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Phenotypic diversity of Sri Lankan rubber clones at their immature stage

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Abstract

Systematic identification of morphological markers useful in the characterization of rubber clones has played a vital role in the crop management system. Although they do not exhibit highly distinct variations, there are certain minor, but more or less stable differences, which can be used for identification. This study was undertaken to estimate morphological diversity present in the Sri Lankan rubber clones at their immature stage and to determine the importance of morphological markers in categorizing different clones into distinct groups. Thirty clones were characterized using 29 standardized morphological descriptors. Principal component analysis (PCA) and Cluster analysis based on first twelve principal components that accounted for 85% of the total phenotypic variation distinguish 30 clones into six major clusters. Out of 29 descriptors, 27 were informative and contributed significantly to the variation among the clones selected. This study identifies the morphological descriptors that are most important for characterization of rubber clones grown in Sri Lanka.

Keywords: characterization, morphological descriptors, principal component analysis, rubber clones

Introduction

Genetic improvement in *Hevea* has been described as one of the outstanding success stories in agriculture of the 20th century. The rapid adoption of indigenously bred new clones has contributed substantially to attain higher yield both in the estate as well as in smallholding sectors of the country. Though the handfuls of improved high-yielding new clones are available, the genetic base of the existing population is very narrow.

Identification of these clones plays a vital role in the crop management system and research. Knowledge of relatively

consistent characters of a clone either singly or in combination will enable to identify different clones recommended for planting. Although clones do not exhibit highly distinct variations, most of them possess certain minor, but more or less stable morphological features, which can be used upon for identification. However, the expression of those characters is the result of the interaction of genotype with an environment in a different magnitude. Hence, it is always desirable to consider those traits that are least influenced.

Use of morphological markers is cost effective when compared to the use of biochemical and molecular markers for preliminary characterization of a large number of accessions (Martinez *et. al*., 2003). All the clones recommended for planting need to be characterized using a standard set of characters. Morphological classification for rubber is done based on the set of descriptors first suggested by Dijkman (1951), then followed by Jayasekara *et.al.* (1984), and Mercykutty *et. al*. (1991). However, the use of minimum but important list of descriptors would help save resources. Therefore, it is necessary to identify highly discriminating descriptors relevant to the clones selected in order to minimize the list of descriptors.

Principal component analysis and cluster analysis are the commonly used multivariate techniques for characterization and genetic diversity analysis of perennial crops such as tea (Wickramaratne, 1981 and Gunasekara *et. al.,* 2001) coconut (Kumaran *et.al*., 2000). However, in rubber, numbers of morphological studies were reported in all rubber producing countries (De Silva and Sachuthananthavale, (1961), Paardikooper, (1965), Jayasekara *et. al*., (1984), Mercykutty *et. al.,* (1991, 2002), Penot and Rasidin, (1994) and Michel and Beningo, (1994)) and all of them were confined to collection of morphological data for identifying the clones. There is no evidence for statistical analysis for characterization of clones based on their morphological descriptors. Therefore, the objective of the present study was to identify key morphological features contributing to the total phenotypic variation among the Sri Lankan rubber clones at their immature stage.

Materials and Methods

Thirty of the Sri Lankan rubber clones in the 100 series, 200 series and 2000 series were selected (Table 1) (Attanayake, 2001).

