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Morphological and genetic diversity of *Irvingia wombolu* Vermoesen accessions for conservation and breeding

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Abstract

Irvingia wombolu (Vermoesen), the cooking type of bush mango, is an economically important but under-studied forest tree in West and Central Africa. This study characterized 88 accessions conserved at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria, to assess morphological, architectural, yield and genetic diversity for conservation and breeding. Morphological traits were evaluated on 73 accessions, yield and yield-related traits on 29 trees over five years (2013–2017), and molecular diversity using RAPD markers on 88 accessions.

The trees showed significant variation in leaf shape, fruit and stone size. Broad-sense heritability ranged from 0.17 (number of fruits per tree) to 0.74 (fresh stone weight), with dry kernel weight (DKW) having moderate heritability estimate ($H^2 = 0.33$) and genetic advance as a percentage of the mean of 15.52%. Path analysis identified dry stone weight as the strongest direct contributor to DKW. Three superior ideotypes (Trees 9, 24 and 28) with high yield and desirable tree architecture were identified. Genetic diversity among the germplasm was high, with an average polymorphism information content value of 0.89 as shown by the RAPD markers. The dendrogram showed seven clusters, with clusters six and seven comprising 30 genotypes each. The presence of significant phenotypic and genotypic diversity among the studied accessions provides opportunities for genetic improvement and could promote domestication and commercialization of the products for farmers to supplement their income.

Keywords: *Irvingia wombolu*, morphological characterization, genetic diversity, RAPD, breeding, domestication

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45 Introduction

46 *Irvingia gabonensis* Aubry-Lecomte ex O'Rorke and *Irvingia wombolu*
47 Vermoesen, collectively known as bush mango, are the most economically
48 important species within the Irvingiaceae family (1,2). These priority non-
49 timber forest product (NTFP) trees are widely distributed in humid lowland
50 forests (3). While *I. gabonensis* produces sweet, edible mesocarp, *I. wombolu*
51 produces sour fruits whose kernels are highly valued as a thickening agent in
52 "ogbono" soup and generate significant income through regional and cross-
53 border trade (4,5). Despite their economic value, both species remain largely
54 undomesticated, while the sourcing of fruits for kernel extraction still relies
55 heavily on harvests from wild forest trees (6).

56 As part of the effort to conserve the gene pool, boost their production and
57 develop them towards a major crop for rural communities in Nigeria, the
58 National Horticultural Research Institute, Ibadan, engaged in intensive
59 germplasm collection activities of *I. wombolu* that have led to the
60 establishment of an ex-situ gene-bank and experimental site in Ibadan,
61 Nigeria. All effort is geared towards proper characterization of the *I. wombolu*
62 accessions for the purpose of enhancing their application in crop improvement
63 and tree domestication. However, the population within the *I. wombolu* family
64 exhibits low morphological and phenological diversity, thereby making their
65 fruits very difficult to distinguish; there is also no clear relation between the
66 biochemical properties of these fruits (6). Due to this overlap of morphological,
67 phenological and biochemical properties, the correct identity of these fruits
68 needs to be investigated. Knowledge on germplasm diversity is very important
69 for conservation purposes and breeding programs aimed at developing
70 improved varieties with higher productivity and stability (7,8).

71 Morphological and molecular markers are useful tools for the identification of
72 crop species (9,10). Limitations of using morphological markers in tropical fruit
73 trees include i) the long time it takes for the tree plants to overcome the
74 juvenility stage and (ii) they are influenced by environmental factors. The use
75 of molecular markers, on the other hand, overcomes these challenges
76 because it reveals diversity at the genome level (11–13). Studies show that
77 molecular marker analysis of tropical fruit trees helps understand the past
78 domestication of taxa, determine effective population sizes and assess the
79 value of a decentralized approach for future domestication (14,15). RAPD
80 markers have been employed for assessing genetic diversity in several plant
81 species. The genetic variation information thus revealed by molecular markers
82 provides better insight for selecting improved genotypes for desired traits and
83 enhances the effective utilization of genetic resources and conservation of *I.*
84 *wombolu*.

85 Therefore, this study aimed to assess the genetic and morphological variation
86 among 88 accessions of *Irvingia wombolu* to characterize the germplasm for
87 effective utilization, genetic improvement and conservation of this important
88 plant genetic resource.

90 **Materials and methods**

91 The eighty-eight accessions of *I. wombolu* used for this experiment were
 92 transplanted to the field in the year 2006 and are being maintained at the
 93 National Horticultural Research Institute (NIHORT). Morphological data
 94 collected from 73 accessions with complete records were used for the
 95 morphometric data analysis. Yield data collected on 29 trees with a complete
 96 dataset over five years (2013–2017) were used for the analysis of yield traits.

97 Morphological data were collected on leaf shape (LS), leaf apex (LAS), leaf
 98 base (LBS), leaf margin (LM), apex curvature (LAC), mature leaf colour (MLC),
 99 young leaf colour (YLC), phenology, mean leaf area (LA), stem girth,
 100 branching density, height to first branch and tree height. Yield traits include:
 101 dry kernel weight (DKW), number of fruits (NFRTS), fresh fruit weight (FFW),
 102 dry stone weight (DSW), stone thickness (ST), fresh stone weight (FSW), fruit
 103 length (FLD), fruit width (FFL), stone length (DSL), dry stone mill weight
 104 (DSMW) and mill weight (MW).

105

106 **DNA extraction and RAPD analysis**

107 Leaf samples were collected from 88 accessions in 2017. Total genomic DNA
 108 was extracted using a modified CTAB method (16). DNA concentrations were
 109 determined, relative to uncut lambda DNA, on 1% agarose gels. The
 110 conditions for RAPD–PCR were identical to those used by (17) and fragments
 111 were resolved on 1.4% agarose gels and sized against a 1-kb ladder (Gibco).
 112 After an initial survey of 30 RAPD primers (all from Operon Technologies, kit
 113 B), using a subsample of material to test for levels of polymorphism and
 114 reproducibility, 11 polymorphic primers were chosen for the full study.

115 **Data Scoring and Statistical Analysis**

116 Since RAPD markers are dominant, we assumed that each band represented
 117 the phenotype at a single allelic locus (18). All distinct bands or fragments
 118 (RAPD markers) were thereby given identification numbers according to their
 119 position on the gel and scored visually based on their presence or absence,
 120 separately for each individual and each primer.

121 Only clear and repeatable amplification products were scored as 1 for present
 122 bands and 0 for absence. The specific bands useful for identifying species
 123 and cultivars were named with a primer number followed by the approximate
 124 size of the amplified fragment in base pairs. Polymorphism was calculated
 125 based on the presence or absence of bands. The dendrogram was
 126 constructed with a distance matrix using the unweighted pair group method
 127 with arithmetic average.

128 Eleven polymorphic RAPD primers were selected after screening. PCR
 129 products were separated on 1.4% agarose gels and scored as present (1) or
 130 absent (0).

Data Analysis

Morphometric data analyses, including frequency distribution, Shannon-Weaver diversity index (H'), multiple correspondence analysis (MCA) and hierarchical clustering, were performed in R (19) using the packages (FactoMineR, factoextra, cluster, rpart, ggplot2 and vegan).

