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RESEARCH ARTICLE

Studies on Diversity of Lemon (*Citrus limon* (L.) Burm.) based on Quantitative Traits under West Bengal Conditions

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A survey was done in 12 districts of West Bengal to identify the elite lemon genotypes resulted in the identification of 52 accessions which were further characterized by using 22 quantitative characters for descriptive analysis, hierarchical cluster analysis, discriminant analysis, correlation co-efficient analysis and principal Component analysis and biplot. Wide variation was observed in 12 quantitative characters*i.e.*fruit weight, fruit rind thickness, juice weight, juice volume, juice percentage, number of seeds per fruit, seed weight, seed length, seed width, TSS: Acid ratio, total sugar and non reducing sugars led to, hierarchical cluster analysis these collections into 20 clusters. Major characters responsible for such clustering by Canonical discriminant analysis were fruit length, juice volume, juice percentage, seed length and TSS: Acid. Principal component analysis (PCA) explained seven components with cumulative variance of 77.432%. However, biplot analysis revealed genotypes present in different quadrant of scoring plot had higher values of quantitative characters remained in corresponding quadrant of loading plot. From this study it is concluded that the variability found in lemon genotypes can be exploited for the selection of elite material for further conservation, detailed evaluation and utilization in the crop improvement programme.

Key Words: Clustering, Dendrogram, Lemon, Principal component analysis

Introduction

In India citrus fruit ranks second in area after mango and third in production after banana and mango, and accounts for 15.41% of the total fruit area and 12.89% of the total fruit production. Lime and lemon being the third most important fruits of citrus, contribute 28.51% share of citrus fruit area and 25.1% of citrus fruit production in India (Saxena, 2018). Lemon fruits have high medicinal and nutritive values as excellent source of vitamin C, beta-carotene and thiamine. Lemon is not usually taken as fresh fruit. The juice is used for making squash refreshing drink and in many culinary purposes, as a garnish of fish and meat. Fruits are used for making pickles and lemon oil. It is also used as stain remover and as a bleaching agent. The health benefits of lemon include treatment of throat infections, indigestion, constipation, dental problems fever, internal bleeding, rheumatism, burns, obesity, respiratory disorders, cholera and high blood pressure,beneficial for hair and skin care. It is very well known for its therapeutic property since generations, lemon helps to strengthen immune system, cleanse stomach, and it is considered as a blood purifier (Organic Facts, 2015).

Therefore, lemon has a great prospect for commercial exploitation despite of its tremendous potentiality; it still has not gained the importance in India. It is mostly grown in homestead and kitchen gardens and very few varieties have been developed which are also not wellaccepted/ adapted 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).

Lemon is heterozygous in nature and thus exhibits a wider variability in seedling population. Information on genetic diversity and phylogeny of cultivars can improve the efficiency of germplasm characterization and its use in breeding programs (Gulsen and Roose, 2001). Importance of clonal selections in crop improvement is well recognized by earlier workers (Badge and Patil,

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1989; Badiyala *et al*., 1992). In West Bengal, diversity of a large sample of lemon genotypes from a wide range of geographic locations has not been reported earlier. Hence, the present investigation of diversity was carried out to know the variability and heterogeneity among different lime collections and locate the elite genotypes possessing desirable fruit characteristics.

Materials and Methods

Fifty two genotypes of lemon were selected by thorough survey and first hand information from growers covering 12 districts of West Bengal during 2015-17. The different collections were named based on code used for different districts (first two letters) and the first letter 'L' of lemon. Thus, different genotypes were named as BNL (collected from Bankura), BRL (Bardhaman), BIL (Birbhum), CBL (Cooch Behar), HGL (Hooghly), HRL (Howrah), NAL (Nadia), PNL (North 24 parganas), PML (Pascim Medinipur), PRL (Purba Medinipur), PUL (Purulia) and PSL (South 24 Parganas). Characterization of selected lemon was performed using 22 quantitative characters chosen from 'Citrus Descriptor' (IPGRI, 1999).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 Department of Bidhan Chandra Krishi Viswavidyalaya for recording quantitative observation. Electronic (digital) balance was used for recording fruit and seed weight. Fruit size, fruit rind thickness, vesicle length and seed size were measured by slide calliperse. Total soluble solids content of fruits was determined with the help of a digital refractometer and calibrated in ºbrix at 20 ^{o}C. Titratable acidity, total sugar, reducing sugar and non-reducing sugars are estimated by following the methods as described in A.O.A.C. (1984). Ascorbic acid was estimated by the method as described by Ranganna (2000).

