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Analysis of leaf phenotypic diversity of some Hevea Accessions/clones conserved at the Institute of Agricultural Research for Development (IRAD) Ekona, Cameroon

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Abstract

This study was carried out to estimate leaf morphological diversity of some accessions/clones from IRRDB 1981 *Hevea* germplasm collection conserved at IRAD Ekona, to determine the importance of leaf morphological descriptors in differentiating accessions/clones. A total of 36 clones/ accessions were characterized using 6 leaf morphological descriptors. Analysis of variance showed that there were significant differences in the leaf morphological parameters for the studied clones. The Principal Component Analysis (PCA) showed that all leaf descriptors were informative and contributed significantly to the variation. The first 2 Principal Component scores (PCs) accounted for 88% of the total variation. The cluster analysis based on significant PCs grouped all accessions and clones in to 6 main clusters at the distance of 1.5. This study permits the characterization of *Hevea* accessions and clones in to diverse groups using leaf morphological descriptors; hence this will be advantageous for production of diverse genotypes during breeding programs to broaden the *Hevea* gene pool.

Keywords: Cluster; Germplasm; *Hevea*; Leaf morphology;

1. Introduction

The rubber tree (*Hevea brasiliensis*) is the only plant species being cultivated for commercial production of rubber in the world. It belongs to the genus *Hevea* of the family Euphorbiaceae and originated from the Amazon basin [1]. The first Cameroon's rubber plantations were established by the Germans and then by the French at the beginning of the 20th century [2]. Like in other rubber producing countries, seedlings were cultivated in Cameroon [3]. Over the years the quest of improvement in rubber breeding has led to the collection of *Hevea* clones and accessions from different rubber producing countries and the International Rubber Research and Development Board (IRRDB) 1981 expedition. The IRRDB expedition covered the three Western states of Brazil, namely Acre (AC), Rondonia (RO) and Mato Grosso (MT), in 16 districts. Wild *Hevea* germplasm was collected from 60 different locations and was distributed among the IRRDB member countries including Cameroon [4, 5]. In Cameroon a germplasm was created in Nkoolong – Kribi – South Region and later on a smaller budwood garden was created in Ekona - South West Region of Cameroon.

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The characterization and evaluation of this germplasm collection is vital in breeding and selection programs. The importance of the broad genetic base and systematically characterized germplasm in the crop improvement has been well recognized. Proper crop improvement depends on the extent of the variability (diversity) in the base population as well as the information on available characters. Consequently, genetic variability studies are crucial during the selection of parents for hybridization [6] and germplasm maintenance. Studies have revealed that, the use of phenotypic characters (Morphological markers) is more cost effective than the use of biochemical and molecular markers for preliminary characterization of large number of accessions /clones to identify phenotypically similar groups [7,8]. Leaf phenotypic characters have been used in studying diversity among plant germplasm; Pyracantha Fortunaeana [9], Sweetpotato (Ipomoea batatas) [10] and *Hevea brasiliensis* [11]. Multivariate statistical tools are extensively used to summarize and describe the inherent variation among genotypes; among them the Principal Component Analysis (PCA) and cluster analysis are commonly use to characterize and analyze genetic diversity of various crops; tea [8], rubber [12] and rice [13]. The IRRDB 1981 Hevea germplasm collection and other clones conserved at IRAD Ekona, Cameroon, have not been characterized to evaluate their diversity. The objective of this study was to estimate the leaf morphological diversity of accessions and clones in the Hevea germplasm to determine the importance of leaf morphological markers for categorizing different accessions and clones in to discrete groups. The more informative and highly causative descriptors can later be used to continue the characterization and evaluation.

2. Material and methods

This study was carried out at the site of IRAD Ekona germplasm collection located at Longitude 090 19.383'; latitude 040 12.504'; altitude 443masl. The Ekona site belongs to the humid forest zone with unimodal rainfall regime and mean precipitation is 3,076mm per year. The temperature varies between 19 and 23°C and the soils are volcanic (andosol) and suitable for rubber cultivation. The germplasm was established in 2012 with bud grafted plants at 1 m x 1 m spacing. The clones and accessions planted are presented in Table 1.