For the yield data, a two-way analysis of variance (ANOVA) was conducted using the linear model:

$$Y_{ijk} = \mu + G_i + Y_j + (G \times Y)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the observation of the k^{th} replicate of the i^{th} genotype in the j^{th} year, μ is the overall mean, G_i is the effect of the i^{th} genotype, Y_j is the effect of the j^{th} year, $(G \times Y)_{ij}$ is the genotype $\times$ year interaction effect and ε_{ijk} is the random error.

Variance components were estimated from the mean squares of ANOVA using the following formulas:

$$\sigma^2g = \text{MSG} - \text{MSG} \times Y / r$$

$$\sigma^2ge = \text{MSG} \times Y - \text{MSe}$$

$$\sigma^2e = \text{MSe}$$

where σ^2g , σ^2ge and σ^2e are the genotypic, genotype $\times$ environment interaction and error variances, respectively; MSG, MSG $\times$ Y and MSe are the mean squares for genotype, genotype $\times$ year interaction and error; and r is the number of years (environments).

Broad-sense heritability (H^2) was calculated as:

$$H^2 = \sigma^2g / (\sigma^2g + \sigma^2ge + \sigma^2e)$$

Genetic advance (GA) and genetic advance as a percentage of the mean (GAM) were estimated at 5% selection intensity ($k = 2.06$) using:

$$GA = k \times \sqrt{\sigma^2g} \times H^2$$

$$GAM (\%) = (GA / \bar{X}) \times 100$$

where $\bar{X}$ is the grand mean of the trait.

Pearson's correlation coefficients, path coefficient analysis and weighted multi-trait architecture indices were also computed. All statistical analyses and visualization were performed in R statistical software version 4.4.0 (19) using the packages (corrplot, lavaan, semPlot and metan).

Results

Significant morphological variation was observed among 73 accessions with complete morphometric data (Fig. 1). Leaf shape was predominantly obovate (70.3%); leaf apex was acute in 98.6% of accessions, while the leaf base and leaf margin were acute in 85.1% and entire in 91.9% of accessions, respectively.

The Shannon-Weaver diversity index (H') varied considerably among traits (Fig. 2). Apex curvature showed the highest diversity ($H' = 1.85$), followed by young leaf colour ($H' = 1.25$), leaf shape ($H' = 0.82$) and mature leaf colour ($H' = 0.66$). Multiple Correspondence Analysis (Fig. 3) revealed that young leaf colour, apex curvature and mature leaf colour were the most discriminatory traits among the accessions. Hierarchical clustering (Fig. 4) grouped the 73 accessions into three morphotypes, with one dominant cluster comprising 95.9% of accessions and two rare morphotypes (2.7% and 1.4%).

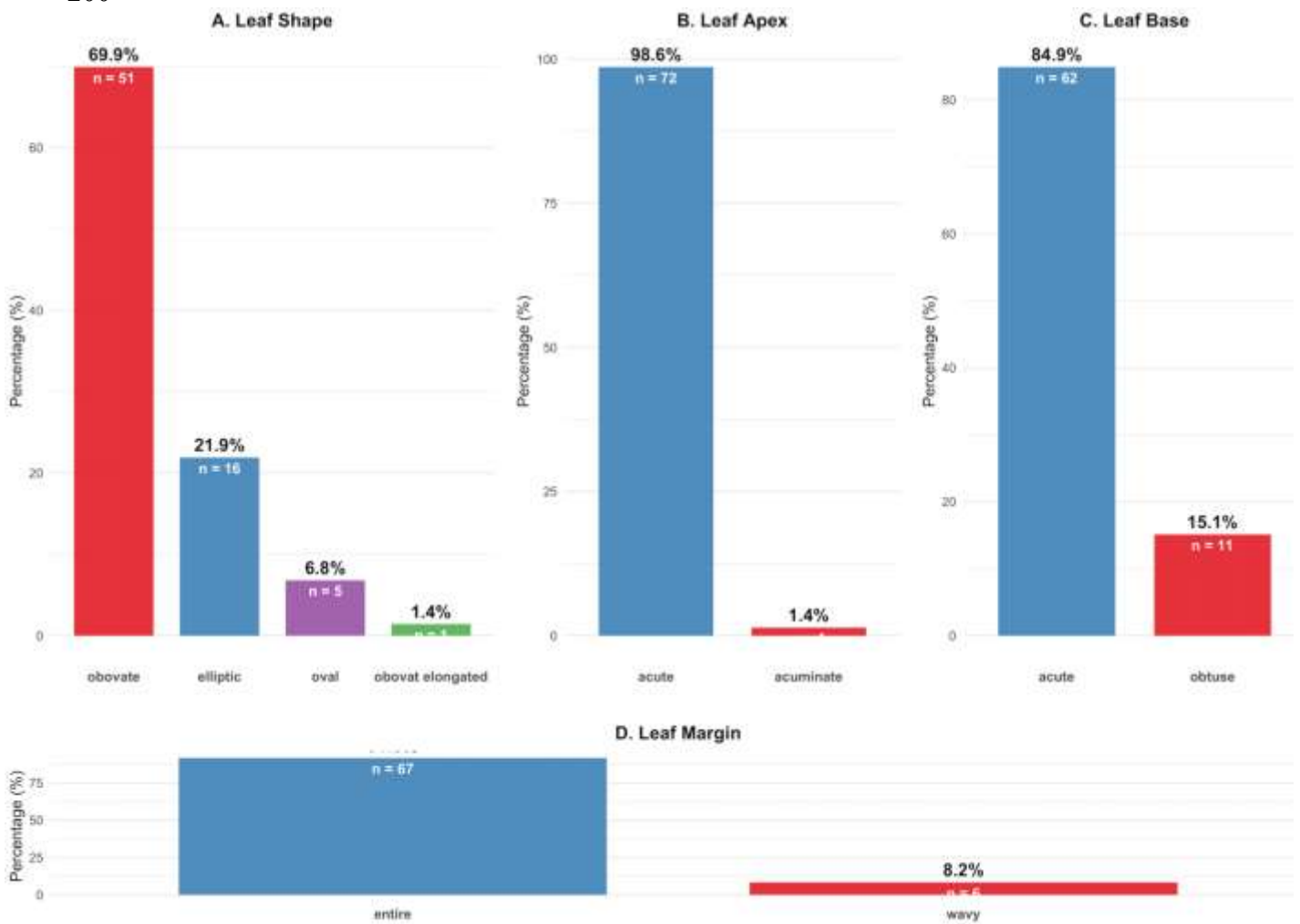


Fig. 1 Frequency Distribution of Leaf Morphological Traits in 73 accessions of *Irvingia wombolu*

