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Characterization and Evaluation of *Hevea brasiliensis* for Genetic Diversity Usin Juvenile Characters

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Abstract

Twenty-six (26) morphological descriptor traits were used to characterize six (6) genotypes of natural rubber at the immature stage using the natural rubber descriptor manual and the traits characterized included petiole, leaf storey and stems. The experiment was set up at the experimental field of Rubber Research Institute of Nigeria (RRIN) Iyanomo, Benin City. The objective of the study was to characterize the juvenile characteristics of natural rubber and to determine the extent of genetic variability that exists among the half-sibs. Data on characterization and agronomic traits were collected and subjected to analysis of variance (ANOVA) using the Generalized Mixed Model to determine the variability that existed among the half-sib genotypes for various traits. Treatment means of the offspring genotypes were separated using Fisher's least significant difference (F-LSD) at a 5% level of probability. The result of the combined analysis of variance (ANOVA) indicated highly significant genotypic differences among the genotypes for all the agronomic characters measured except stem girth, bark thickness and vigour index. The mean square result from ANOVA showed significant variations among the genotypes evaluated in each year as well as the periods and genotype x period interactions for most of the traits measured. In conclusion, the information obtained from this research work showed that considerable genetic variability exists in agro-morphological attributes of natural rubber genotypes. Genotypes with highly similar traits and high-yielding potentials have been identified and characterized.

Keywords: Hevea brasiliensis, Characterization, Genetic Diversity, Juvenile characters and Genotypes

Introduction

The natural rubber tree [*Hevea brasiliensis* (Willd. ex.Adr. de Juss.) Muell Arg.] is native to the Amazonian rainforest. It is the most widely cultivated tree species for producing natural rubber (latex) which has been an undeniably beneficial commodity for the past 100 years (Priyadarshan and Gonçalves, 2013). Natural rubber is known to be one of Nigeria's major agricultural export cash crops, it is a vital raw material for many industries (Haliru *et al*., 2011). If properly developed and managed, natural rubber and other crops can contribute a substantial part of Nigerians' foreign exchange earnings, thereby making the country less dependent on crude oil. Data from the International Rubber Study Group 2011 showed that in 2010, the world production of natural rubber reached 10.3 million tons, of which Brazil only contributed 132 thousand tons, i.e., about 1% of the total. According to (Rao *et al.,* 2011), the genetic base of natural rubber breeding programs has to be expanded to improve breeding efficiency and accelerate the breeding of new genotypes. However, the genetic base is decreasing and little improvement in the productivity of new genotypes is being achieved because of the long period required for interspecific breeding among related species.

In Nigeria, hybridization for genetic improvement of latex commenced in 1965 using many of the first sets of high-yielding genotypes from Malaysia and Sri Lanka and a few high-yielding primary genotypes from Java . Field evaluation and selection programs have been conducted on the hybrids leading to the selection and recommendation of sixteen MG genotypes with a latex yield of 2000 – 3500 kg/ha/yr (Omokhafe *et al.*, 2014). The breeding population of natural rubber in Nigeria includes the primary genotypes such as GTI and TJIR1 from Java, the hybrid genotypes developed in Malaysia and Sri Lanka and the MG genotypes. These genotypes have relatively common parentage which may be characterized by a narrow genetic base of the breeding population. The bleeding and stimulation systems of the natural rubber trees in production are based on the metabolism of each genotype. Therefore, a mixture of genotypes or an error in identifying genotypes when establishing an experimental plantation would cause damage to the plants and such dry notches during the operation may result in low latex yield production. Then to avoid this situation, the identification and selection of genotypes before and after planting are very essential to adopt the best harvesting methods suitable for planted genotypes (Konan *et al*., 2019).

Recently molecular and biochemical methods have been used to identify and characterize genotypes and the methods are expensive and unavailable to farmers and breeders. Thus, to make it easy for farmers and breeders, there is a need to employ morphological criteria which are also very good. Over the years, several new genotypes of natural rubber have been generated in the Plant Breeding Program of the Rubber Research Institute of Nigeria (RRIN), Benin City which has not been characterized and evaluated. Characterization and evaluation of such new genotypes can lead to the identification of promising ones in terms of latex yield potentials and growth attributes. The use of genetically improved high-yielding planting materials of natural rubber in Nigeria has achieved spectacular growth in the areas of rubber plantation establishment and latex production during the past years (Gouvea *et al*., 2013).