Table 1 Clones and Accessions of *Hevea brasiliensis* at IRAD Ekona budwood garden

S/N	Clone	Origin	S/N	Clone	Origin
1	AVROS 2035	Indonesia	19	PB 5/51	Malaysia
2	BR 2	Indonesia	20	PB 619	Malaysia
3	CD 1078	Brazil	21	PB 86	Malaysia
4	GT 1	Indonesia	22	PR107	Indonesia
5	HAR 60	Liberia	23	PR 257	Indonesia
6	IRCA 10	Ivory Coast	24	PR 261	Indonesia
7	IRCA 15	Ivory Coast	25	RO 42	Brazil
8	IRCA 18	Ivory Coast	26	RO 46	Brazil
9	IRCA 27	Ivory Coast	27	RO 54	Brazil
10	MDF 180	Peru	28	RRIC 100	Sri Lanka
11	PB 213	Malaysia	29	RRIC 102	Sri Lanka
12	PB 217	Malaysia	30	RRIM 513	Malaysia
13	PB 235	Malaysia	31	RRIM 527	Malaysia
14	PB 252	Malaysia	32	RRIM 600	Malaysia
15	PB 254	Malaysia	33	RRIM 701	Malaysia
16	PB 255	Malaysia	34	RRIM 703	Malaysia
17	PB 260	Malaysia	35	RRIM 705	Malaysia
18	PB 28/59	Malaysia	36	RRIM 706	Malaysia

The leaf parameters were measured after sampling randomly 10 of intact mature leaves per plant. The leaf length in cm was measured from the base of each leaf to the tip end of the blade using a measuring tape and the average value was recorded. The widths of these leaves were also measured as well as the petiole length in cm. The leaves were separated from the petiole and their individual fresh weights taken. They were latter on dried to constant weight and the leaf and petiole dry weights measured separately using an electronic balance.

2.1. Data Analysis

Data were submitted to an analysis of variance (ANOVA) and principal component analyses (PCA), using the XLSTAT 2008 statistical package. A Dendrogram was generated using cluster analysis on the first 6 principal components (PCs).

3. Results and discussion

3.1. Morphological characterization of leaves

Plant leaf characters are among the characters used to differentiate individuals of a given population [14, 15]. An analysis of variance (ANOVA) was conducted using leaf length, width, leaf fresh and dry weights, petiole length, petiole fresh and dry weights. From the ANOVA it was found that the differences in the means of leaf parameters of the studied accessions and clones were statistically significant at $p \leq 0.01$. This indicates that apart from the leaf dry weight (leaf Dwt), all other studied leaf parameters were significantly different among different clones and accessions. These results confirm the diversity in the studied germplasm. RIMM 706 clone presented the lowest value for petiole length while accession RO 54 had the highest value (Table 2).

