Morphological characterization and evaluation of *Hevea brasiliensis* for genetic diversity and latex quality attributes

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ABSTRACT

Experiment on morphological characterization of natural rubber (*Hevea brasiliensis*) genotypes was carried out during the 2021 cropping season. The experiment was set up at clonal garden of Rubber Research Institute of Nigeria (RRIN) Iyanomo, Benin City. The objective of the study was to characterize available natural rubber genotypes with a view to classifying them into distinct similarity groups using natural rubber descriptor manual. Morphological descriptor traits were used to characterize 16 genotypes of natural rubber at mature stage using RRIN and IPGRI, 2005 descriptor manual for natural rubber. The two parts of the natural rubber trees characterized included the trunk and branching. Data on yield evaluation was also collected using the taping system for all the sampled trees on a half spiral alternate day system donated as S/2d/2 without yield stimulation and the dry rubber yield data collected were subjected to analysis of variance (ANOVA) using the Generalized Mixed Model to determine the variability that existed among the genotypes. Frequency distribution for qualitative characters showed that rubber genotypes had considerable variations for most trunk and branching descriptor traits measured. Combined analysis of variance (ANOVA) indicated highly significant genotypic differences among the genotypes for the agronomic character (latex yield) 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: Morphological characterization, Genetic Diversity, Latex Quality, Genotypes.

INTRODUCTION

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The natural rubber tree is a deciduous, perennial, cross-pollinating and monoecious species of the Euphorbiaceous family (Hamzah *et al.,* 2012). Rubber tree is a sturdy, quick growing, perennial plant that grows to a height of about 25-30m with an economic life span of 30-35 years in the plantations. It has a straight trunk with light grey bark and the branches developed to form an open leafy crown (canopy). The leaves are arranged in groups or stories, each storey with a cluster of spirally arranged trifoliate glabrous leaves and extra floral nectaries present in the region of insertion of the leaflets (Mercy *et al*., 2010). The natural rubber tree is a deciduous tree which sheds its leaves by process of either partial or complete wintering, during December to February followed by refoliation or flowering. The tree is monoecious with unisexual flowers produced in pyramid shaped panicles in the axils of leaves. The panicles bear numerous small male flowers and fewer but bigger female flowers. The female flowers are confined to the tip of the panicles and branchless. Ovary is tricarpellary syncarpous, which on pollination develops into a three lobed dehiscent capsule (regma) with three large mottled seeds (Priyadarshan and Clement-Damage, 2014). Natural rubber tree has a long breeding process, with many selection cycles and difficulties in raising F_2 progenies. These difficulties are as a result of several factors related to the reproductive biology of rubber, such as lack of synchrony in flowering among clones, seasonality in flowering, pollen longevity, low pollination success, and seed recalcitrance (Mercy *et al*., 2010). Therefore, conventional genetic analysis in this crop is difficult and time consuming (Luciana *et al*., 2010).

Natural rubber tree (*Hevea brasiliensis)* is one of Nigeria's prominent industrial and export cash crops which provides raw materials for local industries and also contributes to the export earnings of the nation (Nwaichi & Warigbani, 2013). 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 (Haliru *et al*., 2011). The bleeding and stimulation systems of the natural rubber trees in production are based on the metabolism of each genotype. Therefore, a mixture of genotype 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 to low latex yield production. Then to avoid this situation, the identification and selection of genotypes before and after planting is very essential in order 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 (Luciana *et al*., 2010). Thus, in order to make it easy for farmers and breeders, there is need to employ morphological criteria which are also very good. Over the years, several new genotypes of natural rubber have been generated in Plant Breeding Program of the Rubber Research Institute of Nigeria (RRIN), Benin City which have not been characterized and evaluated. Characterization and evaluation of such new genotypes can lead to identification of promising ones in terms of latex yield potentials and growth attributes (Omokhafe *et al*., 2014). 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).

Because of the very narrow genetic base that exist in the cultivated rubber genotypes, the 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 need to know and understand the potential uses and values of natural rubber genetic resources by characterizing, evaluating and documenting them properly. Methods are still needed to develop, improve and facilitate productive utilization of natural rubber. Although biotechnological methods are now increasingly available to facilitate productive utilization of natural rubber germplasm, but not all countries have the capacity to use such new technologies and as such there is no evidence suggesting that available natural rubber genotypes in RRIN breeding program have been characterized and partitioned into similarity groups or diversity.

The Objectives of this study is to characterize available natural rubber genotypes with a view to classifying them into distinct similarity groups using natural rubber descriptor manual.

MATERIALS AND METHODS

Morphological characterization and evaluation of agronomic trait (latex yield) of sixteen rubber genotypes already established in the rubber nursery of Rubber Research Institute of Nigeria (RRIN) main station at Iyanomo near Benin City was carried out.