Statistical analysis was done for descriptive, hierarchical cluster, discriminant, 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

Results and Discussion

Descriptive analysis of 52 genotypes (Table 1) indicated higher co-efficient of variation (>20) for 12 quantitative

Table 1. Variability study of different quantitative characters of lemon

Characters	Minimum	Maximum	Mean	Std. Deviation	CV (%)
Fruit weight (g)	40.5	170.00	100.15	32.53	32.48
Fruit diameter (mm)	30.50	71.82	50.95	9.37	18.39
Fruit length (mm)	41.63	107.31	73.64	13.94	18.93
Oil glands (/cm2)	32.00	86.00	66.11	11.49	17.38
Rind thickness (mm)	1.45	7.16	3.32	1.33	40.02
Number of segments	7.66	14.00	10.93	1.12	10.28
Vesicle length	3.37	17.22	12.11	2.11	17.44
Juice weight (g)	21.5	64.50	33.72	8.72	25.88
Juice volume (ml)	20.00	65.00	33.26	8.85	26.62
Juice percentage	21.17	58.33	35.14	8.70	24.78
Number of seeds/ fruit	0.00	67.00	27.84	21.58	77.51
Seed weight (g)	0.00	0.35	0.08	0.06	77.34
Seed length (mm)	0.00	12.42	7.08	3.92	55.31
Seed width (mm)	0.00	6.50	3.69	2.09	56.78
Acidity (%)	4.36	7.40	5.73	0.85	14.82
pH	1.74	2.90	2.07	0.26	12.82
TSS (°brix)	4.8	8.40	6.54	0.77	11.84
TSS: Acid	0.78	1.94	1.17	0.25	21.34
Ascorbic acid (mg/100 ml juice)	32.00	55.00	40.83	5.68	13.92
Reducing Sugars (%)	1.00	1.53	1.19	0.12	10.55
Total Sugars (%)	1.58	3.33	2.21	0.52	23.85
Non Reducing Sugars (%)	0.23	2.10	0.96	0.47	49.49

characters like fruit weight, fruit rind thickness, juice weight, juice volume, juice percentage, number of seeds per fruit, seed weight, seed length, seed width, TSS: acid, total sugars and non reducing sugars. Among 12 quantitative characters the co-efficient of variation was remarkably high (>50) in seed characters like number of seeds per fruit (77.51), seed weight (77.34), seed length (55.31) and seed width (56.78). The coefficient of variation was also high in rind thickness (40.02) and non-reducing sugar (49.59). Higher co-efficient of variation revealed higher variability which indicated that superior clones can be identified from the existing variation. Dubey *et al.* (2016) also found higher coefficient of variation in traits like fruit weight, fruit length, juice volume and seed content.

Wide range of values were recorded in few important physical characters of fruits (Table 1) *viz.* fruit weight (40.5 – 170 g), fruit length (41.63 – 107.31 mm), fruit diameter (30.50 – 71.82 mm), rind thickness (1.45 – 7.16 mm), segment number (7.66-14.00), vesicle length (3.37 – 17.22 mm), seed number (0.00 – 67.00) and seed weight (0.00 – 0.35 g). Wide variation of physical characters in lemon fruit was also noticed by Govind and Singh (2002), Singh *et al*. (2009), Singh and Kaur (2009) and Archan *et al.* (2013). Fallahi *et al*. (1990) obtained lesser fruit weight (97.0 to 106.5 g) and rind thickness (3.5 to 4.1 mm) as compared to values obtained in present studies. In contrast, higher range was obtained by Rana *et al*. (2003) in fruit weight (125-525g), fruit length (9.00-13.50 cm), fruit diameter (5.27-9.68 cm) with lesser number of segments (8-12) and number of seeds (0-40). The value was moderate for fruit weight (34.0 - 157.5 g), fruit length (4.08 to 6.87 cm), fruit breadth (4.04 to 5.90 cm), peel thickness (1.9 to 4.1 mm) in 'Baramasi lemon' germplasm at Punjab conditions (Jawandha *et al.,*2012). Akhter *et al*. (2013) also observed variation in fruit weight (28.98-92.53 g), fruit length (4.77-8.03 cm), fruit width (3.23-5.73 cm), seed weight (0.81-2.00 g) and peel thickness (0.18-0.33 cm).