Table 2 Analysis of variance for the leaf parameters

Clone/ Parameter	length	Width	Petiole length	Leaf Fwt	Leaf Dwt	Pet Fw	Pet Dw
RO 54	27.200 cd	10.160 bcd	31.600 d	7.634 abc	4.158 a	4.142 cd	1.559 cd
RO 46	23.800 abcd	11.280 cd	26.000 abcd	11.108 c	5.585 a	4.566 d	1.718 d
RO 42	24.700 abcd	9.700 abcd	27.100 abcd	9.509 bc	4.306 a	3.762 bcd	1.555 cd
PB 235	26.040 abcd	9.480 abcd	28.760 bcd	7.617 abc	3.370 a	3.200 abcd	1.297 bcd
PB 213	27.460 d	9.600 abcd	27.760 abcd	7.944 abc	3.193 a	2.531 abcd	0.979 abcd
RRIM705	24.000 abcd	10.000 bcd	27.800 abcd	7.281 abc	3.134 a	2.710 abcd	0.964 abcd
AVROS 2035	26.800 bcd	12.120 d	24.500 abcd	9.330 bc	3.504 a	2.156 abc	0.792 abc
PR261	23.760 abcd	9.020 abcd	31.140 cd	5.608 ab	2.610 a	2.664 abcd	1.044 abcd
GT1	25.680 abcd	9.060 abcd	23.180 abcd	6.899 abc	3.160 a	2.115 abc	0.818 abcd
RRIM 703	22.000 abcd	9.840 abcd	25.800 abcd	5.180 ab	3.155 a	2.406 abcd	0.944 abcd
PR107	26.800 bcd	8.680 abc	25.400 abcd	6.784 abc	2.812 a	2.008 abc	0.782 abc
PB 255	22.060 abcd	9.760 abcd	20.980 abcd	6.914 abc	2.604 a	2.186 abc	0.843 abcd
RRIM 701	21.000 abcd	8.800 abc	24.080 abcd	6.175 abc	2.841 a	2.341 abcd	0.927 abcd
RRIC 100	24.700 abcd	9.020 abcd	25.800 abcd	6.704 abc	2.488 a	2.112 abc	0.764 abc
RRIM 513	22.760 abcd	8.760 abc	25.360 abcd	6.678 abc	2.505 a	2.175 abc	0.859 abcd
PB 619	22.260 abcd	8.660 abc	25.140 abcd	5.855 ab	2.481 a	2.174 abc	0.820 abcd
RRIM 600	24.580 abcd	8.760 abc	22.400 abcd	7.139 abc	2.837 a	1.662 a b	0.616 ab
RRIM 527	22.160 abcd	8.040 ab	26.300 abcd	5.261 ab	2.315 a	2.172 abc	0.900 abcd
PB252	20.000 abcd	9.040 abcd	23.340 abcd	5.582 ab	2.452 a	1.809 abc	0.782 abc
HAR 60	21.060 abcd	8.160 abc	21.220 abcd	5.623 ab	2.802 a	1.659 ab	0.621 ab

IRCA 10	23.700 abcd	7.520 ab	19.300 abcd	5.945 abc	5.058 a	1.357 a	0.521 ab
PB 217	20.600 abcd	8.320 abc	22.700 abcd	5.278 ab	2.354 a	1.700 ab	0.702 abc
PB 260	20.400 abcd	7.960 ab	24.900 abcd	4.712 ab	2.127 a	1.693 ab	0.689 abc
IRCA 27	20.000 abcd	8.020 ab	18.660 abcd	5.697 ab	2.556 a	1.499 ab	0.603 ab
RRIC 102	20.380 abcd	8.320 abc	19.920 abcd	4.810 ab	2.028 a	1.729 ab	0.654 ab
PB 86	20.600 abcd	7.700 ab	20.400 abcd	5.260 ab	2.149 a	1.424 ab	0.638 ab
PB254	18.260 abc	7.640 ab	17.000 ab	4.619 ab	3.937 a	1.199 a	0.549 ab
BR2	18.840 abcd	8.060 ab	18.220 abcd	5.225 ab	2.268 a	1.199 a	0.566 ab
IRCA18	21.440 abcd	7.480 ab	17.180 ab	4.574 ab	2.137 a	1.246 a	0.458 ab
PR257	20.180 abcd	8.320 abc	17.420 abc	3.712 a	1.829 a	1.194 a	0.508 ab
PB 28/59	17.500 a	7.500 ab	19.600 abcd	3.440 a	1.957 a	1.410 ab	0.592 ab
PB5/51	19.000 abcd	7.960 ab	15.740 ab	4.903 ab	1.884 a	0.890 a	0.373 a
IRCA 15	17.820 ab	7.240 ab	14.840 a	3.999 a	1.984 a	0.978 a	0.485 ab
RRIM 706	17.875 abc	6.725 a	15.750 ab	3.463 a	1.848 a	0.827 a	0.323 a
Pr > F(Model)	<0.0001	<0.0001	<0.0001	<0.0001	0.013	<0.0001	<0.0001