Table 1. *Recommended clones and their parentages*

	Clone	Parentage
01	RRIC 100	RRIC 52 x PB 86
02	RRIC 102	RRIC 52 x RRIC 7
03	RRIC 121	PB 28/59 x IAN 45/873
04	RRIC 130	IAN 45/710 x RRIC 45
05	RRIC 133	IAN 45/710 x RRIC 52
06	RRISL 201	RRIC 103 x HP 8501
07	RRISL 203	RRIC 100 x RRIC 101
08	RRISL 205	82 HP x 82 HP
09	RRISL 206	82 HP x RRIC 101
10	RRISL 208	RRIC 101 x RRIM 600
11	RRISL 210	RRIC 101 x RRIM 600
12	RRISL 211	RRIC 101 x RRIM 600
13	RRISL 215	Illegitimate
14	RRISL 216	Illegitimate
15	RRISL 217	PB 28/59 x RRIC 121
16	RRISL 218	PB 28/59 x RRIC 121
17	RRISL 219	PB 28/59 x RRIC 102
18	RRISL 220	PB 28/59 x RRIC 121
19	RRISL 221	RRIC 52 x PB 28/59
20	RRISL 222	RRIC 102 x IAN 45/710
21	RRISL 223	PB 28/59 x IAN 45/710
22	RRISL 225	RRIC 102 x PB 28/59
23	RRISL 226	RRIC 102 x PB 28/59
24	RRISL 2000	RRIC 100 x RRIC 101
25	RRISL 2001	RRIC 100 x RRIC 101
26	RRISL 2002	RRIC 100 x RRIC 101
27	RRISL 2003	82 HP x RRIC 101
28	RRISL 2004	82 HP x PB 86
29	RRISL 2005	PB 28/59 x IAN 45/710
30	RRISL 2006	PB 28/59 x RRIC 36

The clones raised in the polybags were transplanted into the field with the spacing of 3' x 3' in a completely randomized block design. Characters were recorded at the plant at 12 to 18 months aged from fifteen plants five each randomly selected from three replicates. Data were collected from the top most nature leaf whorl.

All informative morphological data were selected based on the previous studies carried out by early researchers. As the descriptors used in the study consisted of quantitative and qualitative characters such as;

- Nodes - axillary buds and leaf scars
- Leaf storey - shape, separation and external appetence of leaf storey
- Petiole and petiolule - pulvinus, shape, size, orientation, angle and size of petiole
- Leaves - colour, lusture, texture, shape and size, cross-section and longitudinal section appearance, leaf apex, leaf base, leaf margin and degree of separation

were collated.

Non-parametric data were converted on a scale of numeric data to enable them to be used in statistical analysis. Principal Component Analysis (PCA) was carried

out using means of quantitative and modes of qualitative morphological characters. Average linkage cluster analysis was performed subsequently based on the significant number of Principal Components (PC) aiming at grouping of the 30 rubber clones. The analysis was performed using SAS – Version 9.2 (Annon, 2008).

Results and Discussion

Correlation matrices obtained from the PCA of the 29 morphological descriptors shows that Eigenvalues of the first 12 principal components (PCs) were greater than one and they were significantly contributed to the variation existing in the clones studied. Furthermore, those 12 PCs accounted for the 85% of the total variation (Table 2).

Then the eigenvectors generated by the first 12 PC shows that some of the variables (which are highlighted) are comparatively higher than the other variables (Table 2). Although all the twenty-nine variables have contributed to a certain degree to deciding the position of each of first twelve principal components, it is clearly evident that some of the variables play comparatively significant role in selecting the position of each PCs indicating that they are the main contributors in each component.

When explaining the above patterns of the 29 variables, it was clearly evident that 27 variables have contributed significantly to decided the positioning of the first eleven PCs and ultimately to the variation (Table 3). Only two variables were found comparatively less significant. They were the orientation of pulvinus and the nature of veins.

Through the PC analysis, it was able to reduce the number of descriptors (or combinations of descriptors) in the form of principal components. To achieve the main objective of grouping the accessions, cluster analysis was performed based on average linkage on the first twelve principal components. The dendrogram (Fig. 1) indicates that the 30 rubber clones used in this study were grouped into six well-defined clusters at the average distance of 1.0.