Due to the very narrow genetic base that exists in the cultivated rubber genotypes, natural rubber cultivation is under a constant threat of attack by diseases and insect pests due to their genetic vulnerability. Also, the changes in the weather parameters due to the increasing trend in climate change have further complicated the above issues. There is a need to know and understand the potential uses and values of the natural rubber genetic resources by characterizing, evaluating and documenting them properly. The Objectives of this study are to characterize available natural rubber genotypes into distinct similarity groups using the natural rubber descriptor manual, to evaluate the viability of newly developed open-pollinated seeds and to determine the extent of genetic variability that exists among the half-sibs.

Materials and Methods

Location: The experiments were carried out in the 2021 and 2022 cropping seasons at the research field of the Rubber Research Institute of Nigeria (RRIN) main station at Iyanomo near Benin City. The study area is located between longitudes 5o341 – 5o381E; latitude 6o081- 6o111N and at an altitude of 21m above sea level within the humid rainforest agroecological zones of Southern Nigeria. The Mean annual temperature is 26.1^{o}C and the annual rainfall is above 2000mm, distributed in a bimodal pattern with peaks in July and September. The soils of this rainforest belt are mainly leached ultisols with a pH range of 4.5 to 6.5. The soils are deep, porous, non-mottled and non-concretionary red soils with textures ranging from loamy sand at the surface to sandy clay in the sub-soils. The detailed characteristics and classification of the soils in this area have been reported by (Orimoloye, 2011; Orimoloye and Akinbola, 2013).

***Experimental materials, design and field management*:** Six genotypes of NIG 800 series developed at RRIN were characterized and evaluated using juvenile characters. The experimental field was already established in 2018 and the experiment was laid out in a randomized complete block design (RCBD) with four replications. The rubber genotypes were planted at a spacing of 3.4m x 6.7m to give a plant density of 450 trees per hectare. Two years after the plantation establishment, a blanket application of NPK 15-15-15 was carried out at the rate of 400kg/ha. Weeding was done by manual slashing to maintain field sanitation. Data collection was on characterization and the traits that were characterized were leaves and stems. (Leaf lamina**,** petiolule, leaf storey and stem). Three years after planting, the experimental plantation field was evaluated for the following three juvenile characters at 3 months intervals: Stem height, stem girth, number of branches and viguor index. Descriptor data were subjected to multivariate analyses such as: Genetic diversity among and within half-sib families and agronomic data collected were subjected to analysis of variance (ANOVA) and Fisher's least significant difference (F-LSD) at a 5% level of probability was used in comparing the means.

Results and Discussion

Results

Twenty (20) morphological juvenile descriptor traits were used to characterize six (6) genotypes of *Hevea brasiliensis* using the descriptors manual for natural rubber (IRRDB, IPGRI 2005). The various juvenile parts of the natural rubber tree characterized were the leaf (middle leaflet), petiolule and petiole, leaf storey and stems. The results are presented in Tables 1-3

Leaf Traits: The leaf lamina (middle leaflet) traits characterized included shape, colour, lustre, texture, leaf base, leaf apex, leaf margin, longitudinal section, cross-section, and the position of leaflets and pulvinus. Genotypes NIG801, NIG803 and NIG804 exhibited the same shapes (obovate) and genotypes NIG802 and NIG805 had elliptical shapes while NIG 800 had a diamond elliptical shape. The genotypes NIG800, NIG801 and NIG803 exhibited the same colour (dark green) and lustre (dull) while the remaining genotypes varied from each other in terms of colour and lustre. All the genotypes had the same texture (smooth) except NIG805 which had a rough texture. The leaf base of NIG800, NIG803 and NIG804 was cuneate, NIG801 and NIG802 were acute while NIG805 had an attenuate leaf base. Four genotypes NIG800, NIG802, NIG804 and NIG805 exhibited acuminate leaf apex while the remaining two genotypes NIG801 and NIG803 had apiculate and aristate leaf apex respectively. The leaf margin of four genotypes (NIG800, NIG801, NIG802 and NIG805) were entire whereas the other two genotypes (NIG803 and NIG804) were wavy. However, all the genotypes had the same longitudinal section (convex), cross-section (v-shaped), and pulvinus (normal) while the position of leaflets of some genotypes (NIG800, NIG801, NIG804 and NIG805) were just touching, then NIG803 and NIG804 were well separated (Table 1).