(Means of the same letter in a column are not significantly different at p ≤0.01)

3.2. Principal Component Analysis

The Eigen values of the correlation matrices obtained from the PCA of the 7 descriptors are given in Table 3. Eigen values of the first five principal components (PCs) are greater than 0.1, indicating that those 5 PCs contributed more to the variation existing among the clones studied. Furthermore, those 5 PCs accounted for 99 % of the total variation.

Table 3 Results of Principal Component Analysis of 7 characters

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigenvalue	5.302	0.666	0.503	0.346	0.119	0.057	0.007
Variability (%)	75.749	9.520	7.185	4.942	1.694	0.816	0.094
Cumulative %	75.749	85.269	92.454	97.397	99.090	99.906	100.000

Table 4 revealed that the eigenvectors of some of the variables are higher than the others. However, all 7 variables contributed to a certain degree towards deciding the position of each of the first five PCs. It is clear from the table that some of the variables play comparatively more significant role in deciding the position of each PC, indicating that they are the main contributors in each component.

Table 4 Eigen vectors for the first 5 PCs of the 7 morphological characters

Character	PC1	PC2	PC3	PC4	PC5
length	0.364	-0.181	-0.580	-0.517	0.199
width	0.375	0.036	-0.390	0.649	-0.501
Petiole length	0.363	-0.591	0.125	-0.269	-0.395
Leaf Fwt	0.400	0.266	-0.261	0.187	0.553
Leaf Dwt	0.323	0.727	0.181	-0.417	-0.398
Pet Fw	0.412	-0.095	0.390	0.118	0.155
Pet Dw	0.401	-0.086	0.492	0.132	0.255

Leaf Fwt = leaf fresh weight, Leaf Dwt = Leaf dry weight, Pet Fw = Petoile fresh weight, Pet Dw = Petoile dry weight

3.3. Multivariate Cluster Analysis

Average linkage multivariate cluster analysis was done to combine the relationships of each accession/clone for the leaf morphological parameters (Figure 2).