The experimental field was already established in 2009 from a land under prolonged fallow period of over 12 years. The experiment was arranged in a randomized complete block design (RCBD) with four replications. The rubber genotypes were planted at a spacing of 3.4m x 6.7m. Two years after 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. The plantation was opened for tapping in 2014.

DATA COLLECTION AND STATISTICAL ANALYSIS

Characterization

Morphological characterizations of the natural rubber traits were carried out using the Rubber Research Institute of India (RRII) and International Plant Genetic Resources Institute (IPGRI) Descriptor manual for rubber at mature stages. The traits that were characterized includes:

Trunk- girth (good, average, poor), continuity- (persistent, dissolve) height (tall, medium, short), shape (round, oval, fluted), surface (smooth, rough, knobby), colour (light, dark), appearance (straight, crooked, leaning), virgin bark thickness (thick, average, thin), bark hardness (hard, soft), scar (present, absent), bleeding (present, absent), latex colour (white, whitish cream, yellowish cream, yellow).

Branching- position (high, moderate, low), habit (clustered, alternate), spreading (close, widely separated), angle (wide, medium, narrow), continuity (persistent, dissolve) appearance (straight, intermediate, crooked), surface (smooth, intermediate, knobby), density, (heavy, average, light).

Evaluation of latex yield: Data on latex rubber yield were taken throughout the year except for the one month break in February, this is the period of severe defoliation of natural rubber in Nigeria. The taping system for all the sampled trees was carried out on a half spiral alternate day system donated as S/2d/2 without yield stimulation. Tapping was normally carried out between 6.00am-9am on each tapping day. The latex collected from each rubber tree was air dried, monitored and the cumulative dry rubber production was weighed with sensitive weighing balance and recorded monthly. The dry rubber yield data was subjected to Analysis of Variance (ANOVA) using the Generalized Mixed Model to determine the variability that existed among the genotypes.

RESULTS

Morphological characterization of natural rubber genotypes are done at three phases ie natural rubber trees are characterized at juvenile, immature and mature phases. Morphological descriptor traits were used to characterize the rubber genotypes at mature phase using the "Descriptor manual for natural rubber" (IRRDB, IPGRI, 2005). The parts of natural rubber trees characterized were trunk and branching. The results are presented in Tables 4.1-4.2.

Trunk Traits

The trunk traits characterized include the girth, height, shape, surface, colour, appearance, virgin bark thickness, bark hardness, scar, bleeding and latex colour. The trunk girth ranged from poor to average to good. Among the sixteen genotypes, eleven possessed good trunk girth while four possessed average trunk girth and one had poor trunk girth (Table 4.1). Trunk height varied from medium to tall. Nine out of the sixteen genotypes exhibited tall height whereas seven genotypes exhibited medium height. The genotypes were oval, round and fluted for trunk shape while the expression of smooth, rough and knobby traits for trunk surfaces were observed among the sixteen genotypes. In the four traits (trunk girth, height, shape and surface) considered above which were indicators of vigor, most of the genotypes like NIG800 to NIG805, GT1, NIG902, NIG903, RRIM600 and RRIM5/63exhibited good qualities than the other. The trunk colour varied from light to dark. There was minimal variation in the expression of trunk colour but wide variations existed amongst the genotypes in the trunk appearance. Eight genotypes had average virgin bark thickness while five had thick and three had thin virgin bark thickness. Ten genotypes out of the sixteen genotypes exhibited hard back hardness and six had soft back whereas there were presence of scars and bleeding on the trunk of all the genotypes. In this case, there were minimal or no variation in their expression. The trunk latex colour was described as white, whitish cream, yellowish cream and light yellow and all the genotypes had different latex colours. Three genotypes had white latex colour, seven genotypes had whitish cream latex colour while three genotypes each possess yellowish cream latex colour and light yellow latex colour (Table 4.1).

Branching Traits

The traits used in characterizing the genotypes included position, habit, spreading, angle, appearance, surface and density. The position of the branches were diverse among the genotypes. The branching position of seven genotypes were described as moderate, six as high and three as low. The branching habits manifested by the genotypes included clustered and alternate while branch spreading of some genotypes were separated, wide separated and close. Seven genotypes exhibited wide angle while six genotypes had medium angle and three had narrow angles. The wintering type of the genotypes were early, normal and late. Most of the genotypes expressed early and normal wintering while two genotypes (RRIM 600 and RRIM 501) expressed late wintering. The genotypes' wintering patterns ranged from partial to complete wintering. The genotypes were also varied in the manner of expression of their traits (Table 4.2).

Table 4.1: Descriptor traits used for the characterization of the trunk of the 16 genotypes of natural rubber trees at RRIN main Station in 2021 cropping season.

