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 into 6 main clusters at the distance of 1.5. This study permits the characterization of Hevea accessions and clones into 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 Nköoolong – 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 used 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 ≤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).

| 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 |
| RIMM705         | 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        | 24.000 abcd | 10.000 bcd | 27.800 abcd | 7.281 abc | 3.134 a | 2.710 abcd | 0.964 abcd |
| PR107           | 26.800 bcd | 12.120 d | 24.500 abcd | 9.330 bc | 3.504 a | 2.156 abc | 0.792 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 ab | 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 |
### 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 = Petiole fresh weight, Pet Dw = Petiole 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: Dendrogram for 4 accessions and 32 clones based on average linkage multivariate cluster analysis

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 cluster. 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.

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