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Table of Content

Physical, mechanical and acoustic properties of Terminalia superba, Cleistopholis patens and Holarrhena floribunda woods used in sculpture and instrument making in Benin	224-241
Montcho Crépin Hounlonon*, Comlan Aristide Houngan, Clément Adéyèmi Kouchadé, Alexis Enagnon Medehouenou ¹ and Basile Bruno Kounouhéwa ¹	
Seed dispersal of a range restricted and vulnerable species, Guibourtia copallifera Benn. in Sierra Leone	242-249
Jonathan Johnny*, Aiah Libbie ² and Richard Wadsworth	
Screening for complete submergence seedling tolerance in Rice (Oryza sativa L.) genotypes collected from Sudan and South Sudan	250-257
Hanadi El Dessougi, Seif Gasim*, Ishraka Abuanja, Abdelwahab Abdella and Eltayeb Nile	
Molecular characterization of banana genotypes by SSR markers	258-269
Solomon Tamiru Workneh ¹ *, Sisay Kidane Alemu, Gamachu Olani, Abel Debebe, Biruktait Berhanu, Asmare Dagneu and Wegayehu Assefa	

Full Length Research Paper

Physical, mechanical and acoustic properties of *Terminalia superba*, *Cleistopholis patens* and *Holarrhena floribunda* woods used in sculpture and instrument making in Benin

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Wood is important in several fields including sculpture and instrument making. In the last two fields, *Terminalia superba*, *Cleistopholis patens*, and *Holarrhena floribunda* are three of Benin's most used wood species. In this work, we have, on prismatic samples (500 mm × 20 mm × 20 mm) of the wood of these species, used the acoustic method *Beam Identification by Non-destructive Grading* (BING) of CIRAD-Forêt to determine the density ρ , Young's modulus E , shear modulus G and internal friction $\tan \delta$ that allowed the evaluation of the specific stiffness modulus E/ρ and the other acoustic parameters. These tests showed that *T. superba*, *C. patens* and *H. floribunda* wood species have specific moduli of elasticity of 18 ± 3 , 17 ± 2 and 15 ± 1 GPa, respectively; internal friction of the order of 10^{-2} and sound velocities of the order of 4000 m s^{-1} . All these three species have an average acoustic strength above $1.40 \text{ MPa s m}^{-1}$ with acoustic radiation above $7 \text{ m}^4 \text{ kg}^{-1} \text{ s}^{-1}$ for a high ACE above $1000 \text{ m}^4 \text{ kg}^{-1} \text{ s}^{-1}$ for *T. superba* and *C. patens* and low around $674 \text{ m}^4 \text{ kg}^{-1} \text{ s}^{-1}$ for *H. floribunda*. All these species have a medium stability in service and a low propensity to deformation due to their shrinkage anisotropy. In view of these results, the wood of these species can be used in both structural and acoustic works specifically in sound insulation, art sculpture and instrument making.

Key words: Specific modulus, internal friction, sound velocity, acoustic radiation, acoustic impedance, acoustic conversion efficiency, insulation, musical instrument.

INTRODUCTION

Wood is a material of choice that is found in almost all sectors of socio-economic development. The appreciation of its quality is then defined according to its adequacy to the envisaged uses. Each characteristic of wood can thus

answer a given quality provided that it is associated with an application. The notion of wood quality covers all the properties that condition the technological aptitudes. The mechanical, physical, chemical and acoustic properties

as well as the aesthetic aspect still make wood a preferred material in instrument making, the manufacture of art objects, interior constructions (concert halls), sound insulation, the construction of structures entirely of wood (Bucur, 2006; Wegst, 2006; FPL, 2010; Brémaud, 2012). In the special cases of miscellaneous carving and artisanal and semi-industrial manufacture of musical instruments and art objects, not all wood species are eligible (Wegst, 2006). The case of the royal treasures of Abomey returned to Benin in November 2021 and which did not suffer any visible deformation despite the constraints and rigors of the climate is a typical example of the professionalism of the makers and the efficient use of the material wood. In Benin and Africa in general, these trades are threatened because of the progressive disappearance of the holders of endogenous knowledge in the field but also because of the non-availability of appropriate wood species for the realization of these objects and the absence of documentation and reliable scientific information on the species.

Deforestation, climate change, overexploitation, agriculture, demographic pressure, livestock, vegetation fires, etc. are the factors that contribute to the disappearance of these woody species. From 1990 to 1995, a total loss of 298 thousand hectares of Beninese forest cover was recorded (FAO, 2010). For the World Bank Group, citing national statistics, there appears to be a loss of 5.9 to 7.6 million hectares of forest, a decrease in area of 14% and a deforestation rate of 1.4% per year between 2005 and 2015 (World Bank Group, 2020). This decrease in vegetation cover has led to threats to many plant species, some of which are increasingly disappearing from their natural ecosystems. Among these endangered forest species in Africa are those whose wood is used as a basic material for carving, instrument making and other crafts. In studying the importance of biodiversity and tropical forests in South Benin, Adjovi et al. (2017) stratified the different uses of wood in our forests and noted, without being exhaustive, that for South Benin, about 13 species of wood are used in carving, 09 species for furniture making, 04 species of wood in light carpentry, 02 in formwork, 05 for boat building, two for mortar making and three for drumming. This study revealed that among the objects carved or manufactured are: tam-tams, masks, statuettes, mortars, decorative objects, handles, etc. The actors involved in this socio-economic activity include carvers, tradition keepers, dealers, art museum curators, etc. Among the species cited in carving and instrument making in Benin and other African countries (Schmelzer, 2006; Kimpouni, 2009; Lemmens, 2012), are the woods of *Terminalia superba*, *Cleistopholis patens* and

Holarrhena floribunda for which this work determined physical, mechanical and acoustic properties. The main properties determined are density, modulus of elasticity, shear modulus, modulus of specific rigidity, internal friction, speed of sound, acoustic radiation coefficient, acoustic conversion efficiency, and acoustic impedance. The knowledge of these parameters will make it possible to fill the documentary gap which characterizes the Beninese wood species and to better indicate the various uses of these wood species in instrument making.

MATERIALS AND METHODS

Plant

In this study, trees of these species were identified and collected in the Pahou Forest and the Lama in Benin (Table 1). These are dense humid forests specifically of the Guinean-Congolese zone. This zone is the wettest in Benin and is located in its southern part in the Dahomey-Gap and extends from the coast to the latitude of the Commune of Djidja. The climate is sub-equatorial with a bimodal rainfall regime. There are four seasons, including two rainy seasons and two dry seasons. Annual rainfall varies from 900 mm in the west to 1,300 mm in the east. The dominant soil is ferrallitic and deep.

The study area covers the sites of occurrence of the materials and methods. After identification of the species of trees concerned in the field, on each marked and felled individual; a round of wood of 70 cm length was taken by species at man height (1 m 30). For the determination of physical, mechanical and acoustic properties, the sampled logs were cut into bars in the form of prismatic specimens 500 mm x 20 mm x 20 mm in the orthotopic directions of the wood (Photo 1). Thus, we worked on 3 feet of *C. patens*, 2 feet of *T. superba* collected in Pahou and 5 feet of *H. floribunda* collected in Lama in Benin. From these plants we extracted 50 prismatic specimens without defects of *H. floribunda*, 80 specimens of *T. superba* and 85 of *C. patens*.

Vibration measurement

Measurements of physical and mechanical parameters were made by the vibratory method developed by CIRAD-forest and whose operation was described in the work of Brancheriau (2002, 2006), Brancheriau and Baillères (2002), Brémaud (2006, 2008), Traoré et al. (2010), Brémaud et al. (2012), Roohnia (2016), Hounlonon et al. (2017, 2021), Sproßmann et al. (2017), Saadnia et al. (2018a, b) and Ahmed and Adamopoulos (2018). It is based on the spectral analysis of beam vibration natural frequencies and fast Fourier Transforms (FFTs) programmed under the Beam Identification by Non-destructive Grading (BING) software version 9. The test setup is the one presented in the work of Hounlonon et al. (2021), the principle of which is as shown in Figure 1 (Saadnia et al., 2018a, b).

The basic physical, mechanical and acoustic characteristics determined are density, modulus of elasticity and shear, and the internal friction coefficient. The most important physical property

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determined is the density which indirectly gives the density of the sample.

In the range of mechanical properties, we have the modulus of elasticity and shear according to Timoshenko's theory and the modulus of elasticity according to Bernoulli. While it is proven that the most elaborate method is Timoshenko's, the modulus of elasticity according to Timoshenko and the modulus of elasticity according to Bernoulli of 1st order (mode 1) are not very different and are of the same order of magnitude (Brancheriau, 2002; Brémaud, 2006), we focused in terms of mechanical properties on the modulus of elasticity according to Bernoulli mode 1 to extract the acoustic properties (Ahmed and Adamopoulos, 2018). At the end of the vibratory or acoustic properties, the properties of primary interest in structural mechanics such as the speed of sound and internal friction have been determined.

If the acoustic properties of wood, such as the sound level, quality and color of xylophone bars and soundboards, are determined by the mechanical properties of the material from which they are made, this is justified by the fact that sound is produced by the material's own vibration. According to Wegst (2006), the properties on which the acoustic performance depends are mainly the density, the Young's modulus and the damping coefficient. They determine the speed of sound in the material, the natural frequencies and the intensity of acoustic radiation. The most important acoustic properties for the selection of materials with applications in acoustics such as musical instruments and building interiors are the velocity V of sound in the material, the acoustic impedance z , the acoustic radiation coefficient K and the loss coefficient $\tan\delta$ and the acoustic conversion coefficient ACE (Wegst, 2006; Brémaud, 2012; Ahmed and Adamopoulos, 2018).

