INTERNATIONAL JOURNAL OF INNOVATIVE RESEARCH & DEVELOPMENT

ISSN 2278 – 0211 (Online)

# Identification of Phenotypic Traits Associated with Genotypes Tolerance / Resistance and Susceptibility to Lethal Yellowing Disease of Coconut

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***Abstract***
*Parent palms and the first filial generation ($F_1$) progenies consisting of five (5) different single cross hybrids and open pollinated fruits of six (6) exotic and local dwarf and tall coconut accessions of NIFOR breeding coconut populations. Genotypes were evaluated for percent susceptibility to Lethal Yellowing Disease (LYD) as the proportion of susceptible palms to the total number palms planted per genotype from 2000 – 2015 in an LYD prevalent tract. Experimental materials were laid out in a 10-hectare field in completely randomized design of one row of tall variety to two rows of dwarfs while the hybrid varieties (5 treatments) were in 2 hectares in a randomized complete block design of 2 replicates, 8 palms per plot of 4 blocks. Parent palms and their $F_1$ progenies were screened for susceptibility to LYD per cultivar / genotype once in a month for five years 2011- 2015. Data collected were combined with that obtained in years 2000 – 2010 and summarized as the proportion of susceptible palms to the total number of palms planted per genotype expressed in percentage. Cultivars / genotypes that showed maximum mortality of not more than 10 % were classified tolerant or resistant while varieties that showed more than 20 % mortality were classified susceptible. Data were collected on 3-12 random samples of each genotype classified resistant and susceptible respectively. Traits measured were trunk height (m), trunk girth at 20 cm and at 1.5 m above soil level (cm), length of eleven (11) leaf scars at 1.5 m above soil level (cm), number of leaves, length of leaf (m), width of leaf (m), length of petiole (m), width of petiole (cm), thickness of petiole (cm), crown size (m), number of leaflets, length of leaflet (m) and width of leaflet (m). Statistical analysis of the quantitative traits was done by using simple descriptive statistics. Mean, standard deviation, standard error and range of the measurable traits were obtained by using GenStat 10.3 Discovery Edition version 4 (VSN International, 2011). A t-test was used to test for significant mean differences for the measured traits of the resistant and susceptible coconut genotypes / cultivars. Varieties that showed less than 10 % mortality from the effect of LYD after more than two decades of field exposure were the Sri-Lankan brown dwarf, SBD (0 %); Malayan green dwarf, MGD (8.9 %) and Chowghat green dwarf, CGD (9.1 %) hence were classified LYD tolerant or resistant. Genotypes which showed more than 20 % susceptibility were the West African Tall, WAT (98.5 %); Malayan yellow dwarf, MYD (83.7 %); hybrids of MYD x VTT (Vanuatu Tall) (84.4 %); MYD x WAT (75.0 %); SGD x VTT (62.5 %); Malayan orange dwarf, MOD (66.7 %); hybrids of MGD x WAT (31.3 %) and CGD x WAT (31.3 %) and so were susceptible genotypes. There were very highly significant mean differences for the trait means of the susceptible from the resistant genotypes for trunk height, girth at 20 cm and at 1.5 m above soil level, eleven leaf scars length, leaf and petiole lengths and crown size ($P \leq 0.001$).*

***Keywords****: Lethal yellowing disease, resistantgeno types, quantitative traits, t-test, significant mean difference.*