In the present investigation, the variation was quite high in juice volume (20.00 – 65.00 ml), juice weight (21.5-64.50) and juice percentage (21.17-58.33). Fallahi *et al*. (1990) obtained comparatively lesser juice volume (39.1 to 44.4 ml) and juice per cent (40.5 to 43.4) in eight lemon cultivars at Arizona conditions. Earlier findings revealed that the percentage of lemon juice was 16.00 to 51.20 (Rana *et al*., 2003), 49 to 57 in Kachai lemon (Singh *et al*., 2006), 29.7 to 54.6 at Punjab condition (Jawandha *et al.,* 2012) and 37.68 to 41.23 at mid hills of Meghalaya (Mukhim *et al*., 2015).

Prominent variations in bio-chemical characters of fruits like TSS (4.8 – 8.40 °brix), titratable acidity (4.36 – 7.40 %), TSS/acid ratio (0.78-1.94), pH (1.74 – 2.90), ascorbic acid (32 – 55 mg/100 ml juice), reducing sugars (1.00-1.53 %), total sugars (1.58-3.33%) and non reducing sugars (0.23-2.10) were obtained in present study among different lemon collections. Wide variations of chemical composition of fruits were also obtained by different workers (Tisserat *et al.,* 1998; Govind and Singh, 2002; Singh *et al*., 2009; Singh and Kaur, 2009 and Archan *et al.,* 2013). Earlier findings exhibited that the TSS and titratable acid content were 7.26 to 7.66 ^{o}brix and 4.8 to 5.4 per cent, respectively, in the eight lemon cultivars (Fallahi *et al*., 1990) and 7.10-9.50 ^{o}brix and 4.90-5.90 per cent, respectively, in different lemons genotypes (Rana *et al*., 2003). In 'Baramasi lemon' germplasm at Punjab conditions the TSS content ranged from 7.0 to 8.8 °brix, acidity from 4.7 to 7.3 per cent and vitamin C from 25.28 to 92.91 mg/100 ml fruit juice (Jawandha *et al.,* 2012). A moderate range was noticed by Akhter *et al*. (2013) in five lemon germplasm for pH (2.12-2.15), TSS (4.87-6.03 °brix) and vitamin-C (16.61-46.22 mg/100 ml) content. In mid hills of Meghalaya, TSS (≥6.3°Brix), titratable acidity (4.18 to 4.35%), ascorbic acid (≥32.41 mg/100 g) and TSS: acidity (≥1.51) were also good in fruits of Assam lemon (Mukhim *et al*., 2015). It is clear that the variation of biochemical composition noted in the present studies was more or less similar as earlier findings.

Hierarchical cluster analysis grouped 52 lemon collections into 20 clusters considering 22 quantitative characters (Table 2 and Fig 1). Major characters responsible for such clustering by Canonical discriminant analysis were fruit length, juice volume, juice percentage, seed length, TSS: Acid (Table 3). It was found that members of a particular cluster consisted of genotypes from various sampling areas and thus indicated that phenotypic variability was not influenced by their habitat or other environmental factors. Cluster analysis is able to identify physico-chemical variability among different clusters. The variation among clusters might be due to heterozygosity, seedling population and nucella rembryony. Earlier finding of Zandkarimi *et al*. (2011) expressed 5 main clusters from 19 genotypes of lime

Table 2. Clusters of lemon genotypes based on quantitative characters using single linkage clustering methods on squared Euclidean distance matrix

Cluster Number	Cluster Members
1.	BNL 1
2.	BNL 2, BNL 3, BNL 4, BRL 2, BIL 1, HGL 4, HGL 5, NAL 2, NAL 3, NAL 6, PNL 2, PNL 3, PNL 4, PNL 5, PNL 6, PNL 7, PML 1, PML 3, PUL 1, PSL 1, PSL 3, PSL 4, PSL 5
3.	BNL 5, NAL 7, NAL 9
4.	BRL 1
5.	BIL 2
6.	CBL 1
7.	CBL 2
8.	HGL 1
9.	HGL 2
10.	HGL 3
11.	HGL 6
12.	HGL 7, HRL 2, PML 2, PRL 1, PRL 2, PRL 3, PRL 4, PRL 5
13.	NAL 1
14.	NAL 4
15.	NAL 5
16.	NAL 8
17.	PNL 1
18.	PUL 2
19.	PUL 3
20.	PSL 2

Table 3. Canonical Discriminant Function Coefficient based on quantitative characters of lemon