The speed of sound in a structural material depends on the modulus of elasticity and density. In wood, the speed of sound also varies with the direction of the grain because the transverse modulus of elasticity is at least twenty times lower than the longitudinal value. Across the grain, the speed of sound is about one fifth to one third of the longitudinal value (FPL, 2010). It is given, according to the work of Ahmed and Adamopoulos (2018), Saadnia et al. (2018a, b), Roohnia (2016), Brémaud (2006), Wegst (2006), Holz (1996a), and James (1961), by relation (Equation 1).

$$V = \sqrt{\frac{E}{\rho}} = 2lf \tag{1}$$

where V (m s^{-1}) is the speed of sound; E (Pa) is the modulus of elasticity, ρ is the density (kgm^{-3}), l (m) is the length of the specimen, and f (Hz) is the resonant frequency.

E is the modulus of elasticity of the first mode of vibration according to Bernoulli's model (Brancheriau, 2002; Brémaud, 2006). According to this theory, the specific Young's modulus of the n^{th} mode is given by relation (Equation 2).

$$\frac{E}{\rho} = f_n^2 \times \frac{4\pi^2}{m_n^4} \times \frac{AL^2}{I} \tag{2}$$

where A : area of the straight section ($A=b \times h$) L : length; I : moment of inertia ($I = \frac{bh^3}{12}$) for a rectangular section of base b and height

h ; $m_1 = 4.730$; $m_2 = 7.8532$; $m_3 = 10.9956\dots$, with a very large slenderness (L/h) sufficient to neglect the influence of shear and rotational inertia.

The independence of E to the frequency increase (in the audible range) requires taking into account the influence of shear and rotational inertia in model of Timoshenko (Hearmon, 1958; Bordonné, 1989) which is a more elaborate model (Brancheriau, 2002; Brémaud, 2006).

The acoustic impedance or mechanical impedance z (Wegst,

2006; Roohnia, 2016) of the material is given by the product of the velocity V (m s^{-1}) of sound in the material by its density ρ (kg m^{-3}). It can also be expressed as a function of density and Young's modulus. The impedance z is expressed in MPa s m^{-1} by the relation (Equation 3).

$$z = V\rho = \frac{E}{V} = \sqrt{\rho E} \tag{3}$$

The acoustic radiation coefficient K ($\text{m}^4 \text{kg}^{-1} \text{s}^{-1}$) reflects the acoustic radiation damping (Wegst, 2006). This parameter is conceptually different from internal frictional damping (Roohnia, 2016). Noted K , it depends on the elastic modulus (Pa) and density (kgm^{-3}), the influence of which is higher than in the case of specific modulus (Wegst, 2006; Roohnia, 2016). It is expressed by relation (Equation 4).

$$K = \sqrt{\frac{E}{\rho^3}} = \frac{V}{\rho} \tag{4}$$

The inverse of this coefficient is called the anti-vibration parameter according to Yoshikawa (2007).

The Acoustic Conversion Efficiency ACE defines the efficiency with which vibrational energy is transformed into sonic energy and has been accepted as an overall estimate of acoustic properties (Aizawa et al., 1998; Rujinirun et al., 2005; Roohnia et al., 2011). It is the ratio of the radiation ratio to the damping coefficient or internal friction (Brémaud, 2008; Aizawa et al., 1998; Yano et al., 1992; Obataya et al., 2000; Roohnia, 2016) and the acoustic conversion efficiency, expressed in $\text{m}^4 \text{kg}^{-1} \text{s}^{-1}$, is formulated by relation (Equation 5).

$$ACE = \frac{K}{\tan \delta} \tag{5}$$

High radiation damping combined with low internal friction damping means that less sound energy is consumed in the internal friction and more is emitted as sound radiation to the environment - exactly what is expected from musical instrument soundboards.

The transmission parameter or relative acoustic conversion efficiency was also defined which according to Yoshikawa (2007) is given by expression (Equation 6).

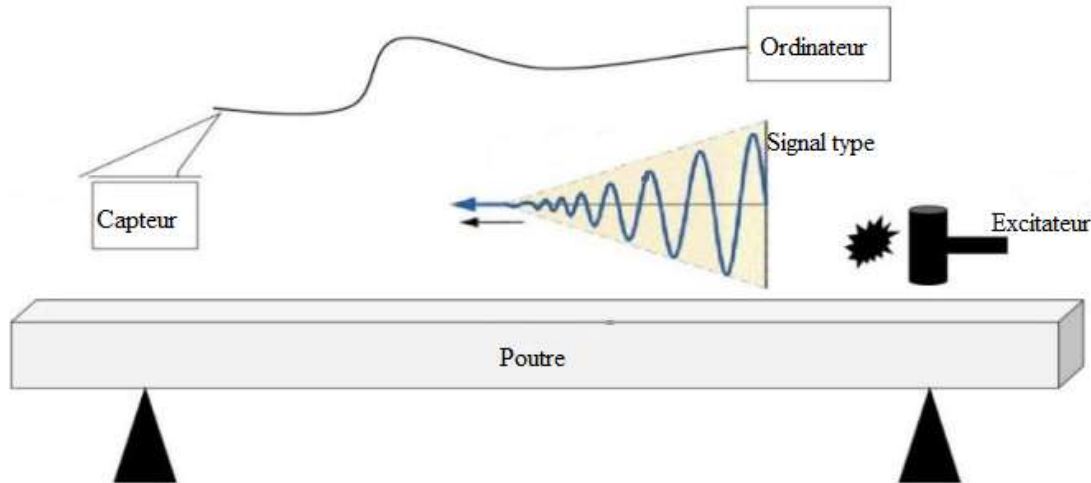
$$\alpha = \frac{\sqrt{E}}{\tan \delta} \tag{6}$$

Internal friction is the term used to refer to the mechanism that causes the dissipation of vibrational energy from a body (FPL, 2010; Roohnia 2016), that is, internal dissipation of acoustic waves (Roohnia, 2016). When a solid is stretched, some of the mechanical energy is dissipated as heat (FPL, 2010). As a result, the damping capacity often referred to by the mechanism that causes it as 'internal friction' is also referred to as 'damping due to internal friction' or 'loss coefficient' (Roohnia et al., 2015; Wegst, 2006; Roohnia, 2016). Internal friction is the property of solid materials that reflects the transformation of mechanical energy into heat under cyclic stress (Brancheriau et al., 2010). The loss coefficient measures the degree to which vibrational energy is dissipated by internal friction in a material. It is given by $\tan\delta = \delta/\pi$ where δ is

Table 1. Observations on the wood species studied.

Plantation	Species	Observations
Pahou	<i>Holarrhena floribunda</i>	Species of varying frequency in the forest. It is normally found in deciduous relics, forest plantations, forest edges, and wet/riverine forests (Orwa et al, 2009).
	<i>Terminalia superba</i>	Exotic species more or less planted in the forest
	<i>Cleistopholis patens</i>	Species confined to the swampy part of the forest
Lama	<i>Holarrhena floribunda</i>	Native species
	<i>Terminalia superba</i>	Common species in the formation
	<i>Cleistopholis patens</i>	The species has not been identified in the forest

Source: Authors

**Figure 1.** Principle of data acquisition.
Source: Adapted from Saadtnia et al. (2018a).

the logarithmic decrement (Newland, 1989; Bodig and Jayne, 1993; Wegst, 2006; Roohnia, 2016). During this study, the internal friction is collected, following the development of the work of Brancheriau (2002) and Brancheriau and Baillères, 2002; Brancheriau et al., 2010, 2011) along with the physical-mechanical and vibrational properties in a raw text file.

The data collected or calculated were tabulated and processed in Excel to give the results of descriptive statistics represented by the mean and standard deviation of each parameter and presented subsequently.

RESULTS AND DISCUSSION

Preliminary investigations among some carvers in the lake regions of Benin, in the communes of Cotonou and surroundings, in Pobè, Kétou, Dassa, Parakou, Djougou and Natitingou have revealed that in carving, the woods

most used are among others: *Ricinodendron heudelotii*, *Tectona grandis*, *H. floribunda*, *T. superba*, *C. patens*, *Triplochiton scleroxylon*, *Milicia excelsa*, *Anogeissus leiocarpa*, *Khaya senegalensis*, *Terminalia catapa*, *Gmelina arborea*, etc.

This finding reflects the work of Adjovi et al. (2017) who found in their work in South Benin that the top 13 most used wood species in carving are *G. arborea*, *Pterocarpus erinaceus*, *T. grandis*, *Diospyros mespiliformis*, *K. senegalensis*, *M. excelsa*, *Eucalyptus* species, *Daniellia oliveri*, *Khaya grandifoliola*, *Azadirachta africana*, *Azadirachta indica*, *Vitellaria paradoxa* and *Triplochiton scleroxylon*. Of these thirteen species, the most used for the manufacture of dugouts is *T. scleroxylon*, *Erythrophleum africanum*, *Cola* spp., *M. excelsa*, *Anarcadium occidentale*.

Table 2. Thickness and diameter of *C. patens*.

Wood	Bark thickness (cm)	Diameter (cm)	Observation
CP1	0.5	18.1-19	Pungent smell, presence of red heartwood in the wine not very differentiated
CP2	0.3	11.2-23.2	Pungent odor
CP3	0.4	23.0-24.8	Pungent odor

Source: Authors

Table 3. Thickness and diameter of *H. floribunda*.