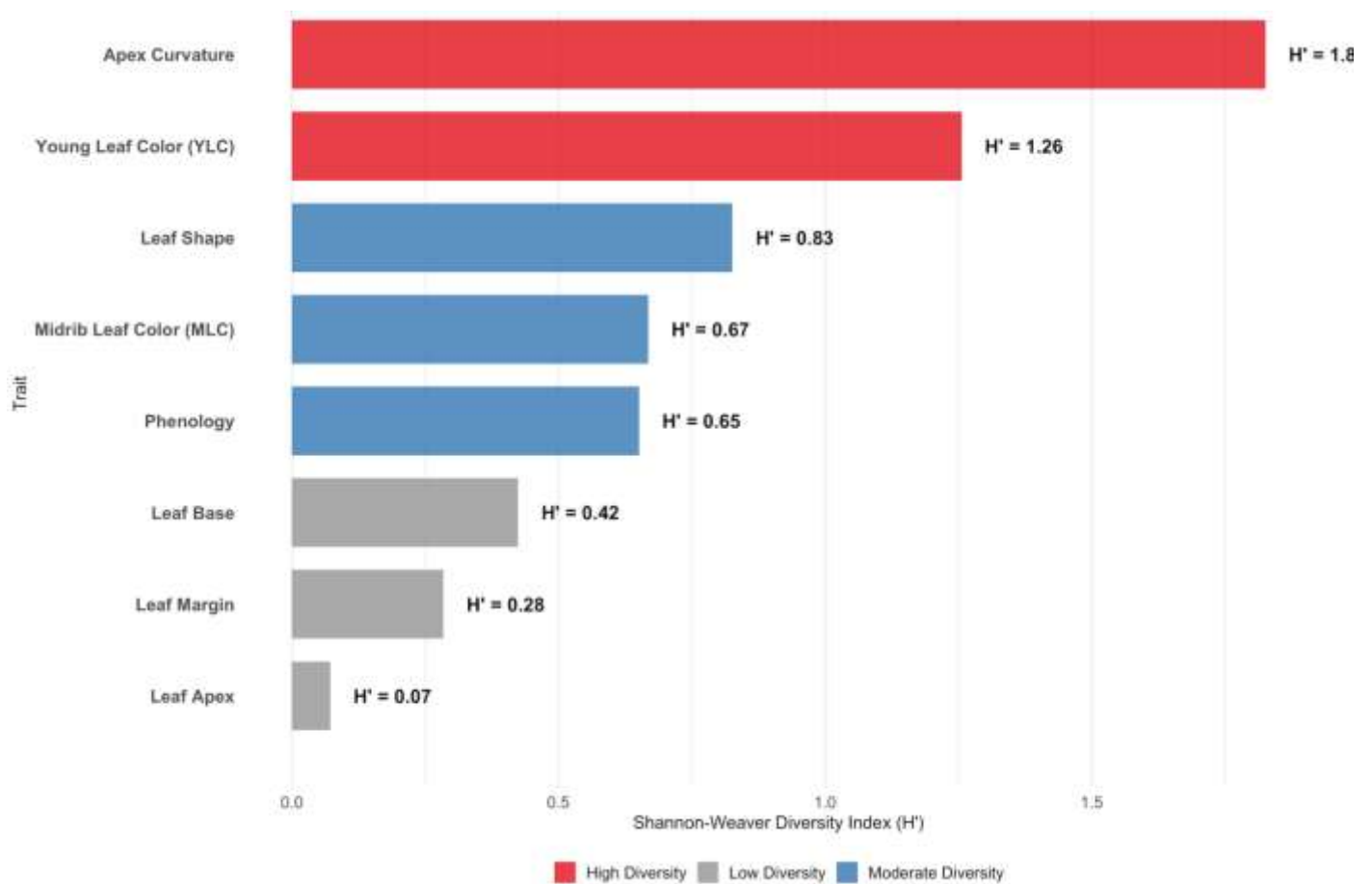


Fig. 2 Shannon-Weaver Diversity Index (H') of Morphological Traits in 73 accessions of *Irvingia wombolu*

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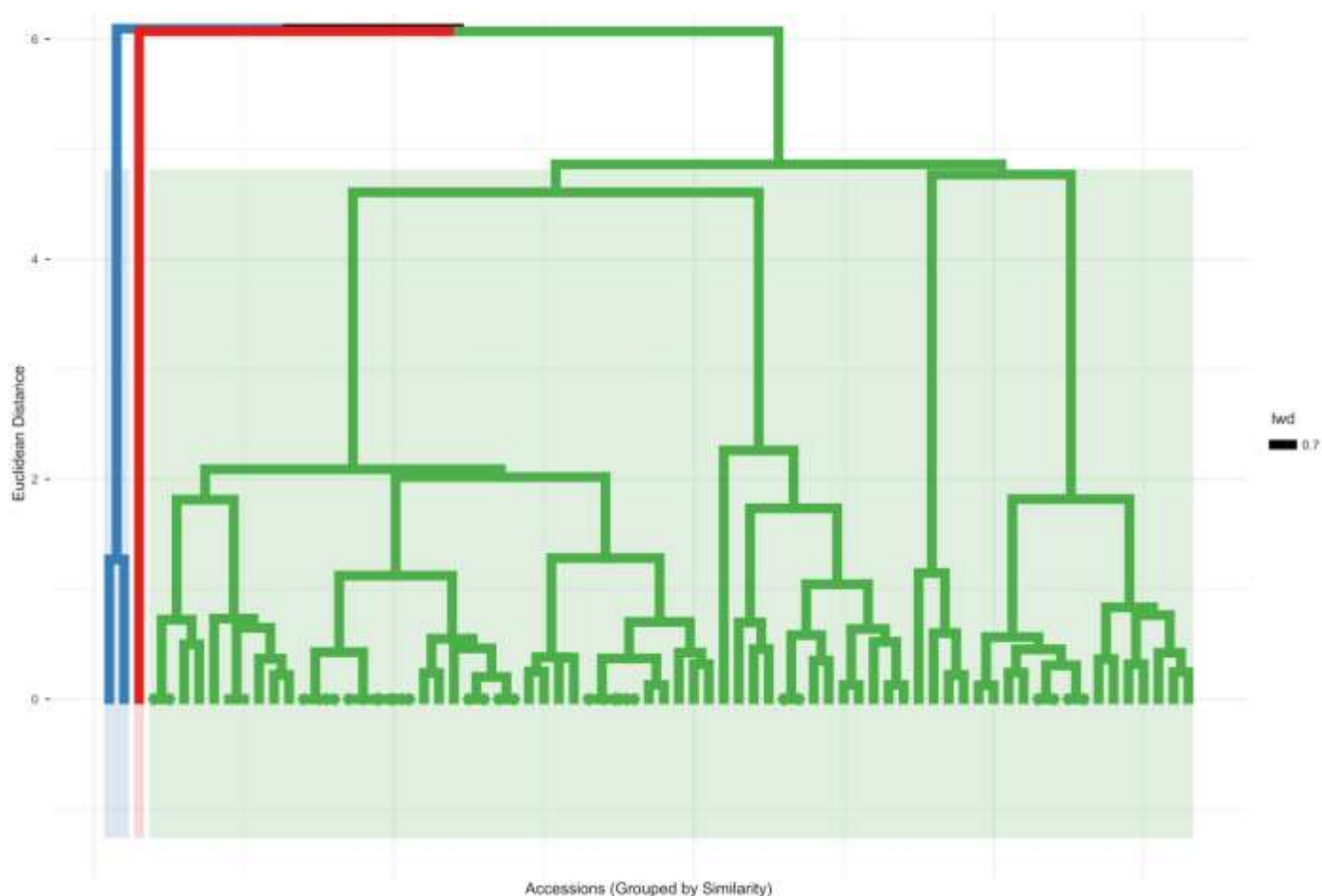


Fig. 4 Hierarchical clustering of 73 accessions of *Irvingia wombolu*

255 Significant genotypic variation was observed for most traits among the 29
256 trees with complete yield data across five years (Table 1). Genotype ×
257 environment (Year) interaction effects were not significant for any of the traits
258 ($P > 0.05$).
259 Broad-sense heritability (H^2) ranged from 0.170 (NFRTS) to 0.744 (FSW)
260 (Table 2). Five traits exhibited high heritability (> 0.60): FSW (0.744), MW
261 (0.687), ST (0.660), FFW (0.658) and DSW (0.640), while four traits showed
262 moderate heritability (0.30–0.60): FLD (0.587), FFL (0.584), DSL (0.582), FSD
263 (0.527) and DKW (0.330). Genetic advance (GA) estimates ranged from 0.28
264 (DKW) to 42.41 (NFRTS) (Table 2). However, when expressed as a
265 percentage of the mean (GAM), MW showed the highest expected genetic
266 gain (34.14%), followed by FSW (28.88%), DSW (27.76%), FFW (23.16%)
267 and ST (22.25%).