## 1. Introduction

The coconut palm (*Cocosnucifera* L.) is a very useful plant in the family *Arecaceae.* Presently more than 75 % of the nation's coconut belt is a continuation of the plantations or groves along the West African coast running from Cote D'Ivoire and the southeast towards Ghana, Togo and Benin to Lagos state in a 1 km wide strip. 95 % of the estimated 36,000 hectares of coconut in the country [i,ii]is cultivated mainly by resource poor farmers with less than 4 hectares each. It is grown mostly in the riverine, coastal and homesteads in southern states of the country, but also found beside streams, banks of rivers and stagnant pools of water in northern parts of Nigeria where favourable agro-climatic conditions exists. About 1.2 million hectares had been identified as suitable for coconut cultivation in Nigeria. Some states and individuals are investing in coconut palm cultivation now by using quality hybrid materials tolerant to Lethal Yellowing Disease, suitable and adapted to local agro-climatic conditions. The coconut palm, despite its predominance along sea coast, is cultivated under diverse types of climate and different soil types in Nigeria – laterite, coastal sandy, alluvial, loamy, reclaimed marshy lowlands and in plain grounds; it tolerates wide range of pH (5.0 – 8.0). It grows well in places with altitude of 0 - 600 m but could be found at height of about 1,000 m above mean sea level. Coconuts tends to grow best in places having mean annual temperature of 25°C – 38°C and an annual well distributed rainfall of 2000 mm [iii].The plant serves as a good source of food, beverages, fibre, pulp, livestock feed, shell for ornamental objects, fuel, activated charcoal, and shell flour [iv]. The most important product of coconut palm is copra [v, vi,vii] from which the coconut oil is derived. The oil is high in saturated fatty acids content of which lauric, myristic and palmitic acids, account for about 77.5% [vi, iv]. It is used for cooking, hair conditioning and industrial purposes [viii, ix, x,xi, iv]. Ball copra, desiccated coconut, coconut chips and yoghurt are food for man [xii, iv]. Besides, the industrial applications of the oil include the manufacture of toilet soaps, detergents, biscuits, margarine and numerous oleochemicals such as coconut-based isopropyl ester, monoethanolamide and lauric acid hexylester [xiii, xii, iv].

Many communities that had abundance of coconut palm trees in Nigeria some decades ago are now left with no trees of this amazingly useful plant. Mature coconut palms were completely destroyed by a disease known as Lethal Yellowing (LYD). To date the disease is known to be recurring in some localities where young susceptible productive palms are killed every year. The disease is regarded as the most significant factor impacting coconut production worldwide [xv]. Surveys conducted in Nigeria had established that LYD is a major cause of decline in the growth of coconut industry in Nigeria [xv,iii, xvi].The inability to culture the LYD pathogen (phytoplasma) in any axenic medium in the laboratory as of now means that evaluation for LYD resistance in coconut varieties cannot be done by inoculation of samples with the pathogen and moreover, the long latent period before manifestation of disease symptoms in palms of productive age from time transplantation to field poses a huge challenge to breeding for resistance in coconut. Presently, the only means of evaluating coconut palm varieties for tolerance / resistance to LYD is by exposing genotypes to the disease in the field in disease endemic zones under natural agro-climatic conditions for many years. Fortunately, the south-western part of Nigeria which had been the home of coconut is relatively free from the disease. Breeding for LYD resistance at the main station began in the 1990s when it became obvious that the only effective means of control was by planting resistant varieties of coconut. Secondly, farmers in localities where the disease was endemic were losing interest in coconut cultivation thereby rendering such areas perpetually devoid of coconut palms. Farmers do collect seednuts from localities they visit irrespective of the physical characteristics the parent palms may possess. The use of disease resistant coconut genotypes is a prerequisite for sustainable production and productivity. The $F_1$ coconut progenies and their parent palms on field trials in NIFOR located in an LYD – prevalent tract had showed variable responses to the disease using phenotypic assessment. Some of the mature palms had survived this cyclical attack for more than two decades. The study was conducted on palms that had spent more than two decades under field exposure to the agro-climatic conditions with the objective of identifying some significant measurable morphological traits associated with the genotypes that had high percentage survival which is indicative of tolerance / resistance to lethal yellowing disease. These identified resistant genotypes would be used as parents for hybridization as well as source of planting materials in disease endemic communities that had been devastated by LYD. Furthermore, farmers could use these traits as a guide for most likely tolerant / resistant parent palms in future collection of seednuts from other areas to be planted in LYD ravaged communities.