Genotypes	Girth	Continuity	Height	Shape	Surface	Colour	Appearance	VBT	BH	Scar	Bleeding	Latex colour
NIG800	good	Persistent	medium	Oval	Smooth	Dark	Straight	Average	hard	present	Present	whitish cream
NIG805	good	Persistent	Tall	Round	Rough	Light	Crooked	Thick	hard	present	Present	whitish cream
NIG801	good	Persistent	medium	Oval	Smooth	Dark	Straight	Average	hard	present	present	whitish cream
GT1	good	Dissolved	Tall	Fluted	Knobby	Light	Leaning	Thin	soft	present	present	yellowish cream
NIG803	good	Persistent	medium	Oval	Smooth	Dark	Straight	Average	hard	present	present	whitish cream
NIG802	good	Persistent	medium	Round	Rough	Dark	Straight	Thick	hard	present	present	whitish cream
NIG804	good	Dissolved	medium	Oval	Smooth	Light	Crooked	Thick	hard	present	present	whitish cream
RRIM600	good	Persistent	Tall	Round	Rough	Light	Straight	Average	soft	present	present	White
PR107	average	Persistent	Tall	Fluted	Knobby	Light	Leaning	Thin	soft	present	present	yellow cream

NIG901	average	Persistent	Tall	Round	Smooth	Dark	Straight	Average	hard	present	present	White
NIG902	good	Persistent	Tall	Round	Smooth	Dark	Straight	Average	hard	present	present	White
NIG903	good	Dissolved	Tall	Round	Smooth	Dark	Straight	Average	hard	present	present	white cream
RRIM5/63	good	Dissolved	Tall	Round	Smooth	Light	Crooked	Thick	soft	present	present	light yellow
PB28/59	average	Dissolved	medium	Round	Smooth	Light	Crooked	Thick	soft	present	present	light yellow
RRIM628	poor	Persistent	medium	Round	Rough	Light	Leaning	Thin	soft	present	present	light yellow
RRIM501	average	Persistent	Tall	Oval	Rough	Dark	Leaning	Average	hard	present	present	creamy yellow

Key:
VBT-Virgin Bark Thickness
BH- Bark Hardness

Table 4.2: Descriptor traits used for the characterization of the branches of the 16 genotypes of natural rubber trees at RRIN main Station in 2021 cropping season.

Genotypes	Position	Habit	Spreading	Angle	Continuity	Appearance	Surface	Density
NIG800	Moderate	Clustered	widely separated	Wide	persistent	intermediate	intermediate	Light
NIG805	High	Clustered	Close	Medium	dissolve	Crooked	Smooth	Average
NIG801	Moderate	Clustered	widely separated	Wide	persistent	intermediate	intermediate	Light
GT1	High	Alternate	Close	Narrow	dissolve	Straight	Smooth	Average
NIG803	Moderate	Clustered	widely separated	Wide	persistent	intermediate	intermediate	Light
NIG802	High	Alternate	widely separated	Wide	persistent	intermediate	Smooth	Average
NIG804	Low	Clustered	widely separated	Medium	persistent	Crooked	Smooth	Average
RRIM600	High	Clustered	Close	Medium	dissolve	intermediate	Knobby	Light
PR107	High	Alternate	Close	Narrow	dissolve	Straight	Knobby	Heavy
NIG901	Moderate	Clustered	widely separated	Wide	persistent	Straight	Smooth	Light
NIG902	Moderate	Clustered	Close	Wide	persistent	Straight	Smooth	Light
NIG903	Moderate	Alternate	Separated	Medium	dissolve	intermediate	intermediate	average
RRIM5/63	Low	Alternate	Separated	Medium	dissolve	Straight	Knobby	Heavy
PB28/59	Low	Clustered	Close	Narrow	dissolve	Crooked	Smooth	Heavy
RRIM628	High	Alternate	Close	Medium	persistent	Straight	Knobby	average
RRIM501	Moderate	Alternate	Close	Wide	persistent	Straight	Smooth	Heavy

Dry Rubber Yield

Table 4.3: Showed the mean dry rubber yield of 16 genotypes of natural rubber evaluated in the year 2021 and 2022 cropping seasons.