268

269 Table 1. Analysis of variance (ANOVA) of yield-related traits in 29 accessions of *Irvingia wombolu* across five environments (years)

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Source	DF	DKW	NFRTS	FFW	DSW	ST	FSW	FLD	FFL	DSL	DSMW	DFSW	FSD	MW
Year (ENV)	4	2.49**	830200.5***	295.70**	4.53ns	0.55*	51.50***	9.63ns	13.73ns	19.05ns	314.13***	17.03ns	67.13**	7887.60***
Genotype (GEN)	28	1.65*	198558.5ns	1529.64***	33.74***	2.07***	104.75***	73.32***	139.16***	99.09***	41.48ns	22.78ns	99.53***	674.62***
GEN × ENV	112	0.49ns	97972.2ns	143.88ns	3.42ns	0.19ns	6.76ns	9.04ns	17.37ns	12.43ns	14.71ns	10.73ns	15.13ns	56.23ns
Residuals	116	0.5	97972	143.9	3.42	0.19	6.76	9.04	17.37	12.43	14.71	10.73	15.13	56.2

271 Reported values are mean squares. Significance codes: *** p < 0.001, ** p < 0.01, * p < 0.05, ns = not significant.

272 DKW - Dry Kernel Weight (g), NFRTS - Number of Fruits per Tree, FFW - Fresh Fruit Weight (g), DSW - Dry Stone Weight (g), ST - Stone Thickness (mm), FSW - Fresh Stone
 273 Weight (g), FLD - Fruit Length (mm), FFL - Fruit Width (mm), DSL - Stone Length (mm), DSMW - Dry Stone Mill Weight (g), DFSW - . dry fruit small width (mm) and MW - Mill
 274 Weight (g).

275
276 Table 2: Genetic parameters for yield-related traits in 29 accessions of *Irvingia wombolu*
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Trait	Mean	σ^2g	σ^2p	H ²	GA	GAM	GCV(%)	PCV (%)
DKW (g)	2.1	0.23	0.71	0.33	0.33	15.52	23	40.12
NFRTS	279.43	20117.26	118089.49	0.17	49.67	17.78	50.76	123
FFW (g)	83.04	277.152	421.03	0.66	22.63	27.26	20.05	24.71
DSW (g)	9.96	6.07	9.48	0.64	3.25	32.61	24.73	30.93
ST (mm)	3.19	0.37	0.57	0.66	0.83	25.93	19.2	23.62
FSW (g)	20	19.6	26.36	0.74	6.75	33.74	22.14	25.67
FLD (mm)	50.53	12.86	21.89	0.59	4.36	8.63	7.1	9.26
FFL (mm)	56.54	24.36	41.73	0.58	5.9	10.43	8.73	11.42
DSL (mm)	38.49	17.33	29.76	0.58	4.97	12.92	10.82	14.18
DSMW (g)	28.85	5.35	20.06	0.27	1.29	4.46	8.02	15.53
DFSW	19.12	2.41	13.14	0.18	0.58	3.01	8.12	18.96
FSD	47.11	16.88	32.01	0.53	4.49	9.52	8.72	12.01
MW (g)	39.3	123.68	179.91	0.69	15.81	40.22	28.3	34.14

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279 σ^2g = Genotypic variance; σ^2p = Phenotypic variance; H² = Broad-sense heritability; GA = Genetic Advance; GAM = Genetic Advance as % of Mean; GCV = Genotypic
280 coefficient of variation; PCV = Phenotypic coefficient of variation. DKW - Dry Kernel Weight (g), NFRTS - Number of Fruits per Tree, FFW - Fresh Fruit Weight (g), DSW - Dry
281 Stone Weight (g), ST - Stone Thickness (mm), FSW - Fresh Stone Weight (g), FLD - Fruit Length (mm), FFL - Fruit Width (mm), DSL - Stone Length (mm), DSMW - Dry Stone
282 Mill Weight (g), DFW - dry fruit small width (mm) and MW - Mill Weight (g).

Strong positive correlations were observed among fruit and stone traits: FFW ↔ DSW ($r = 0.75$), FFW ↔ FSW ($r = 0.69$), FFW ↔ MW ($r = 0.67$) and DSW ↔ FSW ($r = 0.81$). NFRTS showed negative correlations with most other traits, particularly with FSW ($r = -0.41$) and DSW ($r = -0.28$) (Fig. 5). The trait correlation network positioned DKW as a central hub with dense positive connections to fruit traits (FFW, FLD, FFL, MW) and stone traits (DSW, FSW, DSL, DSMW), while a few negative associations (NFRTS and ST) were observed (Fig. 6). Path coefficient analysis revealed that DSW was the only statistically significant direct predictor of DKW ($\beta = 0.99$, $P = 0.01$) (Fig. 7). All other predictors (FFW, FSW, ST, NFRTS and MW) showed non-significant direct effects ($P > 0.01$).