### *1.1. Experimental Site*

The experiment was conducted at the main station of Nigerian Institute for Oil Palm Research (NIFOR), Latitude 6 $^{o}$ 37'N, Longitude 5$^{o}$ 37' E, elevation 149.4 m above mean sea level in a rain forest region of Nigeria.

### *1.2. Plant Material*

The experimental materials were parents and the first filial generation($F_1$) progenies of five (5) single crosses and open pollinated fruits of six (6) exotic and local dwarf and tall coconut accessions of NIFOR breeding coconut populations. Genotypes screened were: West African Tall (WAT), Malayan Green Dwarf (MGD), Malayan Orange Dwarf (MOD), Malayan Yellow Dwarf (MYD), Sri Lankan Brown Dwarf (SBD), Chowghat Green Dwarf (CGD), Malayan Green Dwarf x West African Tall (MGD x WAT), Chowghat Green Dwarf x West African Tall (CGD x WAT), Malayan Yellow Dwarf x West African Tall (MYD x WAT), Malayan Yellow Dwarf x Vanuatu Tall (MYD x VTT) and Sri Lankan Green Dwarf x Vanuatu Tall (SGD x VTT).

### *1.3. Experimental Design*

Palm were laid out in a 10-hectare field in completely randomized design of one row of tall variety to two rows of dwarfs while the NIFOR and exotic hybrid varieties (5 treatments)were in 2 hectares of a randomized complete block design of 2 replicates,8 palms per plot of 4 blocks.

## 2. Materials and Methods

All the palms of the eleven coconut genotypes were visually observed individually for disease symptoms manifestation and death once in a month for five years (2011 - 2015). The number of palms that succumbed to the disease each year was recorded for each genotype in November. Genotypes screened were: West African Tall (WAT), Malayan Green Dwarf (MGD), Malayan Orange Dwarf (MOD), Malayan Yellow Dwarf (MYD), Sri Lankan Brown Dwarf (SBD), Chowghat Green Dwarf (CGD), Malayan Green Dwarf x West African Tall (MGD x WAT), Chowghat Green Dwarf x West African Tall (CGD x WAT), Malayan Yellow Dwarf x West African Tall (MYD x WAT), Malayan Yellow Dwarf x Vanuatu Tall (MYD x VTT) and Sri Lankan Green Dwarf x Vanuatu Tall (SGD x VTT). The characteristic symptoms observed were: dropping of immature nuts which starts with the youngest to the oldest nuts in quick succession; yellowing, browning and shedding of leaves (from oldest to youngest) and death of palm within six months of symptoms manifestation. Data were collected on the total number of palms infected and killed by LYD per genotype, recorded every year and collated at the end of five years. The five-year data was combined with the data gathered since year 2000 to obtain the total number of palms killed per genotype by LYD after over two decades of field exposure. Data was analysed by determining percent (%) mortalityper cultivar / hybrid [xvii] as the proportion of the palms killed by the disease to the total number of palms planted for each genotype expressed in percentage. Disease severity on each cultivar / hybrid was rated using 0 – 100 % rating scale. Cultivars / genotypes that showed mortality not more than 10 % were classified tolerant or resistant while varieties that showed more than 20 % mortality were classified susceptible. Measurements were taken on fourteen (14) Quantitative traits on three (3) to twelve (12) randomly selected palms from the tolerant / resistant group and the susceptible varieties respectively. Data were collected on trunk height (m), trunk girth at 20 cm and at 1.5 m above soil level (cm), length of eleven (11) leaf scars at 1.5 m above soil level (cm), number of leaves, length of leaf (m), width of leaf (m), length of petiole (m), width of petiole (cm), thickness of petiole (cm), crown size (m), number of leaflets, length of leaflet (m) and width of leaflet (m).