Table 2. *Eigenvectors and eigenvalues of the first twelve principal components of twenty-nine quantitative traits of 30 Sri Lankan rubber clones*

Characters	Eigenvectors											
	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**	**PC 11**	**PC12**
1	-0.1383	-0.1875	-0.2483	-0.0027	-0.0945	-0.2132	0.2801	0.0120	**0.4183**	0.1542	0.1855	-0.0330
2	-0.1003	0.2320	-0.1700	0.0451	-0.1039	0.2478	0.0688	0.2613	0.2030	-0.1586	0.0703	**-0.4351**
3	0.0578	0.0706	**-0.4895**	0.0747	0.0961	0.1149	-0.2057	-0.0403	-0.0132	-0.0229	-0.2346	0.2717
4	-0.1593	-0.0768	0.2220	0.0973	**0.4359**	0.1200	-0.1923	-0.1258	-0.0473	0.0836	0.0583	-0.1127
5	-0.2259	-0.1829	0.0283	**-0.3552**	0.1279	0.0836	0.0112	-0.2205	0.0372	0.0898	0.1219	-0.0073
6	0.1482	0.1799	0.0739	**-0.4603**	0.0414	-0.2085	0.1217	-0.0514	-0.0098	-0.1599	0.2341	-0.0999
7	0.0950	0.2420	-0.2380	0.2657	**0.3297**	0.1277	0.1764	-0.0680	0.1230	0.0393	0.0823	-0.0619
8	0.2406	-0.2095	0.1173	0.0611	0.2992	-0.1127	0.2790	0.1880	0.0741	0.0255	-0.0611	0.0242
9	-0.1353	0.1162	**0.3607**	0.2583	-0.1918	0.1979	0.0153	0.1357	0.2249	0.0421	-0.0934	-0.0589
10	0.2580	0.1217	0.1022	0.0623	**0.3672**	0.0364	0.1949	0.0752	0.2730	0.2160	0.0049	0.1461
11	-0.1821	0.2611	-0.0013	0.0496	0.2185	-0.0590	0.1541	**-0.3611**	-0.2693	-0.0669	0.0935	0.2183
12	-0.0148	**-0.4186**	0.0432	0.0438	0.1892	-0.1076	-0.0810	0.2006	0.1539	-0.0145	-0.2297	0.0365
13	0.0178	0.1724	0.1021	0.1810	0.0844	**-0.3065**	0.1124	**0.4047**	-0.3412	0.0936	0.1443	0.1927
14	0.1340	**0.3411**	0.0843	-0.2690	0.0677	-0.0578	-0.1534	0.0662	0.2688	0.1480	0.1457	0.0760
15	0.2306	-0.1851	0.2350	0.0652	0.0962	0.2663	-0.0475	-0.1664	0.1811	**-0.3471**	-0.0465	0.1579
16	**0.3440**	-0.0264	0.2171	-0.1801	-0.0639	0.1006	0.0697	0.1896	0.0114	-0.0050	0.0950	-0.0734
17	**0.3033**	-0.0416	-0.1349	-0.2170	-0.0804	-0.0919	-0.2628	-0.0775	-0.0706	**0.3096**	-0.1793	-0.2123
18	**0.3551**	-0.0705	-0.0752	0.0380	-0.0757	0.1058	0.0933	-0.1514	-0.1329	0.0854	**-0.3155**	-0.2046
19	0.1627	0.0444	0.1113	0.3228	0.0856	**-0.3398**	**-0.3482**	-0.1187	-0.0559	0.0031	-0.0134	-0.1904
20	0.2208	-0.0827	0.1749	0.1380	**-0.3754**	-0.1042	0.1884	-0.1120	-0.1856	0.1827	0.1076	0.1684
21	0.1915	0.0719	-0.0945	0.2804	-0.2527	**0.3383**	0.0403	-0.1024	0.0177	0.1273	0.2901	0.1566
22	-0.1782	0.2656	**0.3979**	-0.0717	-0.0184	0.1744	-0.0324	0.0259	-0.0962	0.0054	-0.2751	-0.0962
23	0.2076	0.1184	-0.0513	-0.1438	-0.0612	-0.0082	-0.1084	0.1236	0.0770	**-0.5501**	-0.0920	**0.3779**
24	-0.1324	0.0792	0.0521	-0.1375	-0.0909	-0.0023	**0.4500**	-0.1578	0.0622	0.1514	**-0.5142**	0.1972

