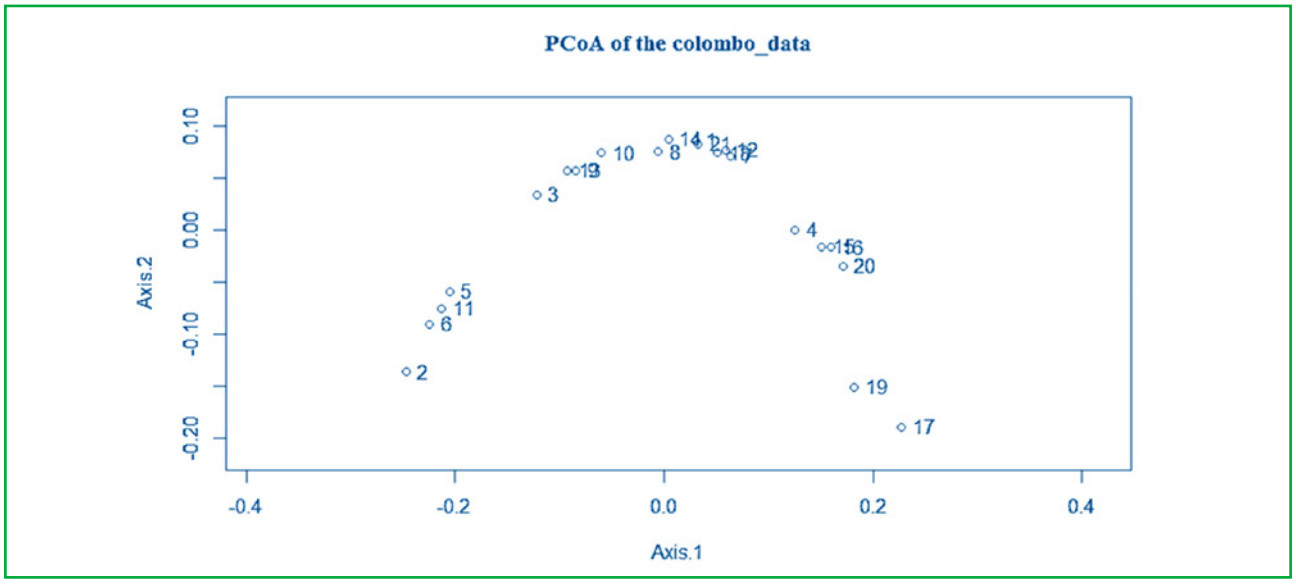

Figure 3 Principal coordinate analysis plot represents the distribution of 21 colombo lemon genotypes

Table 4 Ten higher and lower inter-genotypic distance (D2) of colombo lemon genotypes

10 higher D^2 values	Genotypes combination	10 lower D^2 values	Genotypes combination
13.82	CL 002 and CL 017	2.50	CL 008 and CL 014
13.33	CL 002 and CL 019	2.81	CL 018 and CL 021
13.05	CL 006 and CL 017	3.17	CL 015 and CL 020
12.85	CL 011 and CL 017	3.20	CL 007 and CL 018
12.76	CL 006 and CL 019	3.23	CL 007 and CL 021
12.67	CL 002 and CL 016	3.25	CL 009 and CL 010
12.53	CL 002 and CL 020	3.29	CL 003 and CL 013
12.46	CL 011 and CL 019	3.31	CL 010 and CL 013
12.44	CL 005 and CL 017	3.42	CL 007 and CL 012
12.36	CL 002 and CL 004	3.46	CL 001 and CL 012

Table 5 Intra (Bold) and intercluster distances average of 21 colombo lemon genotypes

Cluster	I	II	III	IV
I	11.73			
II	68.91	13.86		
III	101.86	44.84	10.62	
IV	165.64	110.23	74.50	23.33

intracluster distance were not always proportional. Kong et al. (2012) found fruit length and fruit girth dominated in PC1 and PC2 respectively when he studied on principal coordinate analysis in the Mediterranean and South Asian area.

3.6 Canonical variate analysis (CVA)

To obtain the inter-cluster distance (Mahalanobis' D2 values) canonical variate analysis was functioned. The values of intra- and inter-cluster distance (D2) (Nalla et al., 2014) are presented in Table 5. Surrounded by the cluster genetic heterogeneity index were defined by the statistical gap. Among the genotypes of different clusters extensive genetic diversity was advocated because of larger inter-cluster distances than intra-cluster distances. The maximum inter-cluster distance was recorded between clusters I and IV (165.64) followed by clusters II and IV (110.23) at the same time the minimum distance was found between clusters II and III (44.84) followed by cluster I and II (68.91) (Table 5). The Maximal inter-cluster distance value marked that the genotype which associated to the cluster were far departed. A close relationship between the genotypes of any cluster is called homogeneity which recommended the minimum cluster distance between the genotypes of any clusters. For attaining an ample mutation among the segregate the genotypes of far distance would be considered for crossing. Uddin et al. (2014), as well as Swain and Dikshit (1997), were made similar reports. Bauer et al. (2010) found heterosis when genotypes with greater inter-cluster distance were crossed. For greater heterotic reply it would be recommended that hybridization could have pertained between genotypes associated faraway cluster. Thus hybridization was implied between clusters I and IV (165.64) because of greater inter-cluster distance. So finally hybridization was conducted for obtaining the higher heterotic response of the desirable genotypes of the mentioned two clusters.

4 Conclusions

Inter- and intra-cluster distance, cluster mean and quantitative performance deliberately explored genetic diversity among the genotypes 'CL002', 'CL005', 'CL006' and 'CL011' from cluster IV. Hence it can be concluded that the genotypes would be suitable for productive crossing program to promote high yielding colombo lemon variety(s).

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