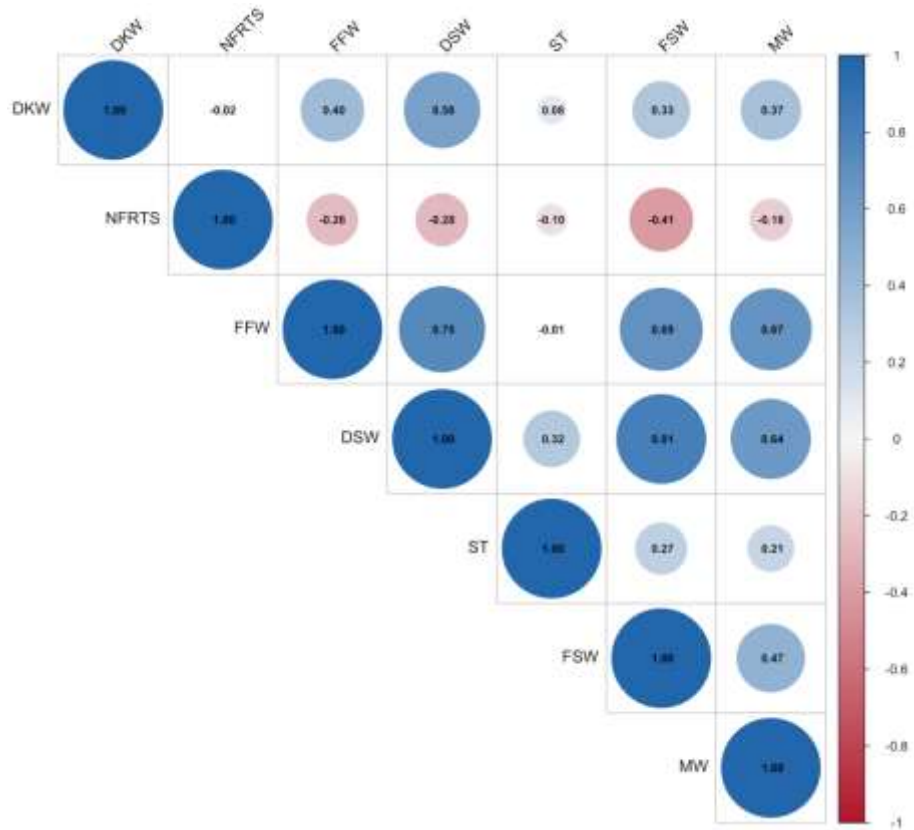
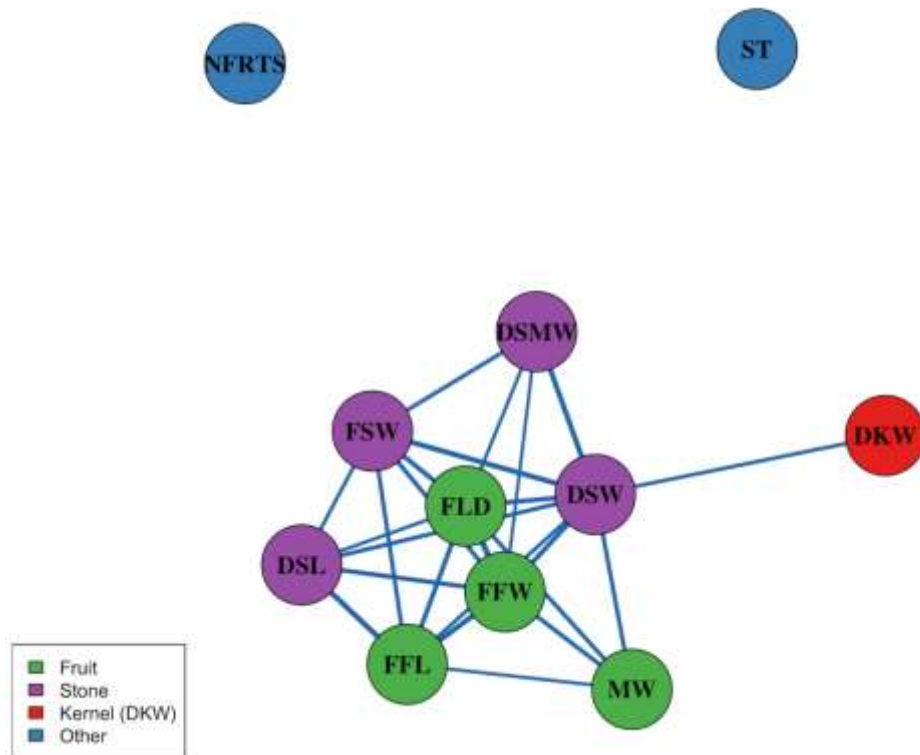


Fig. 5 Correlation Plot for Yield Traits among 29 accessions of *Irvingia wombolu*

DKW - Dry Kernel Weight (g), NFRTS - Number of Fruits per Tree, FFW - Fresh Fruit Weight (g), DSW - Dry Stone Weight (g), ST - Stone Thickness (mm), FSW - Fresh Stone Weight (g), FLD - Fruit Length (mm), FFL - Fruit Width (mm), DSL - Stone Length (mm), DSMW - Dry Stone Mill Weight (g) and MW - Mill Weight (g)

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Fig. 6 Trait Correlation Network for Yield Traits among 29 accessions of *Irvingia wombolu*

DKW - Dry Kernel Weight (g), NFRTS - Number of Fruits per Tree, FFW - Fresh Fruit Weight (g), DSW - Dry Stone Weight (g), ST - Stone Thickness (mm), FSW - Fresh Stone Weight (g), FLD - Fruit Length (mm), FFL - Fruit Width (mm), DSL - Stone Length (mm), DSMW - Dry Stone Mill Weight (g) and MW - Mill Weight (g)

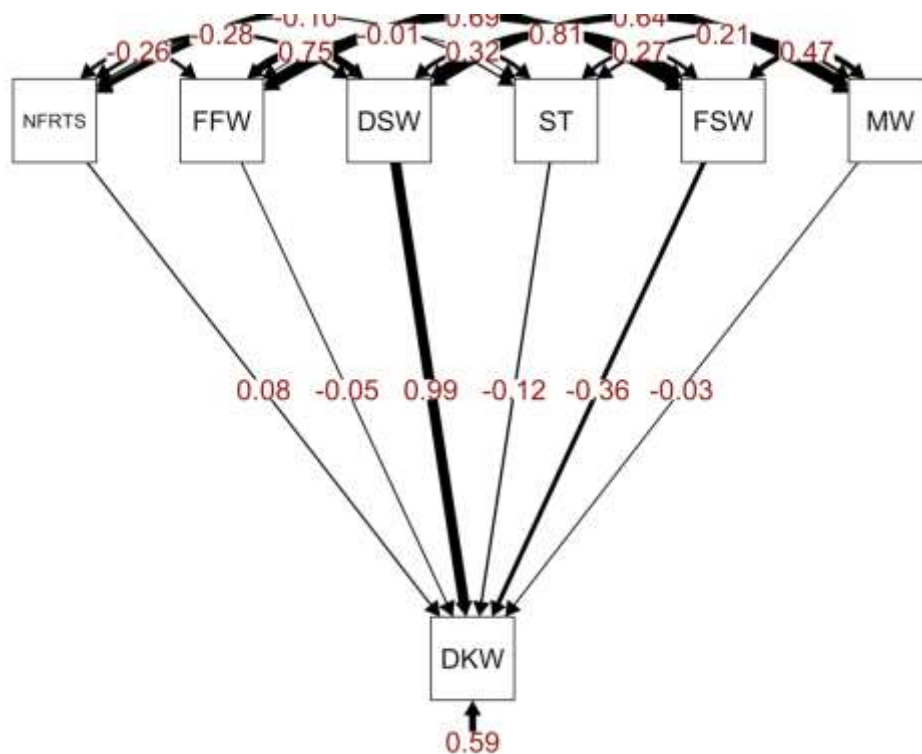
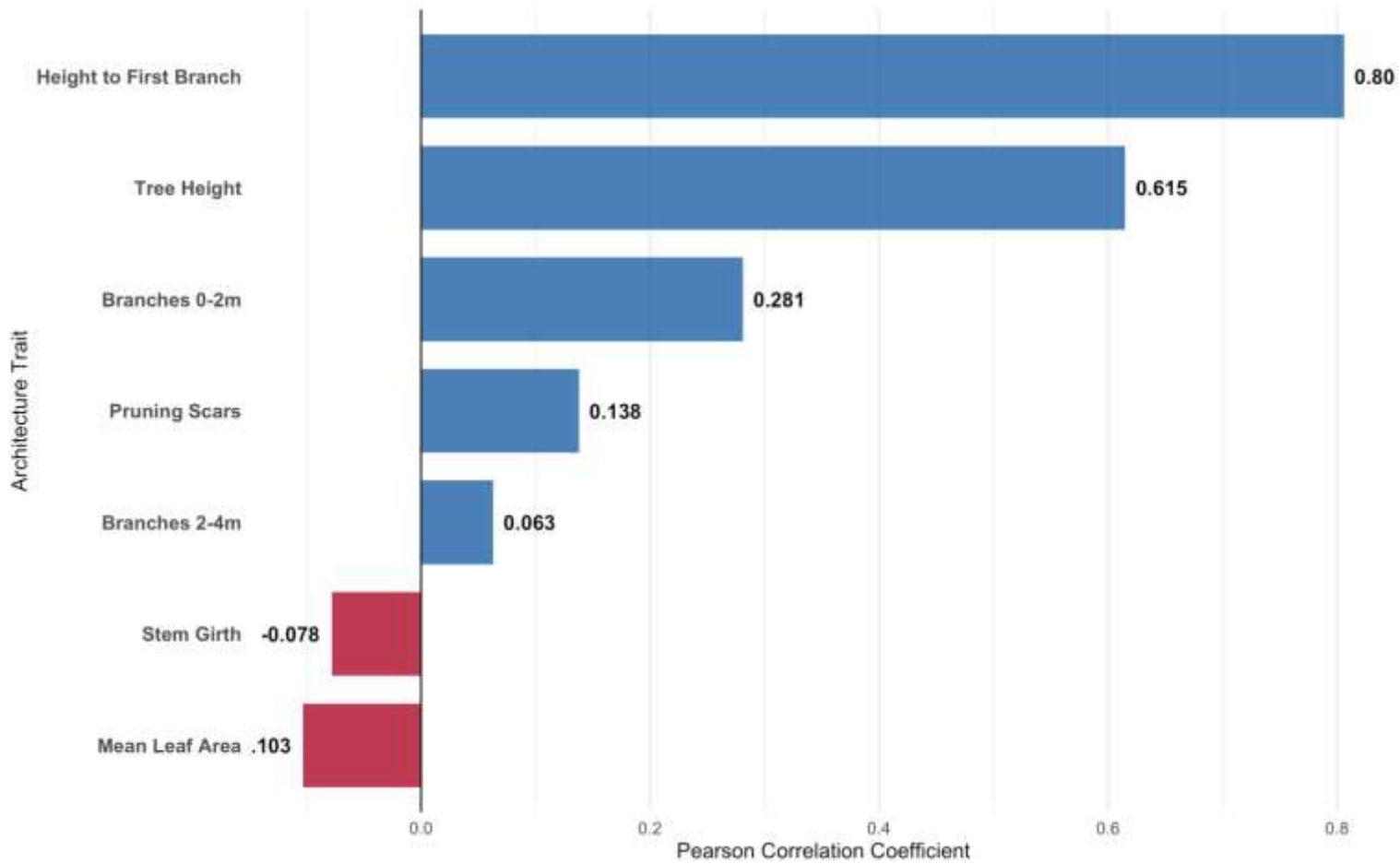