Petiolule and petiole traits: The petiolule and petiole traits used in characterizing the genotypes included the orientation, length, angles between petioles, pulvinus, shape and nectar gland. The length of the petiole of all the genotypes ranged from medium to long, the genotypes NIG800- NIG803 exhibited the same orientations (incline), and angle between petioles (acute) and shapes (straight) while NIG804 and NIG805 had different orientations, angle between petioles and shapes. The pulvinus and nectar glands of all the genotypes were described as normal (Table 2).

Leaf storey traits: The leaf-storey descriptors that were used to characterize the genotypes (NIG800 to NIG805) were the leaf-storey shape, the distance between leaf storey and external appearance. The shape of the leaf-storey and the distance between the leaf storey were diverse across the genotypes. The leaf-storey external appearance of the genotypes could be described as open, medium and dense. However, the phenotypic characterization of natural rubber genotypes brings about genetic variability which is important in the hybridization program (Table 3).

Stem traits: The stem traits characterized were appearance, surface, leaf scar size, leaf scar shape, latex colour and girth. There were no differences among the stem appearance, surface and leaf scar shape for all the genotypes, all of them showed the same stem appearance (straight), surface (smooth) and leaf scar shape (normal). There were variations in the expression of a leaf stem scar size, latex colour and girth (Table 3).

Stem height and stem girth

The mean performances of stem height and stem girth of 6 genotypes of natural rubber evaluated in 2021 and 2022 are presented in Table 4. The result of analysis of variance (ANOVA) showed that the mean plant height of natural rubber genotypes evaluated at the juvenile stage varied very highly significantly among the genotypes ($P<0.001$) and across the years ($P<0.001$). The overall mean stem height over the two years was 2.53m. In both years, the highest stem height was obtained from NIG804 with a mean stem height of 2.69m and NIG803 with a mean stem height of 2.62m. The mean stem height of these two genotypes was significantly higher than the overall mean stem height.

Stem Girth: The combined analysis of variance (ANOVA) indicated no significant variations in stem girth among the *Hevea brasiliensis* genotypes ($P>0.05$). The year had no significant effect ($P>0.05$) but genotypes x year interaction was highly significant ($P<0.01$). Mean stem girth over the years was 13.32cm and it ranged from 13.07cm to 13.59cm. Even though there was no statistical difference among the genotypes and between the years evaluated, stem girth ranged from 12.51cm in NIG802 to 14.26cm in NIG804 in 2021 whereas in 2022 stem girth varied from 12.92cm in NIG804 to 13.98cm in NIG800. Genotype NIG804 produced the highest stem girth of 14.26cm in both years. (Table 4).

Number of branches and vigour index

Table 5 showed the mean performance of the **number of branches** per plant and **vigor index** of 6 rubber genotypes evaluated in the 2021 and 2022 cropping seasons. The result of the combined analysis of variance (ANOVA) indicated that the number of branches per genotype was very highly significantly ($P<0.001$) affected by the genotypes, year ($P<0.001$) and highly significantly by the genotypes x year interaction ($P<0.01$). Among the genotypes, the mean number of branches over the two years was 6.89 with the number of branches per plant ranging from 6.15 to 8.27. The genotype NIG804 produced the most number of branches per plant with a mean of 8.27 and NIG803 produced the least number of branches of 6.32. The rubber genotypes evaluated in 2022 produced significantly more branches per plant (7.49) than those in 2021 (6.29) (Table 5).