Bark thickness (cm)	Diameter (cm)
0.1	9-9.5
0.1	8.7-9
0.1	10.4-10.9
0.1	9.8-10.2
0.2	9.7-10.9

Source: Authors

Table 4. Density, average modulus of elasticity and shear modulus of the studied species.

Wood species	Density (kg m ⁻³)	Modulus of elasticity Bernoulli mode 1 (MPa)	Timoshenko modulus of elasticity (MPa)	Timoshenko shear modulus (MPa)
<i>Terminalia superba</i>	467±41	8305±1716	8504±1783	1008±681
<i>Cleistopholis patens</i>	360±44	6126±1014	6254±1036	850±823
<i>Holarrhena floribunda</i>	536±36	7957±929	8181±980	915±729

Source: Authors

Dendrometric parameters of *C. patens*, *T. superba*, and *H. floribunda*

The bark thickness and diameters of the three *C. patens* woods are summarized in Table 2. The two *T. superba* trees used show good stiffness with straight shafts, white wood. The heartwood and sapwood are not differentiated. The bark thickness of these trees is 0.2 cm with a diameter between 21.5 and 22.8 cm for one and 22.9 and 23.2 cm for the other. For the five feet of *H. floribunda* wood, the bark thickness varies between 0.1 and 0.2 cm shown in Table 3.

Physical, mechanical and acoustic properties of the wood species studied

The physical-mechanical properties of the materials used in instrument making determine the acoustic properties of the instruments. With wood being a multifunctional material, certain physical-mechanical properties determine the acoustic performance. Among these parameters is density, Young's modulus, and the loss or damping coefficient or internal friction (Wegst, 2006). By combining

the basic physical-mechanical properties (Table 4) with the acoustic models, we have determined the acoustic properties shown in Table 5.

Table 4 shows a similarity of Timoshenko and Bernoulli mode 1 moduli of elasticity confirming the observations of Brancheriau (2002) and Brémaud (2006). Compared to *Ricinodendron heudelotii*, all three species have higher densities, moduli of elasticity and shear. *R. heudelotii* is a light wood with a density of 130 to 300 kg m⁻³, soft and brittle with a modulus of elasticity of 3700 to 4800 MPa and a shear of 2.2 to 3.2 MPa (Tchoundjeu and Atangana, 2007). This species is highly prized in the sculpture of Guèlèdè masks, various statuettes and other art objects such as canes and tom-toms (Akpovo and Fandohan, 2021).

The comparison of Bernoulli mode 1 specific moduli and Timoshenko specific dynamic modulus, for all our samples of slenderness L/h of the order of 25, presents in the case of our three species good correlations (Figure 2) confirming the observations of Brémaud et al. (2012), Brémaud (2006) and Brancheriau (2002).

The results of the internal friction obtained (Table 5) for these three species are in the range of the values obtained by Brancheriau et al. (2006) for species of good

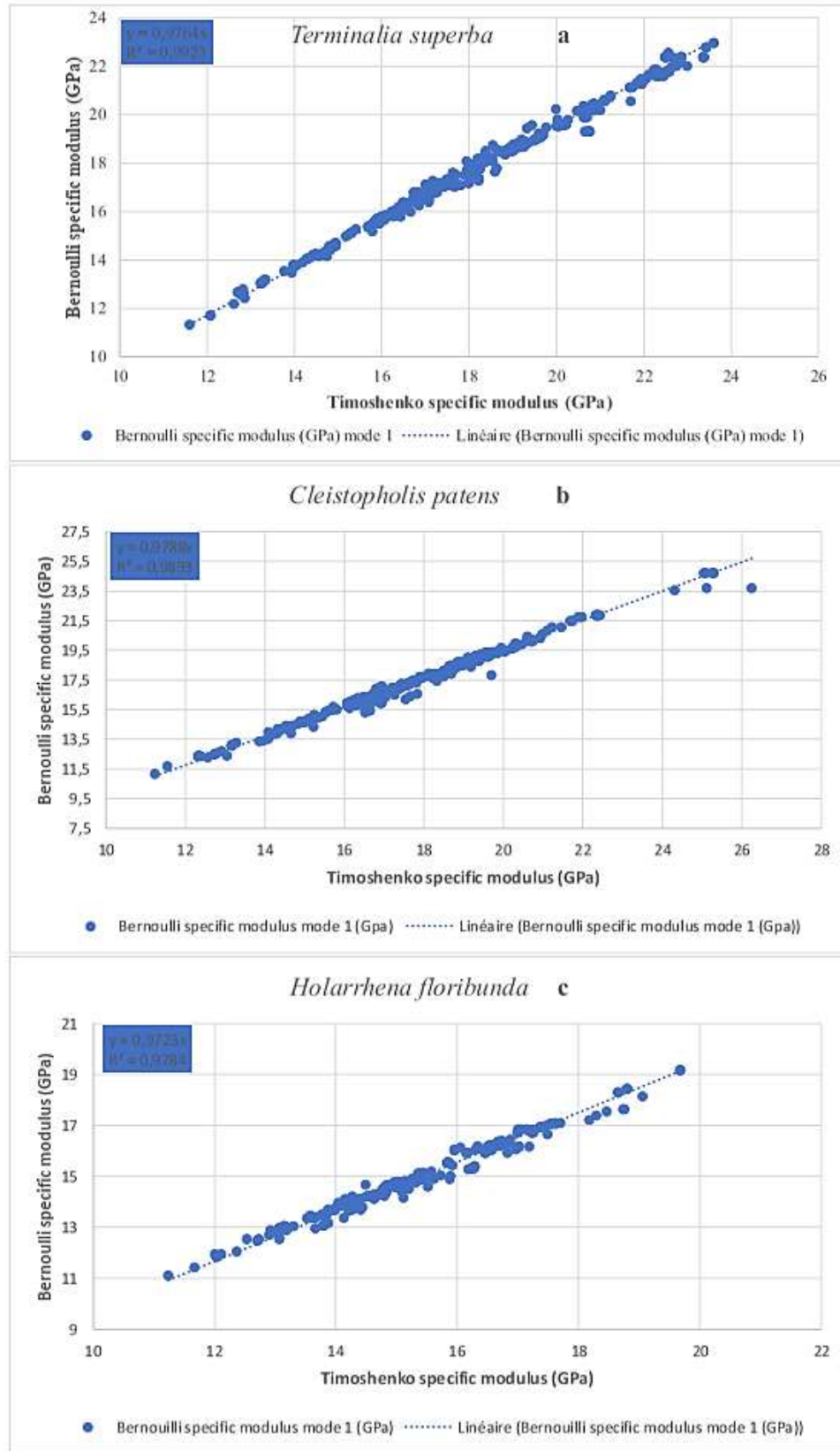


Figure 2. Comparison of Bernoulli mode 1 specific moduli to Timoshenko specific modulus for *T. superba* (a); *C. patens* (b); *H. floribunda* (c).
Source: Authors

Table 5. Specific modulus and average acoustic properties of the studied species.

Wood species	Specific modulus (MPa m ³ kg ⁻¹ or GPa)	Internal friction tan δ (10 ⁻²)	Speed V (m s ⁻¹)	Impedance z (MPa s m ⁻¹)	Sound radiation coefficient K (m ⁴ kg ⁻¹ s ⁻¹)	Acoustic conversion efficiency ACE (m ⁴ kg ⁻¹ s ⁻¹)
<i>Terminalia superba</i>	18±3	1.05±0.50	4188±322	1.96±0.29	9.00±0.69	1039±454
<i>Cleistopholis patens</i>	17±2	1.10±0.43	4141±279	1.47±0.18	11.81±1.79	1242±506
<i>Holarrhena floribunda</i>	15±1	1.23±0.46	3868±178	2.07±0.14	7.25±0.67	674±267

Source: Authors

acoustic quality whose internal friction is located between 0.82 and 1.26×10^{-2} . From Table 5, it appears that, *C. patens* has the highest value of ACE and K, *T. superba* has the highest specific stiffness and the highest speed of sound while *H. floribunda* has the highest values of internal friction and acoustic impedance.

Density and modulus of elasticity

The modulus of elasticity and density are important acoustic parameters (Brémaud, 2012; Wegst, 2006; Roohnia, 2016; Ahmed and Adamopoulos, 2018) especially since they are determinant in the specific modulus of stiffness. The modulus of elasticity increases globally with density as already found in the work of Sproßmann et al. (2017). While the species *T. superba* correlates well with both parameters, *C. patens* and *H. floribunda* correlate less well as shown in Figure 3.

The density of wood used in instrument making covers a wide range from 300 to 1400 kg m⁻³ (Wegst, 2006). For example, the mass of the back and sides of a body or part of the body of an instrument is a function of the density of the wood used and can be controlled by acting on the thickness of the panels. A guitar body with high panel mass reduces the resonant frequency and increases the radiating area in phase, acoustically improving the midrange and extending the duration of the sound (Gore, 2011).