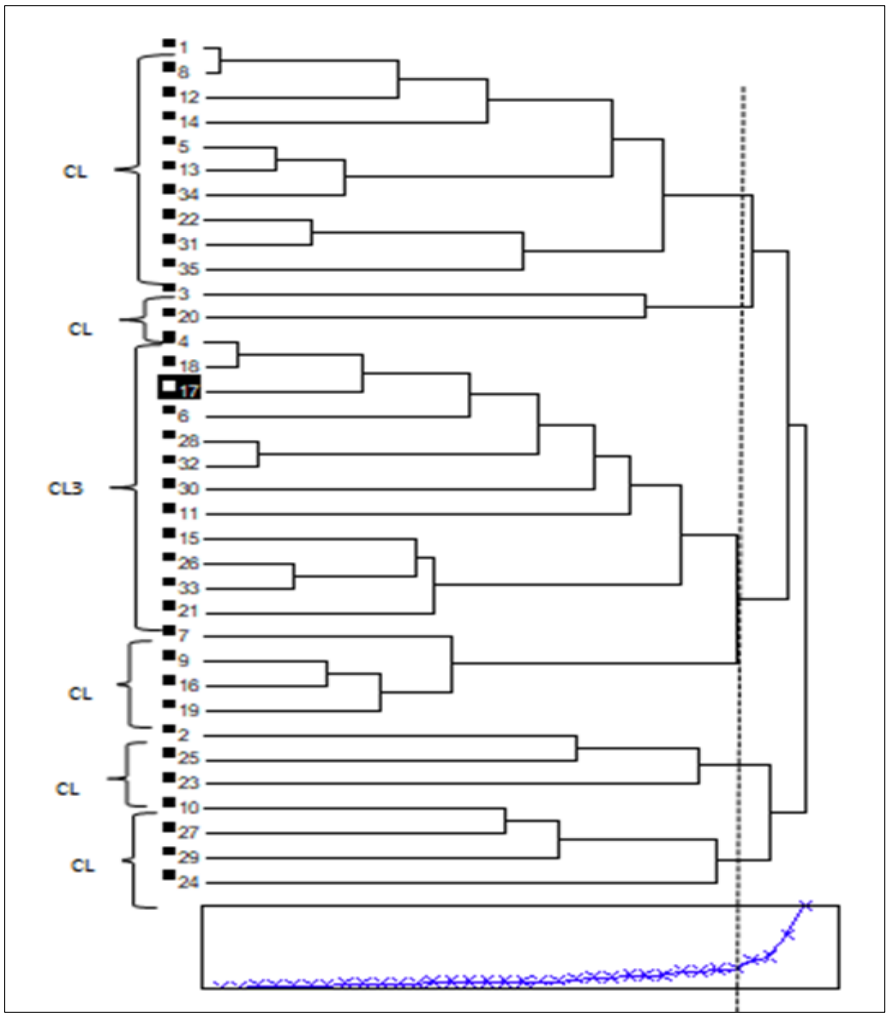

Figure 1 Dendogram for 4 accessions and 32 clones based on average linkage multivariate cluster analysis

1= RRIC 102, 2= R0 54, 3= PB 253, 4= RRIM 513, 5= IRCA 27, 6= RRIM703, 7 = RRIM 600, 8 = PB 5/51, 9 = PR107, 10 = RRIM 705, 11 = PR261, 12 = PR257, 13 = BR2, 14 = IRCA18, 15 = PB252, 16 = GT1, 17 = RRIM701, 18 = PB619, 19 = RRIC100, 20 = IRCA 10, 21 = PB260, 22 = RRIM706, 23 = RO46, 24 = AVROS 2035, 25 = RO42, 26 = HAR60, 27 = PB213, 28 = RRIM527, 29 = PB235, 30 = PB255, 31 = IRCA15, 32= RRIC 102, 33= PB217, 34 = PB86, 35= PB 28/59

The dendrogram indicates that the 4 accessions and 32 clones used in this study were grouped into 6 main clusters based on the average distance of 1.5. A detailed cluster composition is given in table 3. Most of the IRCA clones were found in cluster 1 showing their genetic relatedness with just one accession (BR 2) found in this cluster. Two clones were found in cluster 2 while cluster 3 was made up of 12 clones; the largest luster. Cluster 5 was made up only of the accessions from Rodonia showing that they were genetically very similar. This study is in agreement with other studies where it was concluded that characters of leaf petiole were the most discriminating descriptors in distinguishing the clones into phenotypically diverse groups [12].

Table 3 Cluster composition of different accessions and clones based on the leaf morphological descriptors

Cluster No.	Number of clones/accessions	Clone/accession name
1	10	RRIC 102, PB 5/51, PR 257, IRCA 18, IRCA 27, BR2, PB 86, RRIM 706, IRCA 15, PB 28/59
2	2	PB 254, IRCA10
3	12	RRIM 513, PB 619, RRIM 701, RRIM 703, RRIM 527, RRIC102, PB 255, PR 261, PB252, HAR 60, PB217, PB 260
4	4	RRIM600, PR107, GT1, RRIM 701
5	3	RO 54, RO 42, RO 46
6	4	RRIM 705, PB 213, PB 235, AVROS 2035

4. Conclusion

There were significant variations in leaf characters used between the studied accessions and clones. This study classified 4 accessions and 32 rubber clones in the germplasm into 6 well-defined groups. All the studied leaf parameters contributed to the diversity of the clones and accessions. An analysis of some leaf characters provides the basis for broad classification of this germplasm and continued evaluation.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Oktavia F, Kuswanhadi, Dinarty D, Widodo, Sudarsono. Genetic diversity and population structure of IRRDB 1981 and Wickham rubber germplasm based on EST-SSR. Journal of Agricultural Science. 2017; 39(3): 239–251.