Variable coefficients	Function				
	1	2	3	4	5
Fruit length	-0.046	-0.104	0.098	-0.035	0.072
Juice volume	0.127	0.256	-0.066	-0.022	-0.002
Juice percentage	0.031	0.002	0.170	0.099	0.043
Seed length	0.844	-0.167	0.010	0.034	-0.014
TSS: Acid	-2.160	0.745	-4.292	3.036	2.781
(Constant)	-5.349	-0.533	-6.078	-3.978	-9.906
Eigen value	18.629a	4.316a	3.644a	2.108a	1.347a
% of Variance	62.0	14.4	12.1	7.0	4.5
Cumulative %	62.0	76.4	88.5	95.5	100.0
Canonical Correlation	0.974	0.901	0.886	0.824	0.758
Unstandardized coefficients					

and lemon. Similarly only 5 clusters were obtained 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 lemon genotypes from different agro-climatic zones.

Principal component analysis explained 7 components using 22 quantitative characters with cumulative variance of 77.432 per cent with reference to eigen value more than 1. Zandkarimi *et al*. (2011) obtained 7 main factors using 31 characters with 85.99% of the total variance where as Dubey *et al.* (2016) obtained 4 components using 11 physico-chemical characters. In the present studies, out of 22 characters fruit weight, fruit diameter, fruit length, rind thickness, juice weight, juice volume, juice percentage, number of seed, seed width as observed in the first two components of PCA contributed more to the total variation. Higher contribution of fruit weight, fruit length and fruit width as revealed from PCA of pummelo genotypes was also obtained by Singh *et al.* (2015).