### *2.1. Data Analysis*

Statistical analysis of the quantitative traits was done by using simple descriptive statistics. Mean, standard deviation and standard error of the measurable traits were obtained by using GenStat 10.3 Discovery Edition version 4 [xviii]. A t-test was used to test for significant mean difference for the measured traits of the resistant and susceptible coconut genotypes / cultivars.

### *2.2. Results*

Varieties that showed less than 10 % mortality from the attack by Lethal Yellowing Disease after more than two decades of field exposure were the Sri-Lankan brown dwarf, SBD (0 %);Malayan green dwarf, MGD (8.9 %) and Chowghat green dwarf, CGD (9.1 %). Consequently, they were classified LYD tolerant or resistant. Genotypes which showed more than 20 % susceptibility were the West African Tall, WAT (98.5 %); Malayan yellow dwarf, MYD (83.7 %); hybrids of MYD x VTT (Vanuatu Tall) (84.4 %); MYD xWAT (75.0 %); SGD x VTT (62.5 %); Malayan orange dwarf, MOD (66.7 %); hybrids of MGD x WAT (31.3 %) and CGD x WAT (31.3 %) and were grouped as susceptible varieties. There were very highly significant mean differences for the trait means of the susceptible from the resistant genotypes for trunk height, girth at 20 cm and at 1.5 m above soil level, eleven leaf scars length, leaf and petiole lengths and crown size ($P \leq 0.001$).

| Coconut variety | Origin | Total number of palms Planted | Infected in 5years | 27 years | Mortality (%) | Phenotypic classification |
|---|---|---|---|---|---|---|
| Sri-Lankan Brown Dwarf (SBD) | Sri-Lanka | 3 | 0 | 0 | 0 | HR |
| Malayan Green Dwarf (MGD) | Malaysia | 336 | 1 | 30 | 8.9 | MR |
| Malayan Orange Dwarf (MOD) | Malaysia | 42 | 2 | 28 | 66.7 | HS |
| Malayan Yellow Dwarf (MYD) | Malaysia | 406 | 6 | 340 | 83.7 | HS |
| Chowghat Green Dwarf (CGD) | India | 442 | 1 | 40 | 9.1 | MR |
| West African Tall (WAT) | Nigeria | 546 | 2 | 538 | 98.5 | HS |
| MGD x WAT | NIFOR(Nigeria) | 16 | 1 | 5 | 31.3 | S |
| CGD x WAT | NIFOR(Nigeria) | 16 | 1 | 5 | 31.3 | S |
| MYD x WAT | NIFOR(Nigeria) | 16 | 1 | 12 | 75.0 | HS |
| SGD x VTT | Ghana | 16 | 1 | 10 | 62.5 | HS |
| MYD x VTT | Ghana | 32 | 2 | 27 | 84.4 | HS |
| **Total** | | 1871 | 19 | 1035 | - | - |

*Table 1: Percent Martality of Coconut Genotypesfrom Lyd Attack*

HS = Highly resistant / tolerant, MS = Moderately resistant, S = Susceptible, HS = Highly susceptible, VTT= Vanuatu Tall, SGD = Sri-Lankan Green Dwarf.