[2] International Rubber Study Group (IRSG). 2020.

[3] Chambon B, Thierry Michels. Typology of rubber based farming systems in Cameroon: lessons for future plantings. Forum international sur le caoutchouc, International Rubber Study Group (IRSG). 29-30 March 2004; Limbe, Cameroon.

[4] Onokpise OU. Natural rubber Hevea brasiliensis germplasm (Wild. Ex A. Juss.) Mull. Arg, collection in the Amazon Basin, Brazil. A retrospective. Journal of Economic Botany. 2004; 58: 544-555.

[5] Lam LV, Thanh T, Trang LTT, Truong VV, Lam HB, Tuy LM. Hevea Germplasm in Vietnam: Conservation, Characterization, Evaluation and Utilization, Genetic Diversity of Plants. Rubber Research Institute of Vietnam, Ho Chi Minh City. 2012; 22: 433-456.

[6] Chakravorty A, Ghosh PD, Sahu PK. Multivariate analysis of phenotypic diversity of: landraces of rice of West Bengal. American Journal of Experimental Agriculture. 2013; 3(1): 110-123.

[7] Martinez L, Masuelli R, Rodriguez J. Evaluation of diversity among Argentine grapevine (Vitis vinifera L.) varieties using morphological data and AFLP markers. Journal of Biotechnology. 2003; 6(3): 242-250.

[8] Piyasundara JHN, Gunasekara MTK, Wickramasinghe IP. Identification of discriminating morphological descriptors for characterization of Tea (Camellia sinensis L.) germplasm in Sri Lanka. Tropical Agricultural Research. 2008; 20: 193-199.

[9] Lei Li, Qunxian Deng, Xiaoqing Wang, Juan Liu, Lu Liu, Qin Yang, Shifeng Luo. Diversity analysis of leaf phenotypic characters of Pyracantha fortunaeana. IOP Conf. Series: Earth and Environmental Science. 2018; 199: 022011.

[10] Jackson DM, Harrison HF. Phenotypic Variation in Leaf Morphology of the USDA, ARS Sweetpotato (Ipomoea batatas) Germplasm Collection. HORTSCIENCE. 2020; 55(4): 465–475.

[11] Anushka PVA, Liyanage KK, Withanage SP, Baddewithana BWAN. Analysis of Phenotypic Diversity of selected Hevea accessions from IRRDB 1981 germplasm collection conserved in Sri Lanka. Conference Paper• September 2018.

[12] Liyanage KK, Sumanasinghe VA, Attanayake DPTGS, Baddewithanan BWAN. Phenotypic diversity of rubber clones grown in Sri Lanka at their immature stage based on the available morphological descriptors. Tropical Agricultural Research. 2013; 24(2): 112-127.

[13] Chakravorty A, Ghosh PD, Sahu PK. 2013. Multivariate analysis of phenotypic diversity of land races of rice of West Bengal. American Journal of Experimental Agriculture. 2013; 3(1): 110–123.

[14] Occhiuto PN, Peralt IE, Asprelli PD, Galmarini CR. Characterization of Capsicum germplasm collected in Northwestern Argentina based on morphological and quality traits. AGRISCIENTIA 2014; 31(2): 63-73.

[15] Bianchi Paola Alvares, Iris Petronilia Dutra, Monique Moreira Moulin, Jardel Oliveira Santos, Alexandre Cristiano Santos Júnior (2016). Morphological characterization and analysis of genetic variability among pepper accessions. Ciência Rural, Santa Maria. 2016; 46(7): 1151-1157.