Our three species tested here have a density of 467 ± 41 kg m⁻³ for *T. superba*, 360 ± 44 kg m⁻³ for *C. patens*, 536 ± 36 kg m⁻³ for *H. floribunda*. Values that remain, for example, in the realm of soundboard resonance woods whose density varies 300 to 500 kg m⁻³ (Wegst, 2006; Ahmed and Adamopoulos, 2018). It is true that according to the same authors the woods of the bottoms and sides of violin and piano action have densities of 600 to 900 kg m⁻³; woods of high density for wind instruments, xylophone bars and violin hump; woods of high density of the order of 750 kg m⁻³ are used as electric guitar neck (Wegst, 2006; Ahmed and Adamopoulos, 2018). The back and sides of guitars, for example, are traditionally made of wood of the same species of wood for aesthetic reasons regardless of the stiffness and density of the material (Gore, 2011). According to Gore (2011), cited by Ncube and Masilinga (2017), wood species on the sides

should be heat flexible with a density of 550 to 800 kg m⁻³; the density of 550 kg m⁻³, is more favorable for the back and sides of guitars. The properties for example required for guitar neck woods are strength, stiffness and dimensional stability over time against moisture gradient (Sproßmann et al., 2017). In view of these authors, *H. floribunda* and *T. superba* in their range of variation would offer usable samples in guitar making.

Indeed, depending on the age and level of harvesting, the density of the wood, as well as other wood parameters, of each of these species can vary widely. The values of the densities found remain within the range of the data of the Tropix 7 database which obtained 540 ± 70 kg m⁻³; of PROTA quoting Kimpouni (2009), 370 to 730 kg m⁻³ for *T. superba*. *C. patens* also has its average density close to that of PROTA citing Lemmens (2012) who found a density ranging from 290 to 500 kg m⁻³.

Of the three species studied, *H. floribunda* has the best density. However, the results of the literature remain almost empty on its physical-mechanical characteristics. Indeed, the work done so far remains in the field of life and earth sciences (Schmelzer, 2006; Bayala et al., 2006; Orwa et al., 2009). However, it is also used in kitchen framing (Leciak and Bah, 2008). According to Schmelzer (2006) quoted by PROTA, this wood species is used in Benin to build granaries, beds or children's cribs; its wood is resistant to attack by *Prostephanus truncatus*. For carving, stools and other carved seats, *H. floribunda* is considered the best wood (Schmelzer, 2006; Orwa et al., 2009). Recent work in Ghana by Antwi et al. (2022) showed that branch and trunk wood have similar densities close to those in our work. This study found a density of 467.53 ± 70.87 kg m⁻³ for branch wood and 457.40 ± 70.46 kg.m⁻³ for *H. floribunda* trunk wood.

The average Bernoulli dynamic modulus of each of these species as presented in Table 4 shows that they have moduli in the range of instrument making that runs from 6000 to 40000 MPa (Wegst, 2006; Ahmed and Adamopoulos, 2018).

Indeed, *T. superba* has a mean modulus of 8305 ± 1716 MPa; *C. patens* (6126 ± 1014 MPa) and *H. floribunda* (7957 ± 929 MPa).

If it is true that the acoustic properties are decisive in the choice of a type of wood in acoustics, it will be taken into account the dimensional stability of the species studied, *T. superba* has the highest modulus of elasticity

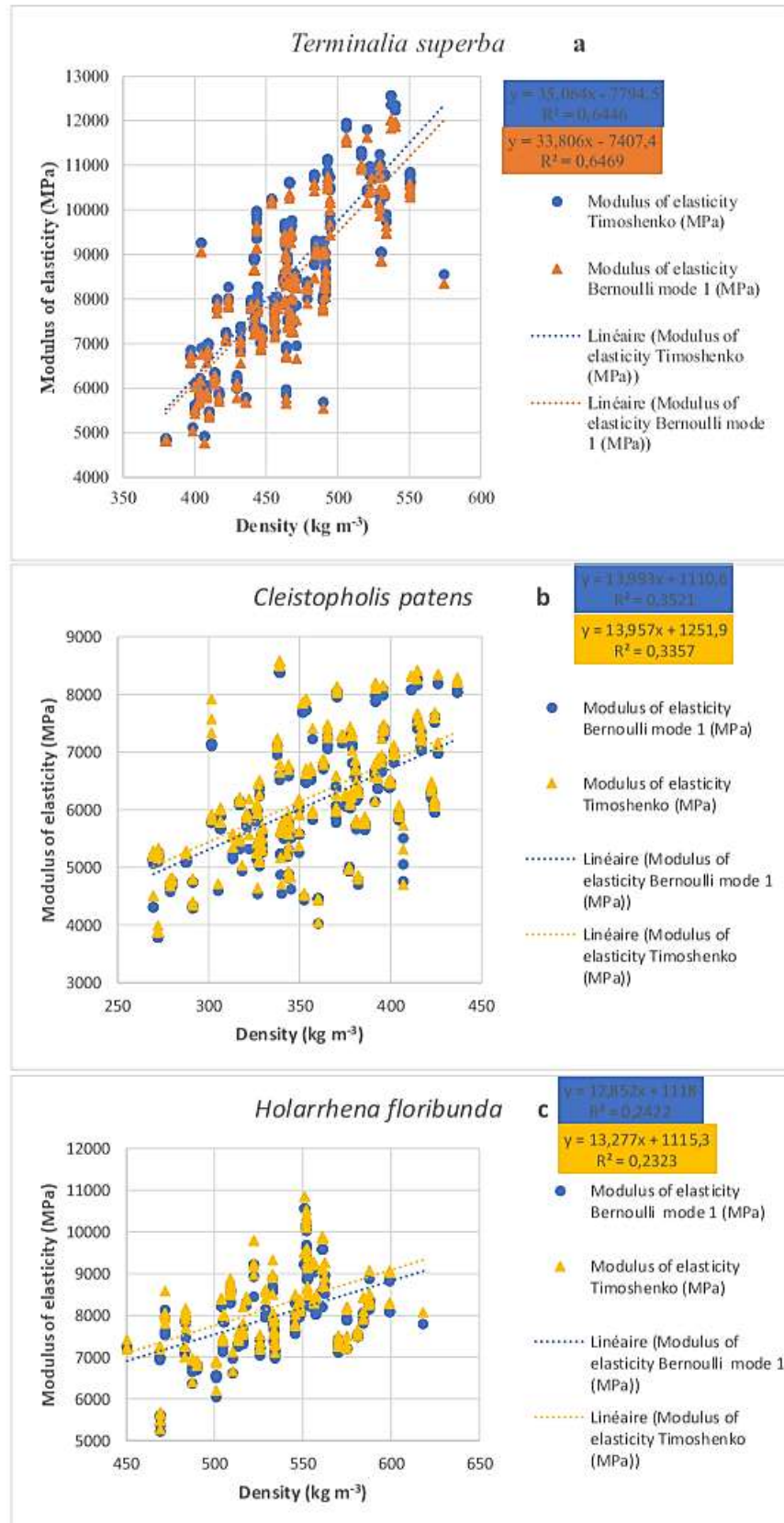


Figure 3. Variation of the modulus of elasticity with density for *T. superba* (a); *C. patens* (b); *H. floribunda* (c).
 Source: Authors

and shear.

Regarding modulus of elasticity *T. superba* has lower moduli than Tropix 7 (11750 ± 2480 MPa) but in the range of PROTA (Kimpouni, 2009) which varies from 3625 to 16660 MPa. *C. patens* has, according to PROTA citing Lemmens (2012), a modulus of elasticity in the range of 6080 to 8230 MPa which is not much different from our values for the same species. *H. floribunda*, according to the work of Antwi et al. (2022), has a static modulus of elasticity of 8213.83 ± 116.23 MPa for the branches and 8654.96 ± 108.30 for the trunk. These values show that the mechanical stiffness of *H. floribunda* wood from Benin is similar to that from Ghana.

Treated woods, as well as, pure species can be suitable for the manufacture of soundboards for moduli of about 6000 to 19000 MPa; violin backs and sides for a modulus of about 8000 to 19000 MPa, wind instruments for about 7000 to 18000 MPa, piano mechanics for about 14000 to 19000 MPa and for xylophone bars from 12000 to 20000 MPa (Wegst, 2006; Ahmed and Adamopoulos, 2018). Therefore, all our woods can be used in soundboard making; *T. superba* and *H. floribunda* for violin backs and sides and wind instruments.

Also, coupling density and stiffness modulus, *T. superba* and *H. floribunda* can be used as back and side woods for guitars following the classification of Sproßmann et al. (2017) which categorized woods from 700 to 1100 kg m⁻³ and 9000 to 18000 MPa as back and side woods, and woods from 1000 to 13000 kg m⁻³ and 14000 to 26000 MPa as guitar fret woods.

Yoshikawa (2007), in Japan, working on the wood species traditionally used for making stringed instruments (*Picea abies*, *Picea sitchensis*, *Paulownia tomentosa*, *Morus alba*, *Acer platanoides*, *Acer* sp., *Pterocarpus indicus* and *Dalbergia nigra*) and substitute species used in the same manufacture (*Pinus albicaulis*, *Tsuga* species, *Sequoia sempervirens*, *Thuja plicata*, *Cinnamomum camphora*, *Zelkova serrata*, *Cupressus sempervirens*, *Pyrus communis*, *Prunus serotina*, *Juglans nigra*, *Pterocarpus dalbergioides*, and *Ochroma pyramidale*) density, modulus of elasticity, and velocity values for the two groups of 260 to 873 kg m⁻³; 6300 to 20000 MPa; 3130 to 5300 m s⁻¹ and 380 to 710 kg m⁻³; 2800 to 22000 MPa; 3500 to 5400 m s⁻¹. These values crossed with our average values recorded in the two previous tables predispose our wood species to certain potentialities in instrument making of stringed instruments whereas in the light of the work of Traoré et al. (2010) on veneer (*Pterocarpus erinaceus* Poir.) the density and the modulus of elasticity of these studied species remain weak and unsuitable for the manufacture of xylophone bars.