Fig. 7 Path Diagram of Contributions of Stone and Fruit Traits to Dry Kernel Weight of *Irvingia wombolu*

DKW - Dry Kernel Weight (g), NFRTS - Number of Fruits per Tree, FFW - Fresh Fruit Weight (g), DSW - Dry Stone Weight (g), ST - Stone Thickness (mm), FSW - Fresh Stone Weight (g), FLD - Fruit Length (mm), FFL - Fruit Width (mm), DSL - Stone Length (mm), DSMW - Dry Stone Mill Weight (g) and MW - Mill Weight (g).

A weighted multi-trait index was calculated for 15 trees with complete architecture and yield data to identify trees with superior architecture for yield potential. Superior idiotypes were identified using trait weights derived from correlations between architecture traits and DKW. Height to first branch ($r = 0.80$) and tree height ($r = 0.615$) received the highest weights, while stem girth and mean leaf area received zero weights due to negative correlations (Fig. 8). Tree 9 achieved the highest architecture score (1.44), followed by Tree 8 (1.25) and Tree 13 (1.16). The radar chart (Fig. 9) of the top three trees revealed that these accessions exhibited superior height to first branch (>1.5 SD above the mean) and above-average tree height (>1.2 SD above the mean).

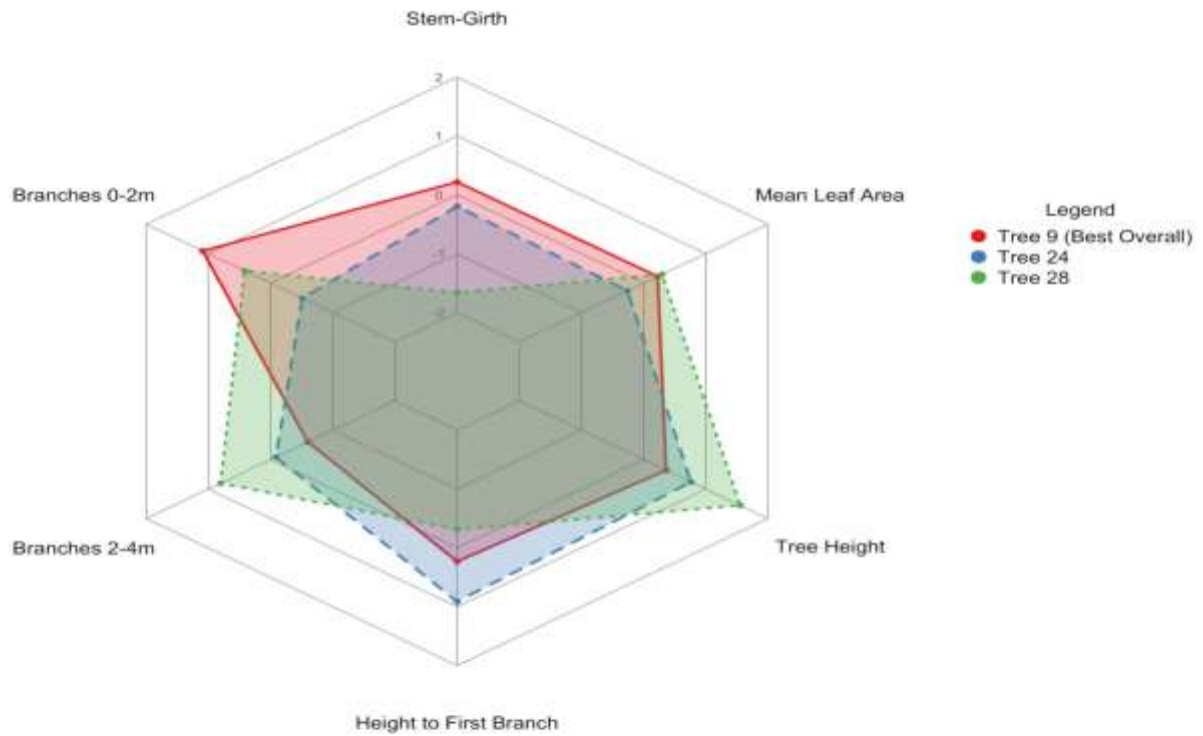
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Fig. 8 Correlations between Architecture Traits and Dry Kernel Weight in *Irvingia wombolu*

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Fig. 9 Ideal Tree Architecture Profile of Top 3 accessions of *Irvingia wombolu*