| Quantitative | Resistant | Genotype | | Susceptible | Genotype | |
|---|---|---|---|---|---|---|
| Trait | (Mean ± SD) | SE | Range | (Mean ± SD) | SE | Range |
| TH (m) | 7.775 ± 1.199 | 0.346 | 6.5–11.0 | 10.246 ± 1.237 | 0.357 | 7.8–12.5 |
| G1 (cm) | 81.80 ± 18.34 | 5.295 | 70 - 120 | 142.8 ± 12.34 | 3.563 | 120 – 159 |
| G2 (cm) | 71.00 ± 2.374 | 0.685 | 67 - 77 | 82.42 ± 5.23 | 1.510 | 70 – 86 |
| ELSL (cm) | 48.50 ± 8.15 | 2.353 | 41 - 67 | 88.67 ± 12.5 | 3.608 | 73 - 110 |
| TLN | 27.83 ± 1.337 | 0.386 | 26 - 30 | 27.17 ± 1.642 | 0.474 | 25 – 30 |
| LL (m) | 3.839 ± 0.402 | 0.116 | 3.52–4.9 | 35.075 ± 0.344 | 0.099 | 4.8 – 5.6 |
| LW (m) | 1.625 ± 0.018 | 0.039 | 1.3 – 1.9 | 1.677 ± 0.014 | 0.034 | 1.6 – 1.9 |
| PL (m) | 0.836 ± 0.235 | 0.068 | 0.63–1. | 181.324 ± 0.059 | 0.017 | 1.2 – 1.4 |
| PW (cm) | 6.075 ± 0.559 | 0.162 | 5.4 – 7.5 | 7.025 ± 1.209 | 0.349 | 4.8 – 9.3 |
| PT (cm) | 2.467 ± 0.150 | 0.043 | 2.1 – 2.8 | 2.675 ± 0.347 | 0.100 | 1.9 – 3.6 |
| CS (m) | 5.942 ± 0.639 | 0.184 | 5.2 – 7.3 | 7.792 ± 0.626 | 0.180 | 5.2 – 8.4 |
| LTN | 196.2 ± 16.54 | 4.775 | 185 - 240 | 213.0 ± 26.04 | 7.518 | 196 -260 |
| LTL (m) | 1.095 ± 0.100 | 0.029 | 1.0 -1.3 | 1.191 ± 0.083 | 0.024 | 1.1 – 1.3 |
| LTW (cm) | 4.875 ± 0.603 | 0.174 | 4.2 – 6.05 | .208 ± 0.254 | 0.073 | 4.8 – 5.6 |

*Table 2: Mean, Standard Error and Range for 14 Quantitative Trait of Resistant and Suscptible Coconut Genotypes*

TH = Trunk height, G1= Girth at 20 cm above soil level, G2 = Girth at 1.5 m above soil level, ELSL = Eleven leaf scars length, TLN = Total number of leaves, LL = Leaf length, LW = Leaf width, PL= Petiole length, PW = Petiole width, PT = Petiole thickness, CS = Crown size, LTN = Number of leaflets, LTL= Leaflet length, LTW = Leaflet width, SD = Standard deviation, SE = Standard error.

| Quantitative Trait | Resistant Genotype (Mean ± SD) | Susceptible Genotype (Mean ± SD) | $t_{critical}$ ($t_{0.001(2), 22}$) | $t_{calculated}$ |
|---|---|---|---|---|
| TH (m) | 7.775 ± 1.199 | 10.246 ± 1.237 | 3.792 | 4.97*** |
| G1 (cm) | 81.8 ± 18.34 | 142.8 ± 12.34 | 3.792 | 9.57*** |
| G2 (cm) | 71.0 ± 2.374 | 82.42 ± 5.23 | 3.792 | 6.89*** |
| ELSL (cm) | 48.5 ± 8.15 | 88.67 ± 12.5 | 3.792 | 9.32*** |
| TLN | 27.83 ± 1.337 | 27.17 ± 1.642 | P = 0.287 | 1.09 ns |
| LL (m) | 3.839 ± 0.402 | 5.075 ± 0.344 | ($t_{0.001(2), 22}$) | 8.09*** |
| LW (m) | 1.625 ± 0.018 | 1.677 ± 0.014 | P = 0.331 | 0.99 ns |
| PL (m) | 0.836 ± 0.235 | 1.324 ± 0.059 | 3.792 | 6.99*** |
| PW (cm) | 6.075 ± 0.559 | 7.025 ± 1.209 | 3.792 | 2.47 ns |
| PT (cm) | 2.467 ± 0.150 | 2.675 ± 0.347 | P = 0.075 | 1.91 ns |
| CS (m) | 5.942 ± 0.639 | 792 ± 0.626 | 3.792 | 7.17*** |
| LTN | 196.2 ± 16.54 | 213.0 ± 26.04 | P = 0.072 | 1.89 ns |
| LTL (m) | 1.095 ± 0.100 | 1.191 ± 0.083 | P = 0.018 | 2.55 ns |
| LTW (cm) | 4.875 ± 0.603 | 5.208 ± 0.254 | P = 0.098 | 1.76 ns |