Speed of sound

The speed of sound is directly related to density and

modulus of elasticity. The speed of sound in wood and any hygroscopic material decreases with increasing temperature or humidity and is proportionally influenced by density and modulus of elasticity (Tsoumis, 1991). The velocity of sound, in wood, reflects the speed of transmission of the energy received thus woods with high velocity and low damping coefficient better facilitate the transmission of vibration energy (Ahmed and Adamopoulos, 2018). Materials with high sound propagation velocities, that is, low densities for high relative stiffness or low internal friction are the best facilitators of energy transfer (Bourgeois, 1994; Gore, 2011).

For the wood samples we tested, *T. Superba* has a velocity (4188 ± 322 m s⁻¹); *C. patens* (4141 ± 279 m s⁻¹); *H. floribunda* (3868 ± 178 m s⁻¹). In the literature, it is reported that, the highest velocities of sound propagation, are obtained in the longitudinal direction followed significantly by a lower velocity in the radial direction and by a lower velocity in the tangential direction (Kúdela and Kunštár, 2011). Several acoustic parameters are necessary for wood selection in instrument making. In the case of velocity, the range of desired values for the woods used varies from 3000 to 6500 m s⁻¹. Often woods of high celerity are used as resonance woods (Wegst, 2006). Woods for piano action, violin bows, xylophone bars, violin backs and sides, and wind instruments have low velocities (Ahmed and Adamopoulos, 2018). Thus, all three species studied have velocities that are in the range of velocities (Wegst, 2006) thus showing suitability for making different musical instruments. The speed of sound, of *P. kesiya* and *Pterocarpus angolensis*, used in guitar making in Zambia, is 4481 and 3799 m s⁻¹, respectively, while that of *Picea abies* is 4894 m s⁻¹ (Ncube and Masilinga, 2017). Hilde et al. (2014) found a velocity of 5600 m s⁻¹ (with variation from 5200 to 6300 m s⁻¹) for spruce and 3500 m s⁻¹ for pine. The high modulus of rigidity of *P. sitchensis* and its relatively low density prevail of a high sound velocity (Bourgeois 1994). It is expected that resonance woods have a high sound velocity around 3000 m s⁻¹ although velocities of 4000 to 6500 m s⁻¹ are preferred for soundboards (Wegst 2008). In this range, all our three wood species have good resonance potential. For instruments with impulsive excitation, the parameters of sound decay are particularly important (Hase, 1987; Holz, 1996b; Brancheriau et al., 2006; Aramaki et al., 2007; Chaigne and Doutaut, 1997; Wegst, 2006). Spruce, often used as a high quality wood in instrument making, has a low density of 450 kg m⁻³ and a very high specific modulus of elasticity of 26 to 36 GPa, arguing for a sound speed above 6000 m s⁻¹ (Haines, 2000; Bucur, 2006; Yano et al., 1990, 1992). Combining speed with density and, referring to Figure 4 (Wegst, 2006) of sound speed versus density, *T. superba* and *C. patens* can be used in the manufacture of soundboards while *H. floribunda* would be a candidate for the manufacture of violin backs and sides or wind

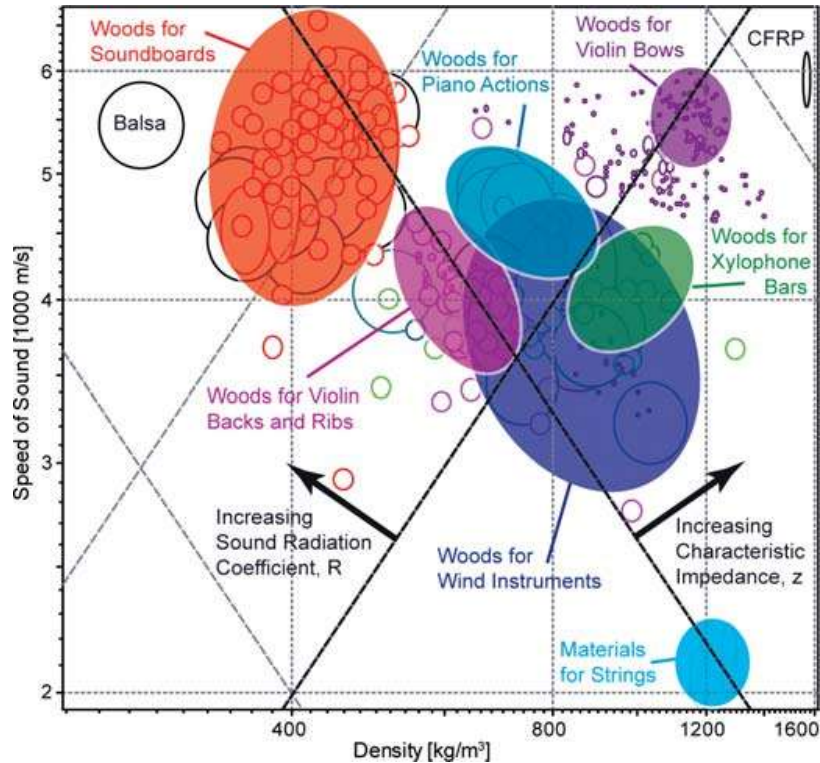


Figure 4. Speed of sound as a function of wood density, graph illustrating the selection of wood in instrument making. Source: Wegst (2006).

instruments. The case of *C. patens* is illustrated by its uses which, according to Lemmens (2012) reported by PROTA, vary from use in carpentry (roofing, doors, beams, dugouts, furniture, veneers, plywood, floats...) to carving musical instruments such as making drums.

Sound radiation coefficient K and acoustic impedance z

Figure 5 shows the variations of K and z as a function of the speed of sound. In general, the acoustic radiation coefficient K and the acoustic impedance z increase when the speed of sound increases. It also shows a good correlation between these different parameters for *T. superba*, *C. patens* and *H. floribunda*.

The sound radiation coefficient is a measure of the vibration in wood when damped by sound radiation. High values of the sound radiation coefficient K are desired for materials used to produce loud sound, and as such, it is very important for its resonance quality (Ahmed and Adamopoulos, 2018). A high radiation coefficient means that there is less energy dissipation due to the inertia of the vibrating body. For resonators, it is desired to resonate the sound by using a lightweight resonator body or top plate with a higher modulus of elasticity. This

parameter for wood in soundboards can range from 7 to 16 $\text{m}^4 \text{kg}^{-1} \text{s}^{-1}$ but slightly lower acoustic coefficients can be accepted for other parts of musical instruments (Roohnia, 2016).

The acoustic radiation coefficients, of the species we studied, are: *T. Superba* ($9.00 \pm 0.69 \text{ m}^4 \text{kg}^{-1} \text{s}^{-1}$); *C. patens* ($11.81 \pm 1.79 \text{ m}^4 \text{kg}^{-1} \text{s}^{-1}$); *H. floribunda* ($7.25 \pm 0.67 \text{ m}^4 \text{kg}^{-1} \text{s}^{-1}$). These values are much higher than those of vène (*Pterocarpus erinaceus*) wood. Thus according to Roohnia (2016), these species can be used in soundboard manufacturing although Wegst (2006) advocates values of $K > 8 \text{ m}^4 \text{kg}^{-1} \text{s}^{-1}$ would be ideal for soundboard manufacturing. The radiation coefficient K , for Sitka spruce and western cedar is about $12 \text{ m}^4 \text{kg}^{-1} \text{s}^{-1}$. These two species have a large sound radiation coefficient which is highly appreciated for producing loud sound (Wegst 2006) as does *Cleistopholis patens* with its sound radiation coefficient of about $12 \text{ m}^4 \text{kg}^{-1} \text{s}^{-1}$.

Wood in music will also need to have a high characteristic impedance to allow for the reflection of sound in the surrounding environment as maple does (Wegst 2006). Like the velocity and the acoustic radiation coefficient K , the acoustic impedance z is related to the modulus of elasticity and density. According to the graphs in Figures 6 to 8, the impedance is related to the density by a linear law while the radiation coefficient is