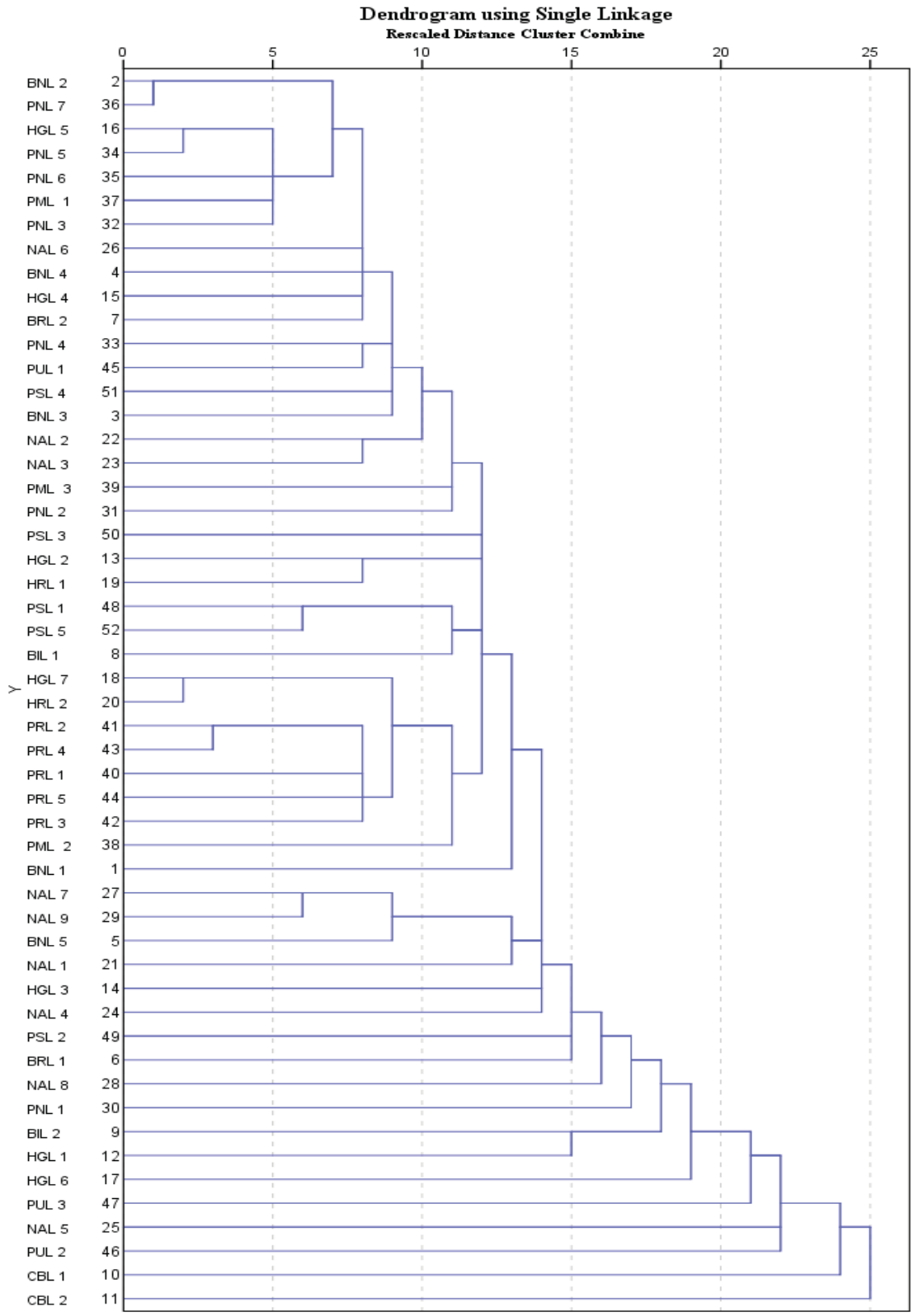

Fig. 1. Dendrogram (by single linkage) of different lemon genotypes using quantitative characters

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

Variables	Components matrix						
	F1	F2	F3	F4	F5	F6	F7
Fruit weight	**0.810**	0.447	0.071	0.067	0.133	-0.002	0.087
Fruit diameter	**0.835**	-0.008	0.172	0.197	-0.048	0.144	-0.197
Fruit length	**0.677**	0.488	-0.168	-0.120	-0.005	-0.064	0.168
Oil glands/cm^2	0.018	**0.602**	-0.174	0.149	0.479	-0.251	-0.039
Rind thickness	**0.518**	-0.130	-0.025	0.008	-0.505	0.297	-0.289
Number of segments	0.391	-0.103	-0.269	**0.464**	0.286	0.349	-0.298
Vesicle length	0.262	0.424	0.261	-0.228	0.365	**0.464**	0.034
Juice weight	**0.749**	0.443	-0.108	-0.185	-0.148	-0.268	-0.123
Juice volume	**0.753**	0.424	-0.149	-0.164	-0.142	-0.260	-0.131
Juice percentage	**-0.532**	-0.150	-0.164	-0.274	-0.302	-0.224	-0.373
Number of Seed	**0.678**	-0.475	0.185	0.109	-0.043	0.040	-0.237
Seed weight	0.405	-0.288	**0.501**	0.138	-0.075	-0.088	0.416
Seed length	0.546	**-0.574**	0.188	0.189	0.093	-0.191	0.188
Seed width	**0.553**	-0.373	0.332	0.226	-0.178	-0.191	0.141
Acidity	-0.279	0.241	**0.759**	0.076	0.085	-0.002	-0.100
pH	0.128	-0.010	-0.254	-0.512	-0.181	0.093	**0.574**
TSS	0.125	-0.383	-0.184	0.145	**0.624**	-0.297	-0.097
TSS: acid	0.288	-0.468	**-0.669**	-0.003	0.337	-0.179	0.097
Ascorbic acid	0.028	-0.111	-0.284	0.119	0.210	**0.599**	0.190
Reducing Sugars	0.206	-0.034	**-0.584**	0.219	-0.511	0.097	0.066
Total Sugars	-0.262	0.321	-0.1624	**0.811**	-0.249	-0.058	0.158
Non Reducing Sugars	-0.355	0.353	-0.0104	**0.772**	-0.146	-0.102	0.145
Eigen value	5.378	2.854	2.302	2.208	1.836	1.300	1.154
Variability (%)	24.447	12.976	10.465	10.040	8.346	5.909	5.246
Cumulative %	24.447	37.424	47.889	57.930	66.276	72.185	77.432

Biplot analysis (Fig. 2 and Fig.3) revealed that genotypes remained in the 1st quadrant of scoring plot (HGL 6, PSL 5, HGL 1, BIL 2, NAL 5, PSL 1, HGL 3, BRL 1 etc.) had obviously higher values of characters loaded in 1st quadrant of loading plot (oil glands/cm^2, vesicle length, fruit length, juice weight, juice volume, fruit weight). Again, 2nd quadrant revealed that genotypes confined in this quadrant (CBL 1, PRL 2, PRL 3, HRL 2, NAL 8, PML 2, HGL 7, PNL 5 etc.) possessed higher values of 3 characters (total sugars, non reducing sugars, acidity). Similarly genotypes (PML 3, PUL 2, HGL 4, PNL 1, NAL 9, NAL 1, BRL 2, BNL 4, PSL 2 etc.) remained in the 3rd quadrant of biplot, contained higher juice percentage and genotypes of 4th quadrant of biplot (NAL 1, NAL 3, PNL 4, HGL 2, BNL 3, PSL 4, PUL 3, BNL 5 etc.) showed higher reducing sugars, pH, ascorbic acid, rind thickness, fruit diameter, number of segments, seed weight, seed width, number of seed, seed length, TSS, TSS: Acid.