*Table 3: A T-Test Table for Tests of Significance Means Difference for 14 Quantitative Traits of Resistant And Susceptible Coconut Genotypes*

Where TH = Trunk height, G1= Girth at 20 cm above soil level, G2 = Girth at 1.5 m above soil level, ELSL = Eleven leaf scars length, TLN = Total number of leaves, LL = Leaf length, LW = Leaf width, PL= Petiole length, PW = Petiole width, PT = Petiole thickness, CS = Crown size, LTN = Number of leaflets, LTL= Leaflet length, LTW = Leaflet width, SD = Standard deviation;

*** = Very highly significant, ns = non-significant effect

$P \leq 0.001$.

## 3. Discussion

The presence of large swollen base, large trunk (stem) girth and annual trunk increment, long leaves and large canopy size in a coconut genotype may be pointers to susceptibility to LYD. Coconut farmers should be very careful not to use planting materials (seednuts and seedlings) obtained entirely from parent palms that exhibit all these traits in a single cultivar or variety especially when they are to be cultivated in LYD endemic zones. Most highly susceptible coconut genotypes to the disease had been found in this study to possess these traits. Breeding for LYD resistance in coconut by evaluation of first filial generation ($F_1$) genotypes and their germplasm parent plants in disease endemic environments for many years is a prerequisite for identifying genotypes which could be used for direct cultivation in disease prone environments or as parents for hybridization. The germplasm is the breeder's tool from which genotypes with the desired characteristics are evolved. This implies that an ideal germplasm should provide the plant breeder with sufficient fixable genetic variability for traits of interest. Genetic variation in resistance response to diseases had been reported in many crops including coconut palms [xix, xx,xxi, xxii,xxiii, xxiv]. Therefore, the choice of germplasm is vital component in screening for disease resistance particularly in coconut being a perennial crop with a very long-life cycle. The breeder cannot afford to make mistake in the choice of genetic material as this may retard progress in selection or lead to total crop failure. It will also permit the breeder to determine the extent of genetic variability in the population for identified resistance related characters. The mode of resistance to LYD in coconut as suggested by a researcher[xxv]was governed by a single dominant gene locus. One scientist[xix]proposed that the trait was polygenic; another researcher[xxvi] observed that it was governed by a single major co-dominant gene locus with influence of minor modifier gene loci. In a review of data collected from field resistance studies of various ecotypes and their hybrids in Jamaica by some workers [xvii], they observed that resistance to lethal yellowing in coconut palms was significantly affected by genotype and location in the field and there was a significant genotype x location interaction. And that the heritability of resistance appeared to be very high with estimates ranging from 0.72 to 0.79 and stated that it could be assumed that major influence on the expression of resistance to the disease was through additive genetic effect. Highly susceptible genotypes to lethal yellowing disease were found to have very highly significantly different means in most of the measured quantitative traits. Irrespective of the fact that the expression of phenotypic traits is governed by inherent genes, the environment and the interaction between the genes and the environment, it does indicate that the resistant genotypes do possess some lethal yellowing resistance factors having lasted decades in areas of cyclical occurrence. Further work would be the analyses of these phenotypically classified resistant genotypes for presence of resistance marker(s) compared with standard LYD resistance and susceptible genotypes as control using microsatellites.

## 4. Conclusion

Sri-Lankan brown dwarf, some true to type genotypes of Malayan green dwarf and chowghat green dwarf are very promising lethal yellowing resistance genotypes that could be used as source of durable materials in LYD prevalent zones of Nigeria. They could as well be used as resistant parents for the production of hybrid materials for further testing for lethal yellowing disease resistance by plant breeders.

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