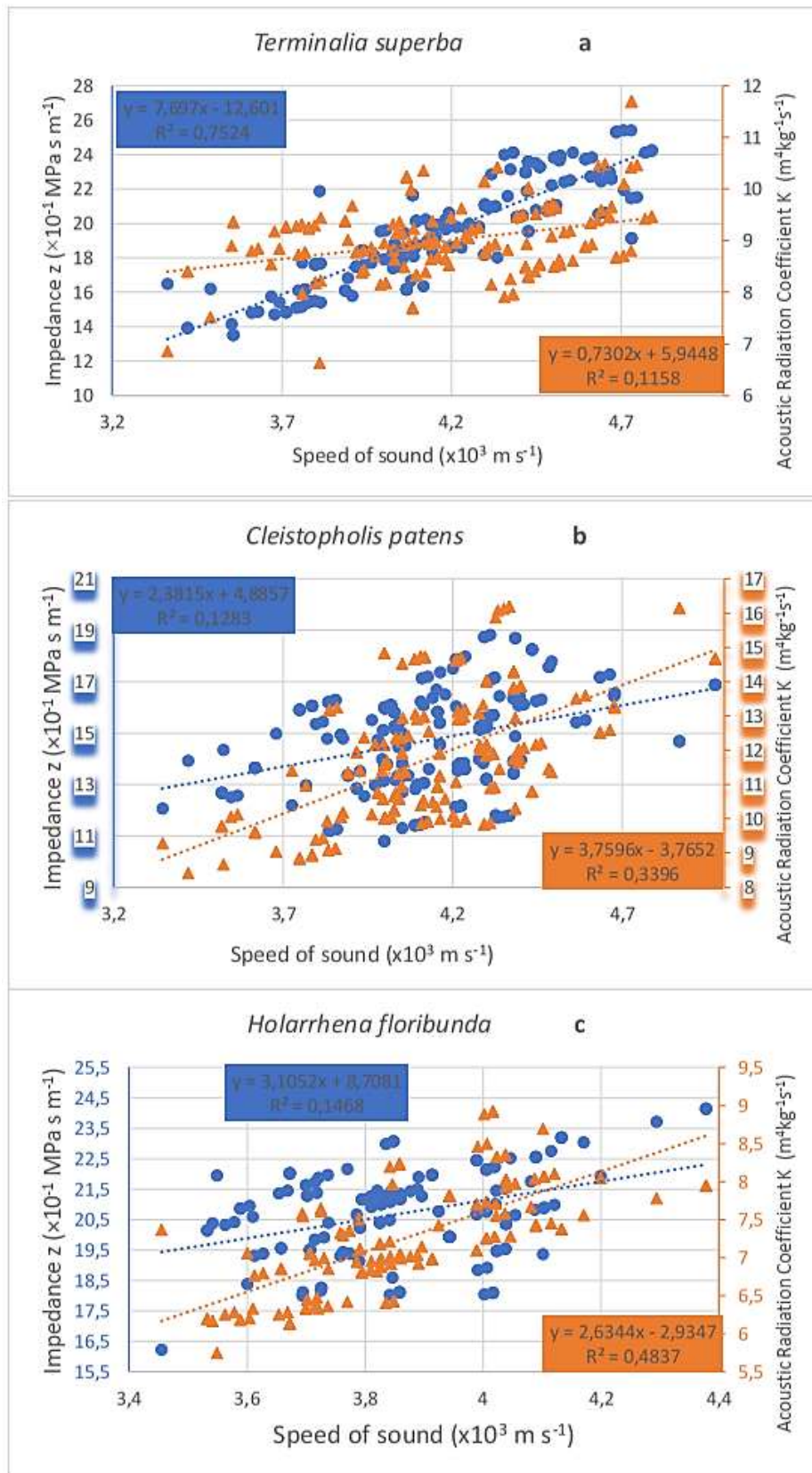


Figure 5. Variation of impedance and acoustic radiation coefficient with speed of sound for *T. superba* (a); *C. patens* (b); *H. floribunda* (c).

Source: Authors

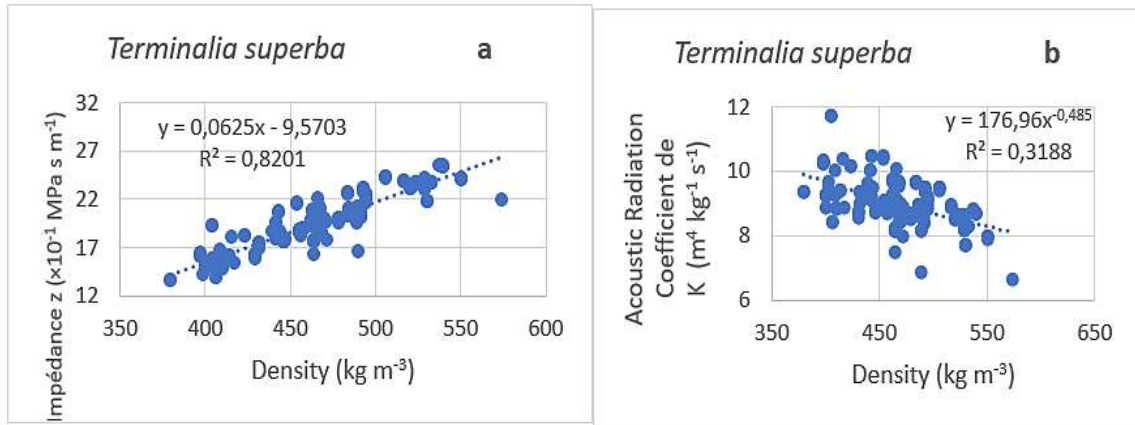


Figure 6. Variation of impedance and acoustic radiation coefficient as a function of density for *T. superba*.
Source: Authors

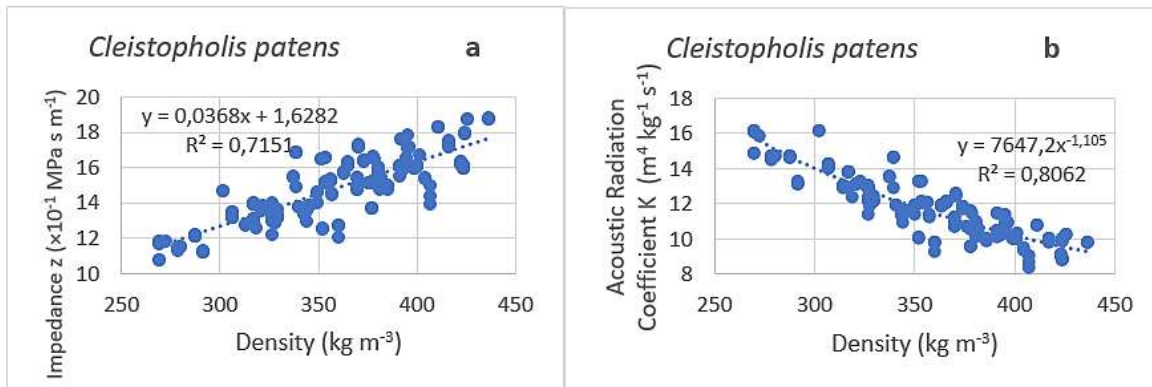


Figure 7. Variation of impedance and acoustic radiation coefficient as a function of density for *C. patens*.
Source: Authors

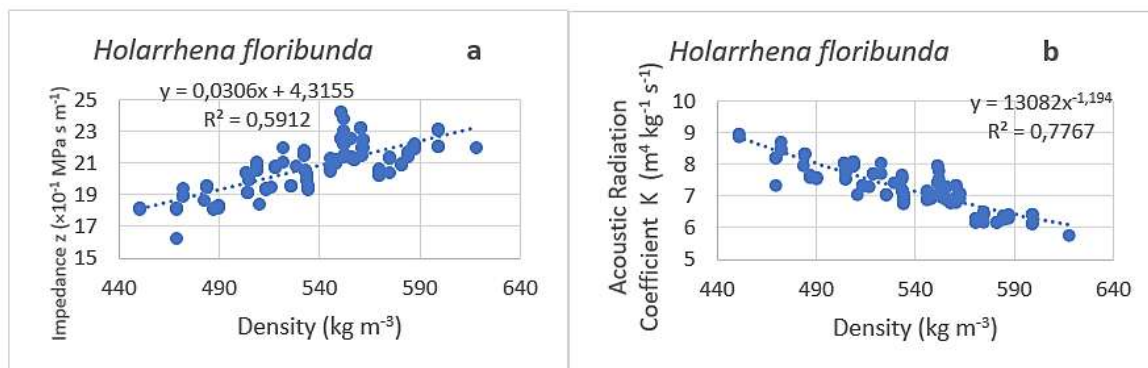


Figure 8. Variation of impedance and acoustic radiation coefficient as a function of density for *H. floribunda*.
Source: Authors

related to the density by a power law with strong correlation in both cases.

Impedance is important when the transmission of vibration is from one medium to another as in the case of

musical instruments. For strings, it is a very important quantity and low impedances are highly valued (Wegst, 2006; Hilde et al., 2014) as in the case of sound transmission in air. Values of z between 1.2 and 3.4 MPa s m⁻¹ are required for stringed instruments and high values required for percussion instruments such as xylophones such that resonance will be over a large time period (Hilde et al., 2014). For the three species that are the subject of our study, the average characteristic impedances are for *T. Superba* (1.96 ± 0.29 MPa s m⁻¹); *C. patens* (1.47 ± 0.18 MPa s m⁻¹); *H. floribunda* (2.07 ± 0.14 MPa s m⁻¹). Thus according to Hilde et al. (2014), these species can be used in the manufacture of ropes. Kollmann and Coté (1968) found sound wave resistance values ranging from 2 to 3.7 MPa s m⁻¹ for wood while they found values of 39.5 MPa s m⁻¹ for steel and 25.8 MPa s m⁻¹ for iron (Roohnia, 2016). Excellent sound radiators such as *T. plicata* and *P. sitchensis*, has a low acoustic impedance, 1.683 and 2.165 MPa s m⁻¹. The acoustic impedance of *P. angolensis*, 2.298 MPa s m⁻¹ is similar to that of *Picea* spp. *Acer saccharum* has a sufficiently high impedance of 2.982 MPa s m⁻¹ that allows the wood to act as a reflector and contribute to sound radiation (Wegst, 2006). In contrast, *P. kesiya* for the soundboard impedance 2.733 MPa s m⁻¹, the slightly lower impedance of *P. angolensis* for the back and sides reduces sound deflection according to Ncube and Masilinga's (2017) guitar study in Zambia. These values prove in a general way therefore the low mechanical impedance of wood compared to materials like some metal alloys and the strong potentiality of our wood species to be used both in instrument making and sound insulation.