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574 Considerable variation in the amplified fragment patterns was observed using
575 different primers. Of the 30 primers tested, 11 were polymorphic and were
576 used for the characterization and genetic diversity analysis of the 88 *I.*
577 *wombolu* accessions. A total of 100 alleles were generated with an average
578 of 9.09 alleles per locus per primer, ranging from a minimum of 6 alleles using
579 primer OPH02 and OPT08 to a maximum of 14 alleles using OPT20 primer
580 (Table 3, Fig. 10). Polymorphic Information Content (PIC) values ranged from
581 0.079 (OPT08) to 0.95 (OPHO5) averaging 0.89 per locus. The matrix of Nei's
582 genetic distances between pairs of populations was used for UPGMA cluster
583 analysis. The 88 populations were classified into seven major groups; groups
584 six and seven contained 30 accessions each, group three contained 16
585 accessions, groups two and four contained 2 accessions each, group five
586 contained 7 accessions and group one contained only 1 accession (Fig. 11).
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Table 3. RAPD primer sequence and polymorphic information observed in 88 *Irvingia wombolu* accessions

Marker	Primer Sequence	MAF	Sample Size	Allele Number	Gene Diversity	PIC
OPB10	OPB – 10 51 – CTG CTG GGA C – 31	0.24	88	8	0.91	0.9
OPT07	OPT – 07 - 51 – GGC AGG CTG T – 31	0.19	88	11	0.93	0.93
OPH07	OPH – 07 - 51 – CTG CAT CGT G – 31	0.25	88	8	0.9	0.89
OPT20	OPT – 20 51 – GAC CAA TGC C – 31	0.26	88	14	0.91	0.91
OPH02	OPH – 02 - 51 – TCG GAC GTG A – 31	0.31	88	6	0.88	0.87
OPT06	OPT – 06 - 51 – CAA GGG CAG A – 31	0.28	88	9	0.9	0.9
OPT01	OPT – 01 - 51 – GGG CCA CTC A – 31	0.24	88	10	0.91	0.91
OPT05	OPT – 05 - 51 – GGG TTT GGC A – 31	0.2	88	9	0.91	0.91
OPT08	OPT – 08 - 51 – AAC GGC GAC A – 31	0.36	88	6	0.79	0.76
OPH05	OPH – 05 - 51 – AGT CGT CCC C – 31	0.13	88	11	0.95	0.95
OPB12	OPB – 12 51 – CCT TGA CGC A – 31	0.35	88	8	0.86	0.86
Total		2.81	968	100	9.85	9.79
Mean		0.26	88	9.09	0.9	0.89

MAF = Major Allele Frequency; PIC = Polymorphic Information Content

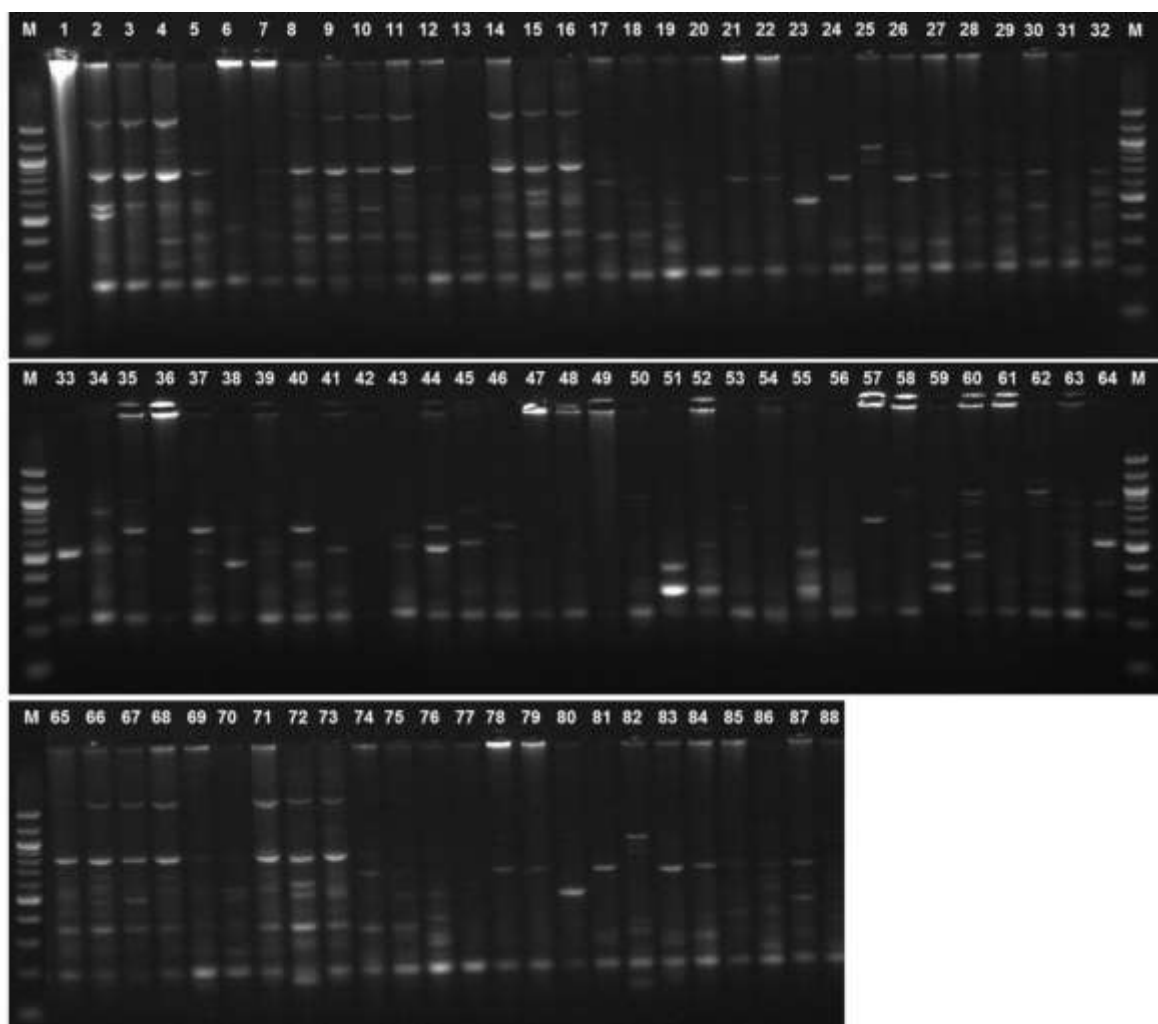


Fig. 10 RAPD profiles of 88 *Irvingia wombolu* accessions using primer 0PB12 (Lane "M" is 1kb ladder marker DNA, Lanes 1-88 are the *Irvingia wombolu* accessions.)