Characters	Eigenvectors											
	PC 1	**PC 2**	**PC 3**	**PC 4**	**PC 5**	**PC 6**	**PC 7**	**PC 8**	**PC 9**	**PC 10**	**PC 11**	**PC12**
25	-0.0582	-0.1114	0.1982	0.1019	-0.1694	-0.1718	-0.1838	**-0.3344**	**0.3557**	0.0208	0.1835	0.1960
26	0.1341	-0.1647	0.0247	-0.1825	0.1287	**0.4479**	0.0054	-0.0527	-0.2188	0.2094	0.2426	0.0583
27	-0.0858	**-0.3379**	-0.0294	0.0595	-0.0032	0.0468	0.2363	0.0681	-0.2454	**-0.3234**	0.1694	-0.1360
28	-0.2065	-0.1158	-0.0295	-0.1048	-0.0462	0.1688	-0.2456	**0.4121**	-0.0430	0.2756	0.0248	**0.3657**
29	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
Eigen value	3.958	3.097	2.411	2.201	2.163	1.739	1.681	1.628	1.438	1.308	1.142	1.037
% of total variance	14.1	11.0	8.6	7.8	7.7	6.2	6.0	5.8	5.1	4.6	4.0	3.7
% cumulative variance	14.1	25.2	33.8	41.6	49.4	55.6	61.6	67.4	72.5	77.2	81.3	**85.0**

1 - Axillary bud,
2 - Leaf scar
3 – Shape of leaf scar
4 - Shape of leaf storey
5 -Separation of leaf storey
6 - Ext. appearance of leaf storey
7 - Size and width of leaf storey
8 – Pulvinus
9 - Petiole shape
10 - Petiole size
11 – Petiole orientation
12 – Petiolue orientation
13 – Petiolule angle
14 – Petiolule size
15 – Petiolule junction appearance
16 – Leaflet colour
17 – Leaflet luster
18 – Leaflet texture
19 – Leaflet shape
20 – Leaf area
21 – Leaflet thickness
22 – Leaf margin
23 – Degree of leaflet separation
24 – Cross-sectional appearance
25 – Longitudinal sectional appearance
26 – Shape of leaf apex
27 – Shape of leaf base
28 – Colour of veins
29 – Nature of veins

Table 3. *Main contributor descriptors for each principal component (PC)*

PC	Main descriptors
1	Leaflet colour, Leaflet luster, Leaflet texture
2	Petiolue orientation, Petiolule size, Shape of leaf base
3	Shape of leaf scar, Petiole shape, Leaf margin
4	Separation of leaf storey, External appearance of leaf storey
5	Shape of leaf storey, Size and width of leaf storey, Petiole size, Leaf area
6	Petiolule angle, Leaflet shape, Leaflet thickness, Shape of leaf apex
7	Leaflet shape, Cross-sectional appearance
8	Petiole orientation, Petiolule angle, Longitudinal sectional appearance, Colour of veins
9	Axillary bud, Longitudinal sectional appearance
10	Petiolule junction appearance, Leaflet luster, Degree of leaflet separation, Shape of leaf base
11	Cross-sectional appearance, Leaflet shape
12	Leaf scar, Degree of leaflet separation, Colour of veins