The impedance of the soundboard is proportional to the acoustic impedance of the material it is made of and also to the square of its thickness. Soundboards of considerable thickness, such as those of pianos for example, have an impedance clearly higher than that of the strings. The achievement of a high sound quality is therefore dependent on a meticulous monitoring of the impedances of the strings and the soundboards (Wegst, 2006). Experimental tests with the dimensioning of different instruments are desirable to further refine the specific observations.

Internal friction tanδ, acoustic conversion efficiency (ACE), and specific stiffness modulus E/ρ

The internal friction or damping coefficient is a parameter that reflects the energy dissipation in the wood. If the characteristic impedance z indicates the transmission of vibration from one medium to another, the acoustic radiation coefficient K describes the amplitude or loudness while the acoustic conversion efficiency represents the maximum response (Barlow, 1997; Wegst, 2006). ACE is the efficiency of converting vibrational

energy into sound energy and for some musical instruments, better wood rhymes with high ACE (Yano and Minato, 1993). A low tanδ is often required as one of the necessary conditions for a wood to be used as soundboards of piano, guitar and other musical instruments (Matsunaga et al., 1999).

For our three species, the internal friction tanδ and the acoustic conversion efficiency ACE are respectively for: *T. superba* (1.05 ± 0.50) · 10⁻² and (1039 ± 454) m⁴ kg⁻¹ s⁻¹; *C. patens* (1.10 ± 0.43) 10⁻² and (1242 ± 506) m⁴ kg⁻¹ s⁻¹; *H. floribunda* (1.23 ± 0.46) 10⁻² and (674 ± 267) m⁴ kg⁻¹ s⁻¹. The specific modulus of stiffness of these three species is as follows: *T. superba* (18 ± 3 GPa); *C. patens* (17 ± 2 GPa); *H. floribunda* (15 ± 1 GPa).

The tanδ values of the species studied are slightly higher than those of Traoré et al. (2010) for veneer used in Mali for xylophone manufacture. Compared to this same species, the ACEs found here are significantly higher. *T. superba* has a higher specific stiffness than vene wood (Traoré et al., 2010), while the other two species have specific stiffnesses of the same order. Xylophone woods have a very low damping coefficient tanδ and a high value of the peak sound radiation ACE, but not of the average sound radiation coefficient K , and harmonic table woods have higher values for ACE and K . (Wegst, 2006). Thus, *T. superba* and *C. patens*, on this basis, are recommendable for soundboards. Working on tropical woods, Brémaud (2012) measured ACE values of 700 to 800 m⁴ kg⁻¹ s⁻¹ generally higher than those of *H. floribunda* but lower than the other two species. Baar et al. (2016), working on the tropical hardwood species *Azelia*, *Intsia*, *Astronium* and *Millettia* species, meeting the criteria for idiophones such as xylophone bars, found a low internal friction of 0.006 to 0.008 and an ACE of about 700 to 800 m⁴ kg⁻¹ s⁻¹ for a specific stiffness around 16.6 to 21.0 GPa. For *Azelia* spp. the internal friction of value 0.0075 found is just like for the other species is lower than the values of our species; its specific rigidity of 16.6 GPa is in the range of that of our species while its ACE of 649 m⁴ kg⁻¹ s⁻¹ is in the range of that of *H. floribunda* but very low compared to those of the two other species here studied.

The internal friction tanδ of these three species is not in the range of poor acoustic quality woods with a damping coefficient between 1.52 × 10⁻² and 2.21 × 10⁻² but rather in the range of good acoustic quality woods with tanδ between 0.82 × 10⁻² and 1.26 × 10⁻² (Brancheriau et al., 2006). The best perceived acoustic material 'quality' was that of very low internal friction even though the variability of the damping coefficient for wood was restricted to its lowest range between 0.25 × 10⁻² and 0.55 × 10⁻² (Hase, 1987; Brémaud, 2012).

Wegst (2006) argues that resonator woods should have a high ACE unlike other parts of musical instruments. Coupling the ACE with the internal friction, and reading the graph in Figure 9a (Wegst, 2006), the average values of our species indicate that *C. patens* can be used in

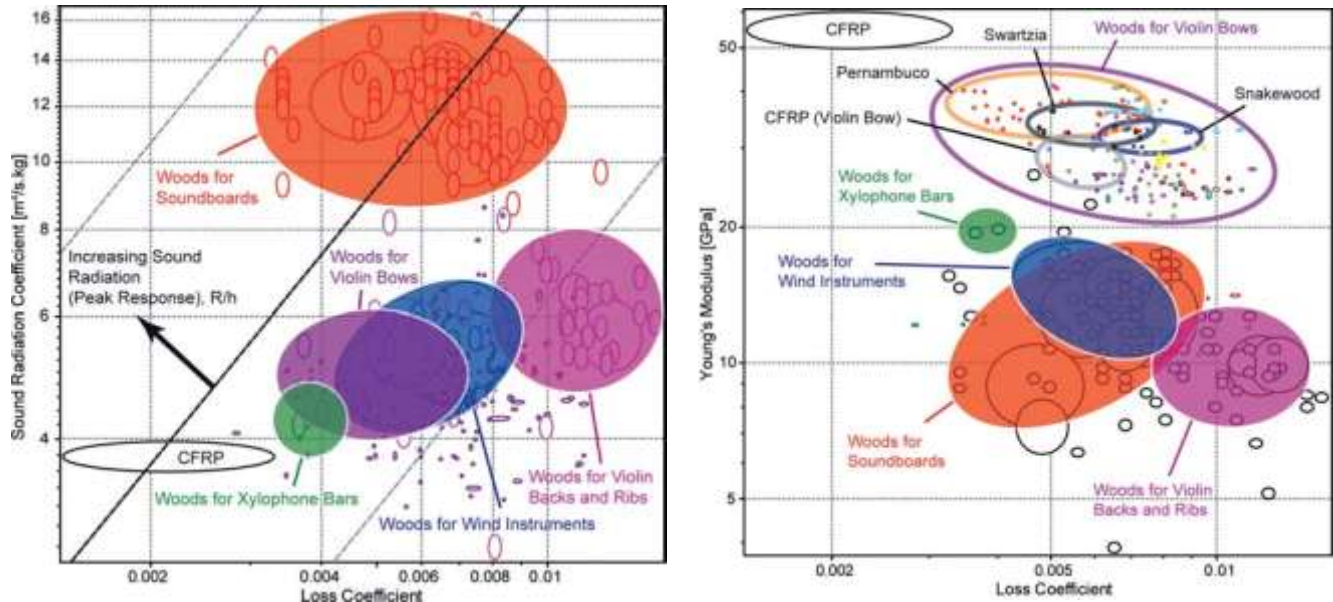


Figure 9. Acoustic radiation coefficient as a function of internal friction (a) and modulus of elasticity as a function of internal friction (b), graphs illustrating the selection of wood in instrument making. Source: Wegst (2006).

making soundboards and *H. floribunda* for violin backs and sides.

According to Wegst (2006), modulus of elasticity can be related to internal friction as shown in Figure 9b. Based on this graph and our average values, the three species studied here would all be candidates for the manufacture of violin backs and sides. Based on the following Figure 9a from Wegst (2006), Roohnia (2016) concluded that light tone woods for resonator soundboards can exhibit ACE beyond 2000 m⁴ kg⁻¹ s⁻¹.

In wood acoustics, these species under certain conditions can be used for the manufacture of musical instruments such as woodwinds, percussion instruments, strings and brass bands (Bucur, 2006). Indeed, by combining the acoustic and mechanical properties of wood, we group wood species under the generic term of resonance wood. Woods of high specific modulus (frequency descriptor) find wide application in the manufacture of resonators for musical instruments requiring minimum weight; that is, the utility of specific stiffness is to find wood parts with minimum weight for bending vibration of the body of a sound producing instrument whose primary design constraint is physical deflection or deformation, rather than loading at a point of failure. There are no better materials or materials similar to wood to be combined with or to substitute for wood in these very important acoustical properties to achieve structural wood quality (Bucur, 2006).

The sound produced by a string is barely audible because a single string is capable of moving a small volume of air. So to produce a sound of satisfactory volume for our ears, the string must be coupled with a

resonator that will transmit the vibration energy of the string and radiate the sound. Generally, high ACE woods are known to be excellent soundboards (Brémaud, 2012). Wegst (2006) finds that xylophone bar woods have fairly high values of peak response (maximum response) ACE but not average loudness K. Both their ACE and K are lower than spruce for string soundboards, which corresponds to less radiation damping, and their higher z-impedance would result in less energy loss through the mounts. Numerical simulation showed that the decay time of xylophone bars was governed primarily by internal friction, but very little by losses through the supports (Chaigne and Doutaut, 1997). A high specific modulus may allow radiation at low frequencies (Yano et al., 1994). Fairly light, spruce, with a specific modulus of about 31 GPa, has a moderate characteristic impedance of 2.683 MPa s m⁻¹ (Bucur, 2006), which can promote the transmission of sound in air (Wegst, 2006).

Figures 10 to 12 show for each species, the variation of ACE as a function of tanδ and then the variation of internal friction tanδ as a function of specific modulus. Overall, ACE follows a power law of internal friction with strong correlation coefficients. The strong dispersion of the specific modulus as a function of internal friction did not allow us to have the exact power law tanδ = 10^{-A} × (Eρ)^{-B} of Ono and Morimoto (1983), which is the reference. Instead, we have a power law of the form tanδ = (Eρ)^{-B} given the high dispersion of our species, which are tropical wood species.