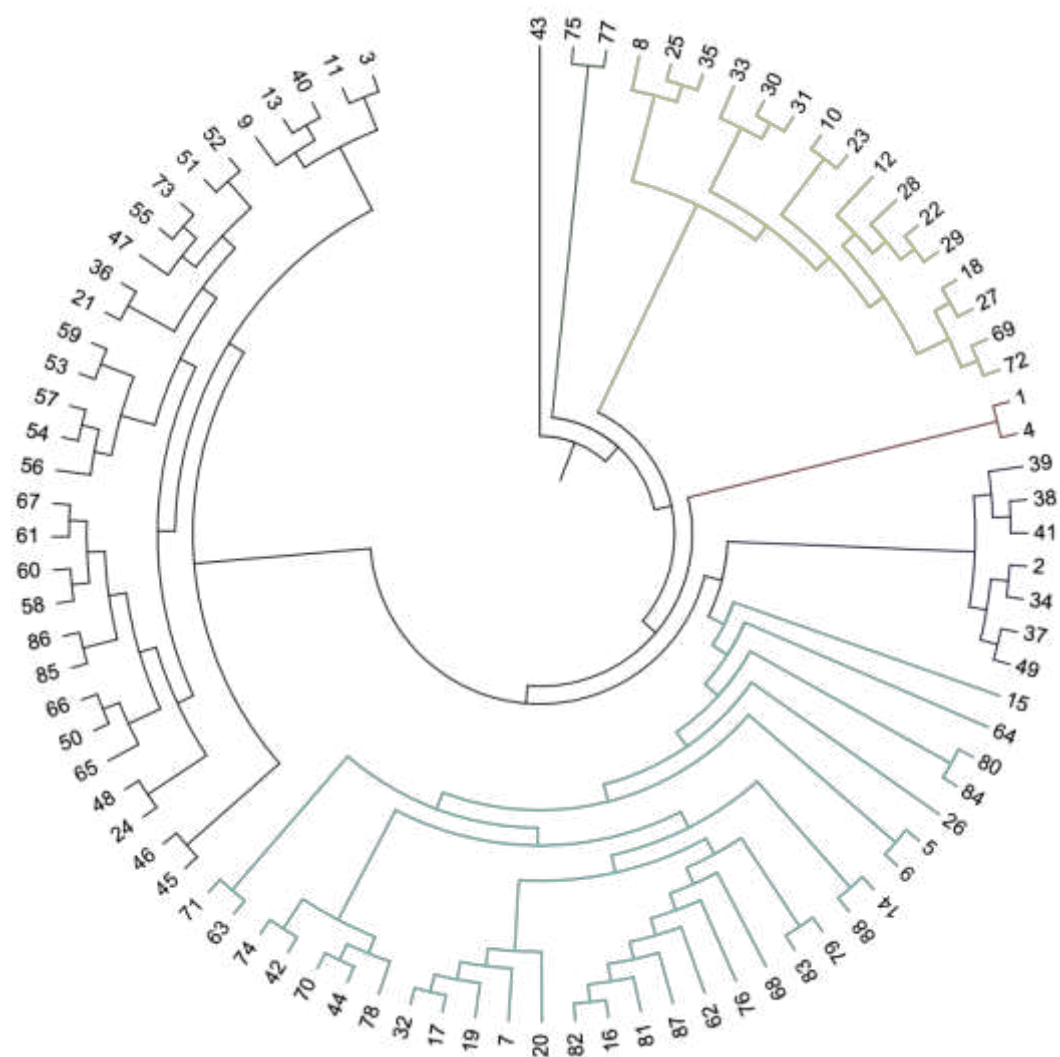


Fig. 11 UPGMA-based dendrogram derived from RAPD analyses of 88 *Irvingia wombolu* accessions

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662 Discussion

663

664 Leaf traits showed dominant patterns across most accessions, but apex
665 curvature and young leaf colour were the most informative traits in classifying
666 the *Irvingia wombolu* accessions into subgroups. This suggests that a
667 relatively small number of morphological descriptors could somewhat
668 discriminate among closely related accessions through a painstaking
669 assessment protocol. This is consistent with earlier reports that *Irvingia*
670 species are morphologically similar and often difficult to distinguish while
671 relying solely on routine field observations (20–22). However, the dominance
672 of a single morphotype alongside a few rare morphotypes suggests that the
673 germplasm contains both broadly shared phenotypes and distinct forms that
674 should be conserved for future improvement of *Irvingia wombolu*. A similar
675 pattern was reported in a study on *I. wombolu* and *I. gabonensis* (20,23). It is
676 imperative to conserve both the common and rare morphotypes to avoid
677 losing potentially useful alleles due to domestication or genetic erosion
678 through loss of habitat.

679 Significant variation in yield was recorded among the 29 accessions of *Irvingia*
680 *wombolu* evaluated over five years. High broad-sense heritability was
681 observed for yield-determining traits such as fresh stone weight, stone
682 thickness, fresh fruit weight and dry stone weight. In contrast, dry kernel
683 weight (DKW), the primary economic trait, showed moderate heritability.

684 Following the classification of (24), who categorized estimates of genetic
685 advance as a percentage of mean (GAM) that were less than 10% as low,
686 those that ranged between 10% and 20% as moderate and those more than
687 20% as high, the observed GAM values in this experiment ranged from
688 moderate to high and it confirms that these traits were not only highly
689 heritable but will also respond favourably to selection. The combination of
690 moderate-to-high estimates of broad-sense heritability and GAM for key traits
691 indicates a strong potential for their genetic improvement through phenotypic
692 selection. For example, selection for dry kernel weight, which has a heritability
693 estimate of 0.33 and GAM value of 13.32%, implies that imposing selection
694 pressure on the top 5% of the base population could result in significant gains
695 of 13% in yield over time. The non-significant genotype $\times$ year interaction for
696 most traits further supports yield stability across seasons.

697 Trait association studies (correlation networks and path analysis) confirmed
698 strong positive relationships among fruit and stone size traits, with dry stone
699 weight emerging as the most important direct contributor to DKW; hence,
700 indirect selection for dry kernel yield through dry stone weight may be more
701 efficient than selecting on fruit traits alone. Similar trait association
702 approaches have been useful in fruit-tree improvement because they help
703 breeders identify easy-to-score surrogate traits that are tightly linked to
704 economic yield (25,26).

705 The weighted multi-trait architecture index successfully identified three
706 superior ideotypes that combine high kernel yield with desirable height at the
707 first branch, tree height and architecture. This suggests that tree architecture
708 may contribute indirectly to yield potential, probably through canopy cover,

light interception and even distribution of fruiting points (27). The identified ideotypes are potential candidates for selection based on their overall phenotypic acceptability, viz ideal tree architecture and productivity (28,29).

The RAPD analysis showed a high level of polymorphism and resolved the 88 accessions into seven clusters, thereby confirming substantial molecular diversity within the collection. This agrees with earlier work that shows *Irvingia* species are genetically differentiated across geographic regions and that RAPD markers are effective for detecting structure in bush mango germplasm (20). More recent marker-based studies using DArTseq markers likewise reported clear separation of *I. wombolu* from *I. gabonensis* and significant population differentiation among country-level collections (23). Collectively, the morphological and molecular results indicate that the NIHORT collection represents a valuable genetic resource for conservation and breeding.

Conclusion

The *Irvingia wombolu* germplasm at NIHORT exhibited substantial morphological and genetic variation. High diversity in leaf traits facilitated the identification of rare morphotypes, while strong associations were observed among fruit and stone traits, many of which showed moderate to high broad-sense heritability. Path coefficient analysis revealed dry stone weight as the direct predictor of dry kernel weight. In addition, three superior ideotypes (Trees 9, 24 and 28) with desirable plant architecture and high kernel yield were identified. The RAPD markers further confirmed considerable genetic diversity among the 88 accessions with seven distinct clusters. Collectively, these findings highlight the value of the NIHORT collection as an important genetic resource and provide a strong foundation for the conservation, selection and genetic improvement of *I. wombolu*.

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752 Data availability statement

753 Data availability: The data and scripts (R) that support the conclusions of this
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756

757 Author Contributions

758 All authors whose names appear on the submission

759 1. made significant contributions to the conception or design of the work; or
760 the acquisition, analysis, or interpretation of data; or the creation of new
761 software used in the work;

762 2. drafted the work or revised it critically for important intellectual content;

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