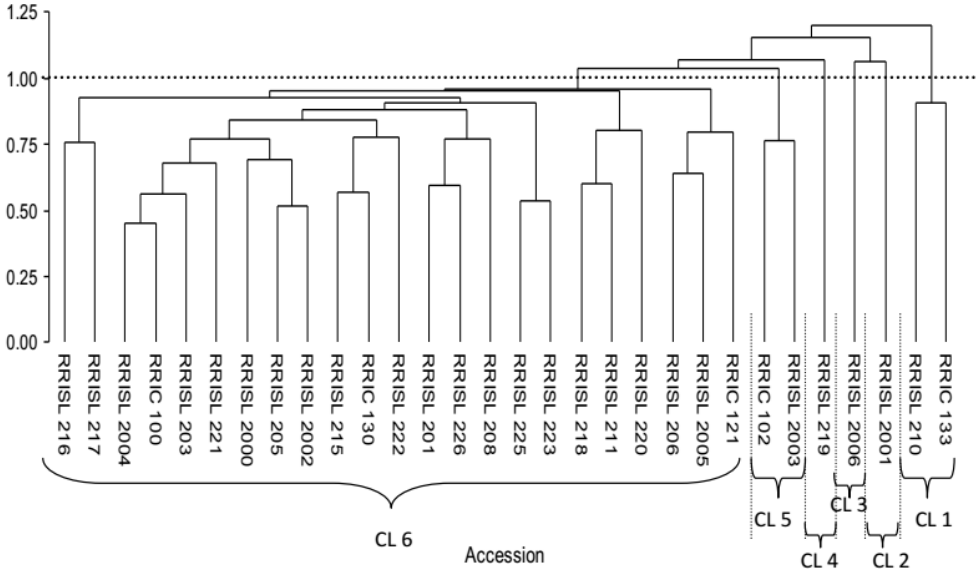

Fig. 1. Dendrogram for 30 rubber clones under studied based on average linkage cluster analysis using 29 morphological descriptors (CL 1 to CL 6 is the different clusters at average distance of 1.0)

Cluster 1 consists of two clones, RRIC 133 and RRISL 210. They possess longer petiolule, dark colour leaflets, glossy luster and smooth texture than other clones that help in the form of a separate cluster.

In cluster 2, consist only one clone RRISL 2001 and it was clearly separated from others because it had a unique morphological feature like circular shape leaf scars and it process some other very important features like broad elliptical shape leaflets, smooth leaf margin, arched shape petiole, apiculate leaf apex and it had leaflets with "S" shape in the longitudinal sectional appearance. Though these characters are not unique to the clone RRISL 2001, by a combination of these characters together it is easy to separate out this clone from others.

The cluster 3 consists only one clone RRISL 2006, and it possessed features of conical shaped leaf story, light green colour leaflets and aristate type leaf apex. The combination of these characters together helped to identify clone RRISL 2006 separately from others.

Cluster 4 includes only one clone RRISL 219 which differs significantly from the upward orientation of petiloule, broad elliptical shape leaf storey, straight appearance of the petiolule junction and touching habit of the leaflet in leaflet separation character. Although those characters are not common to RRISL 219, combinations of them together helped to identify this clone easily.

Cluster 5 includes two clones RRISL 2003 and RRIC 102, and both have common features of conical shape leaf storey, broad elliptical shape leaflets and thin (thickness) leaflets that help to form a separate cluster. Within the cluster, those two can separated from their shape of the petiole, degree of leaflet separation, the shape of the leaf apex and leaf base.

The remaining 23 clones formed a major cluster (Cluster 6) which shows more resemblances in their morphology. This is because that most of the clones possess similar traits due to lower genetic diversity as they were derived from common ancestors.

The present analysis of morphological characters provides the basis for broad classification of rubber clones and PCA identifies the variables contributing to most of phenotypic diversity while clustering helps in grouping of clones based on their degree of relationship to each other based on their common morphological features.

Conclusions

This study classified the 30 rubber clones recommended to grow in Sri Lanka into well-defined phenotypic groups. Principal component analysis (PCA) using 29 morphological descriptors and cluster analysis based on first 12 principal components (PCs) group all 30 clones

into six major clusters. Among all 29 descriptors measured, 27 descriptors highly contributed to the phenotypic diversity of the clones. Although clones do not exhibit highly distinct variations, most of them possess certain minor, but more or less stable morphological features, which can be used upon for identification. Analysis of morphological characters provides the basis for broad classification of rubber clones, and PCA identifies the variables contributing to most of phenotypic diversity of the clones.

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