Vigour index: Combined analysis of variance result of vigour index for both years showed that the trait did not vary significantly ($P>0.05$) across the genotypes but very highly significant variations ($P<0.001$) existed across the years while genotype x year interaction did not differ from one another ($P>0.05$). The mean viguor index of the 6 rubber genotypes ranged from 3.46 to 3.54 with an overall average of 3.50. However, the effect of season on the trait was highly significant; with 2022 producing the highest viguor index of (3.57) than the 2021 cropping season 3.43 (Table 5).

Discussion

Morphological characterization of the genotypes using juvenile characters showed considerable variations for descriptor traits such as 1). Leaf lamina (middle leaflet); leaf shape, leaf colour, leaf lustre, leaf texture, leaf base, leaf apex, leaf margin and position of leaflets. 2). Petioles; petiole length, petiole orientation, angle between petioles and petioles shape. 3). Leaf storey; leaf storey shape, the distance between leaf storey and leaf storey external appearance. 4). Stem; stem leaf scar size, stem latex colour and stem girth. Similar observations have been reported by (Konan *et al*., 2019; Ngomuo *et al*., 2017; Nyadanu *et al*., 2017; Adebo *et al*., 2015 and Benor *et al*., 2012). According to Polhamus (1962), Paardekooper (1965) and Konan *et al*., (2019), the leaves and the stems are the traits that were characterized at a young age because identification of genotypes' leaves and stems at a mature age is rather difficult and not always reliable. The results of the combined analysis of variance (ANOVA) over the 2021 and 2022 cropping seasons showed highly significant genotypic differences within the genotypes for some quantitative agronomic characters measured. The highly genotypic significant variation for some of the characters studied implied the existence of genetic diversity among the genotype of natural rubber tested and this will provide ample scope for further genetic improvement of these materials. However, these significant genotypic variations in the expression of the various agronomic characteristics can also be attributed to the diverse inherent genetic properties of the natural rubber genotypes used in this study and therefore suggest the existence of considerable genetic variations among the genotypes for these attributes. Lam *et al*., (2009) reported considerable variations among the *Hevea* IRRDB 81 collections assessed. Then according to (Sumanth *et al*., 2017), the success of any crop improvement program depends on the extent of genetic variability that exists in the available germplasm of that particular crop. Therefore, this result also implies the potential of these genotypes to be used as a source of germplasm/genetic materials for the improvement of any of these agronomic attributes of natural rubber genotypes.

Conclusion

The result of this study showed that there were significant genotypic variations among the genotypes. The frequency distribution of the genotypes for qualitative characters showed considerable variations for traits characterized. Genotypes NIG804, NIG803, NIG800 and NIG805 gave high mean stem height, stem girth, number of leaf whorls, number of branches, dry rubber yield and number of leaf areas per plant. They are therefore recommended as high-yielding genotypes which can be incorporated into breeding programs geared towards higher dry rubber yield, vegetative growth attributes, timber production and germplasm material for the improvement of the traits in plant breeding programs.

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Table 1: Characterization of the leaf lamina (middle leaflet) of 6 genotypes of natural rubbers at the immature stage in RRIN main station

Genotype	Shape	Colour	Luster	Texture	Leaf base	Leaf apex	Leaf margin	Longitudinal section	Cross section	Position of leaflets	Pulvinus
NIG800	Diamond Elliptical	Dark green	Dull	Smooth	Cuneate	Acuminate	Entire	Convex	V-Shaped	Just touching	Normal
NIG801	Obovate	Dark green	Dull	Smooth	Acute	Apiculate	Entire	Convex	V-Shaped	Just touching	Normal
NIG802	Elliptical	Green	Dull	Smooth	Acute	Acuminate	Entire	Convex	V-Shaped	Well separated	Normal
NIG803	Obovate	Dark green	Glossy	Smooth	Cuneate	Aristate	Wavy	Convex	V-Shaped	Well separated	Normal
NIG804	Obovate	Light green	Glossy	Smooth	Cuneate	Acuminate	Wavy	Convex	V-Shaped	Just touching	Normal
NIG805	Elliptical	Light green	Dull	Rough	Alternative	Acuminate	Entire	Convex	V-Shaped	Just touching	Normal