The combined analysis of variance (ANOVA) result showed that the character of interest (dry rubber yield) exhibited very highly significant difference (P<0.001) among the genotypes over the years under which they are studied. Genotype*year interaction was also highly significant (P<0.01). The overall mean of dry rubber yield of the genotypes in both years was 22.62g/t/t and it ranged from 12.71g/t/t in PR107 to 31.45g/t/t in NIG800. In the year 2021 and 2022, the dry rubber yield varied from 15.10g/t/t in PR107 to 28.08g/t/t in NIG901. In 2021 cropping season, the highest dry rubber yielding genotypes were NIG800 (31.45g/t/t), NIG804 (28.95g/t/t) and NIG805 (27.99g/t/t) with a range of 12.71g/t/t in PR107 to 31.45g/t/t in NIG800. Applying a correction term of (69.3), the yield range was 88.803kg/ha/yr to 2179kg/ha/yr. In 2022, the highest dry rubber yielding clones (genotypes) were NIG901 (28.08), NIG805 (27.23) and NIG804 (27.06) with a range of 15.10 g/t/t (1046kg/ha/yr) in PR107 to 28.08g/t/t (1945kg/ha/yr) in NIG901 while PR107 had the least dry rubber yield in both years. In the combined mean NIG800 also produced the highest dry rubber yield of 28.99g/t/t (2009kg/ha/yr) followed by NIG804 28.00g/t/t (kg/ha/yr) and NIG805 27.61g/t/t (kg/ha/yr) whereas PR107 gave the least dry rubber yield of 13.91g/t/t. There were significant differences (P<0.05) in fluctuation in the latex yield among the genotypes in both years.

Table 4.3: Interaction effect of Genotype x year on dry rubber yield (g/t/t)

Genotype	2021	2022	Genotype Mean
GTI	15.83	16.07	15.95
NIG800	31.45	26.55	28.99
NIG801	25.47	22.42	23.94
NIG802	26.20	23.55	24.87
NIG803	25.33	25.55	25.44
NIG804	28.95	27.06	28.00
NIG805	27.99	27.23	27.61
NIG901	23.92	28.08	25.99
NIG902	26.29	25.79	26.04
NIG903	21.53	26.84	24.18
PB28/59	19.19	15.95	17.57
PR107	12.71	15.10	13.91
RRIM501	22.57	18.55	20.56
RRIM600	21.06	21.20	21.13
RRIM628	18.97	17.79	18.38
RRIM5/63	20.48	18.25	19.36
Year Mean	22.99	22.25	22.62

F-LSD ($_{0.05}$)

Genotype mean = 2.62***

Year mean =0.93^{ns}

Genotype*year mean =7.40**

Discussion

Characterization of genotypes is a very important practice for effective conservation and utilization of the genetic materials. It is a vital component of genotype conservation and other components including documentation, evaluation and maintenance. Morphological characterization helps to identify duplicate genotypes and so helps in drastically reducing number of accessions/genotypes for effective germplasm maintenance (Nwankwo *et al*., 2010). Its breeding implications through prevention of inbreeding depression and enhancement of heterosis are well known. Also characterization descriptions permit relatively easy discrimination between phenotypes. They are usually higher inheritable characters that are easily detected by the naked eye and find expression in all environments (Nwankwo *et al*., 2010).

Among the two descriptor traits (trunk and branches) used to characterize natural rubber genotypes, there were minimum and maximum variations in the expression of their descriptor traits among the genotypes. Trunk colour had minimal variation in their expressions but wide variations existed within the genotypes in the trunk appearance. Genotype GT1 is easily recognized by the trunk girth, GT1 had a very good trunk girth and this is in line with the findings of (Konan *et. al*., 2019) in five recommended clones of Cote d'Ivoire characterized. Ten genotypes out of the sixteen genotypes exhibited hard back hardness and six had soft back whereas there were presence of scars and bleeding on the trunk of all the genotypes. In this case, there were minimal or no variation in their expression.

The mean dry rubber yield of all the 16 genotypes of natural rubber evaluated in this study over both cropping seasons was 22.62g/t/t (1567.6kg/ha/yr) which was very low when compare with the RRIN average of 3500kg/ha/yr This wide difference that was observed in the mean dry rubber yield may be attributed to the high influence of micro-climatic changes (rainfall) on dry rubber yield that was recorded in both years.

According to (Omokhafe *et al*., 2012), higher rainfall reduces latex yield during tapping and this suggests that even though natural rubber is a rain forest tree crop (ie requires enough rainfall for its vegetative growth), excessive rainfall may not be desirable especially during tapping. In India, significant influence of three weather factors (rainfall, temperature and relative humidity) has been reported by (Sailajadevi *et al*., 2008). In Nigeria, only rainfall has been implicated. The significant clonal variations for dry rubber yield among the 16 genotypes suggested that selection within these genotypes to further improvement on dry rubber yield is possible.

CONCLUSION

The clonal garden (gene pool) of the Rubber Research Institute of Nigeria (RRIN) was morphologically characterized for genetic diversity using agro-morphological descriptors for natural rubber and latex quality attributes. Frequency distribution of the genotypes for qualitative characters showed considerable variations for most of the traits studied. There was consistency in the performances of some natural rubber genotypes and some were non-consistent in their performances across the different characters measured. The significant clonal variations for dry rubber yield among the 16 genotypes suggested that selection within these clones/genotypes to further improvement on dry rubber yield is possible.

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