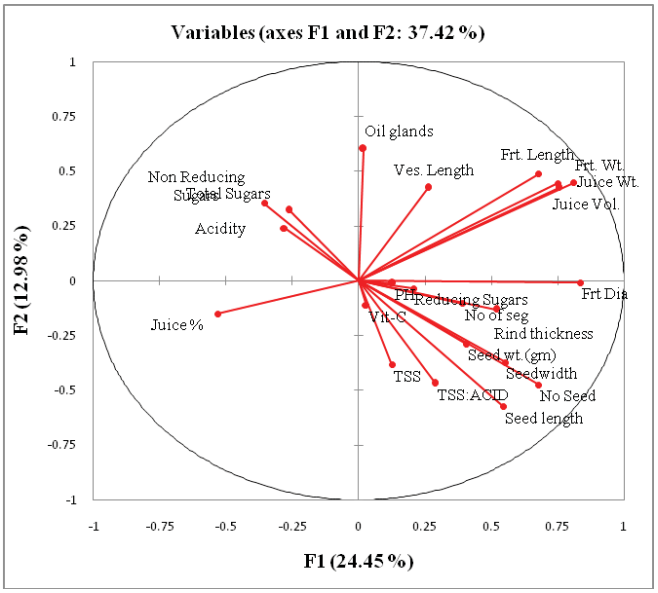

Fig. 2. Loading biplot of PCA (F1 Vs F2) for quantitative characters of lemon

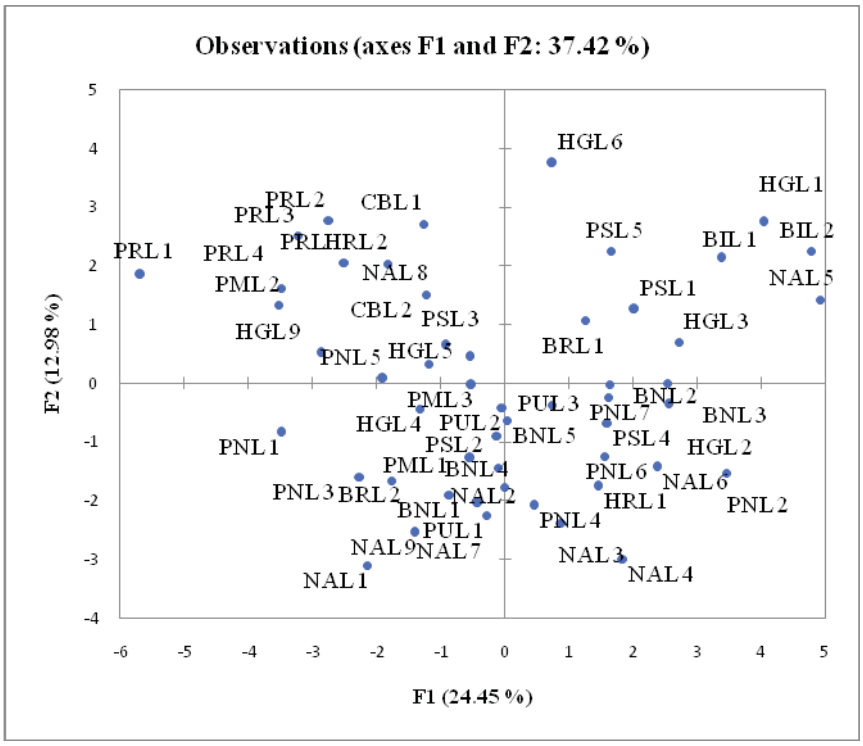

Fig. 3. Scoring biplot of PCA (F1 Vs F2) for lemon genotypes based on quantitative characters

Conclusion

From the above results, it is concluded that there is a profound diversity among acid lime collections and few genotypes may be exploited for various attributes based on consumers acceptance. Few of the genotypes viz., PNL1, PNL3, BRL2, PML2, HGL9, NAL4, HGL1, BIL2, NAL5, PUL3, BNL5, PNL7 etc.may be exploited as breeding material for development of improved varieties in lemon.

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