Table 2: Characterization of the leaf petiolule and petiole of 6 genotypes of natural rubber at immature stage at RRIN main Station

	Petiolule		Petiole			
Genotypes	**Length**	**Orientation**	**Angle b/w petiole**	**Pulvinus**	**Shape**	**Nectare gland**
NIG800	Long	Incline	Acute	Normal	Straight	Normal
NIG801	Medium	Incline	Acute	Normal	Straight	Normal
NIG802	Medium	Incline	Acute	Normal	Straight	Normal
NIG803	Medium	Incline	Acute	Normal	Straight	Normal
NIG804	Medium	Horizontal	Perpendicular	Normal	Concave	Normal
NIG805	Long	Upwards	Obtuse	Normal	Arched	Normal

Table 3: Characterization of the leaf-storey and leaf stem of 6 genotypes of natural rubber at the immature stage in RRIN main Station.

Genotypes	Shape	Distance between leaf storey	External Appearance	Appearance	Surface	Leaf scar size	Leaf scar shape	Latex colour	Girth
NIG800	Hemispherical	Separated	Open	Straight	Smooth	Small	Normal	Whitish Cream	Average
NIG801	Conical	Intermediate	Dense	Straight	Smooth	Normal	Normal	Light yellow	Average
NIG802	Truncate	Intermediate	Medium	Straight	Smooth	Small	Normal	Whitish Cream	Average
NIG803	Bow	Well-separated	Open	Straight	Smooth	Normal	Normal	Light yellow	Average
NIG804	Hemispherical	Intermediate	Medium	Straight	Smooth	Small	Normal	Whitish Cream	Good
NIG805	Bow	Well separated	Open	Straight	Smooth	Normal	Normal	Whitish Cream	Average

Table 4: Mean stem height (m) and stem girth (cm) of 6 genotypes of natural rubber evaluated in the years 2021 and 2022

	Stem height (m)			Stem girth (cm)		
Genotypes	2021	2022	Genotype Mean	2021	2022	Genotype Mean
NIG800	2.22	2.59	2.41	13.19	13.98	13.58
NIG801	2.33	2.68	2.50	13.37	13.39	13.38
NIG802	2.22	2.59	2.40	12.51	13.88	13.19
NIG803	2.45	2.79	2.62	12.68	13.46	13.07
NIG804	2.54	2.84	2.69	14.26	12.92	13.59
NIG805	2.35	2.70	2.53	13.11	13.09	13.09
Year Mean	2.35	2.70	2.53	13.18	13.45	13.32

Stem height: F-LSD $_{(0.05)}$
Genotypes means = 0.10***
Year Mean= 0.06***
Genotype x Year= 0.29^{ns}

Stem girth: F-LSD $_{(0.05)}$
Genotypes means = 1.61^{ns}
Year Mean= 0.93^{ns}
Genotype x Year= 4.55**

Table 5: Mean number of branches and vigour index of 6 genotypes of natural rubber evaluated in the years 2021 and 2022

	Number of branches			Vigour index		
Genotypes	**2021**	**2022**	**Genotype Mean**	**2021**	**2022**	**Genotype Mean**
NIG800	5.65	6.66	6.15	3.41	3.67	3.54
NIG801	5.73	7.01	6.37	3.42	3.54	3.48
NIG802	5.96	6.87	6.41	3.38	3.53	3.46
NIG803	5.37	7.27	6.32	3.47	3.56	3.52
NIG804	7.85	8.66	8.27	3.46	3.61	3.53
NIG805	7.24	8.42	7.83	3.41	3.53	3.47
Year Mean	6.29	7.49	6.89	3.43	3.57	3.50

$F\text{-}LSD_{(0.05)}$ $F\text{-}LSD_{(0.05)}$

Genotypes means =1.29*** | Genotypes means = 0.12^{ns}

Year Mean=0.74*** | Year Mean= 0.07***

Genotype x year= 3.64** | Genotype x year= 0.33^{ns}