In general, woods with high specific stiffness E/ρ combined with low damping coefficient tanδ are accepted for piano, guitar, and violin soundboards (Matsunaga et

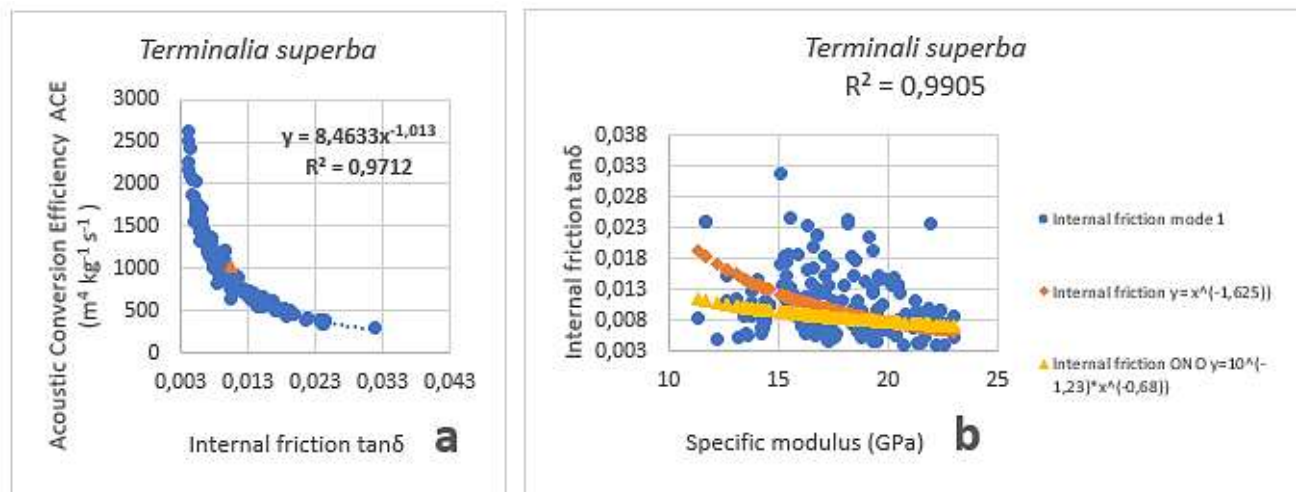


Figure 10. Variation of the Acoustic Conversion Efficiency as a function of internal friction (a) and internal friction as a function of the specific modulus of rigidity (b) for *T. superba*.
Source: Authors

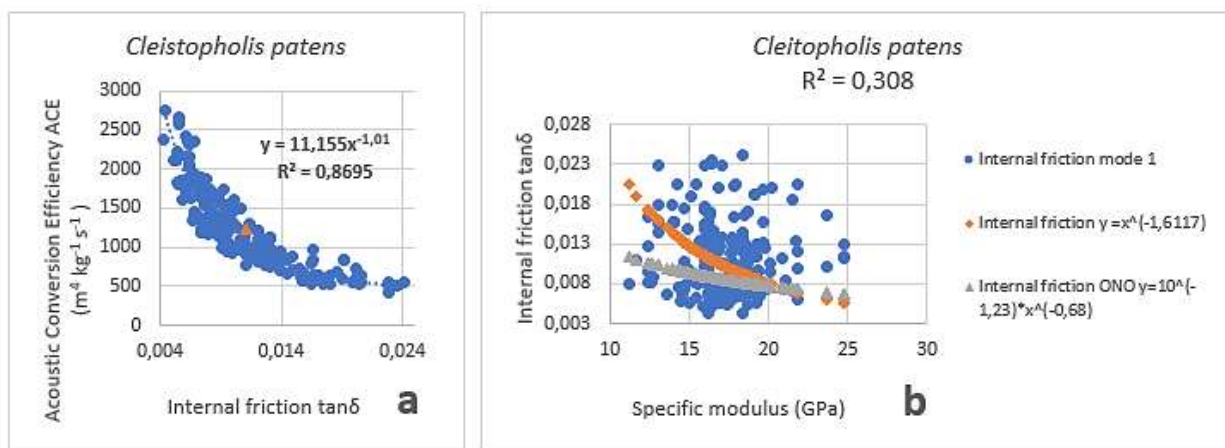


Figure 11. Variation of Acoustic Conversion Efficiency as a function of internal friction (a) and internal friction as a function of specific modulus of rigidity (b) for *C. patens*.
Source: Authors

al., 1996).

The relationship between internal friction $\tan\delta$ and specific stiffness E/ρ is determined by the orientation of the wood elements and can be modeled by considering the microfibril angle or grain angle (Obataya et al., 2000; Brémaud et al., 2011b). Thus the difference observed from the standard form of Ono and Morimoto (1983) can be explained by the fact that our species are tropical species either by the chemical composition or presence of extractives or by the water content of the wood or the presence of compression wood or the structure of the wood (Obataya et al., 2000; Brémaud et al., 2010a, 2011a; Obataya et al., 1998; Sasaki et al., 1988) which

influences the internal friction $\tan\delta$ more than the stiffness modulus E/ρ (Brémaud et al., 2013). Extractables can be without effect as they can decrease, or at times, grow, the internal friction $\tan\delta$ by a coefficient that can reach at most 2 with very little influence on the stiffness modulus E/ρ (Brémaud et al., 2012).

Since the modulus of elasticity generally increases with density, a higher mechanical impedance is recommended for panels of musical instrument body frames or others (Pollens, 1984; Yoshikawa, 2001). Also, high values of the specific modulus of elasticity E/ρ are highly recommended for excellent soundboards (Bucur, 2006; Ono, 1996; Ono and Morimoto, 1984). Because of this,

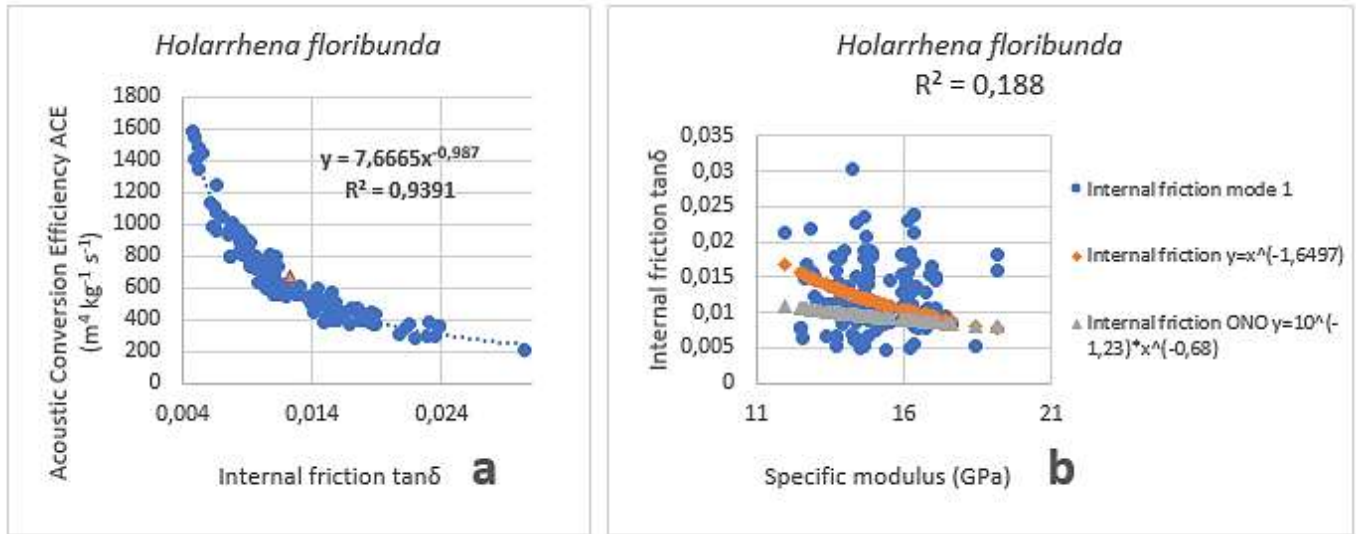


Figure 12. Variation of Acoustic Conversion Efficiency as a function of internal friction (a) and internal friction as a function of specific modulus of stiffness (b) for *H. floribunda*. Source: Authors

the performance of soundboards and cases cannot be predicted solely from the modulus of elasticity and velocity (Yoshikawa, 2007), internal friction, and others. For Western instruments (marimbas and xylophones), the required wood qualities have been defined as high density, modulus of elasticity, and good moisture-related dimensional stability (Holz, 1996b).

Shrinkability

Shrinkability is a parameter influencing the dimensional stability of wood in both structural and instrumental applications. The physical properties of shrinkage and infradensity of the studied species were researched in the literature. Values of volume shrinkage and radial and tangential shrinkage given the R/T ratio indicate that these species are dimensionally stable in the face of moisture gradient. Indeed, according to Tropix 7, *T. superba* has a tangential shrinkage RT of 6.1%; a radial shrinkage RR of 4.3% for RT/RR ratio of 1.4 for a volume shrinkage coefficient of 0.42% and is required to be medium stable in service. PROTA reporting Lemmens (2012) found that *C. patens* has a radial shrinkage RR of 5.0%, a tangential shrinkage RT of 9.6% for a shrinkage anisotropy RT/RR of 1.9; it is a moderately stable wood in service. The literature is silent on the physical, mechanical and acoustic properties of *H. floribunda*, which remains in the domain of natural sciences (Bayala et al., 2006). However, recent work by Antwi et al. (2022) shows that the branch wood of this species has similar properties to the trunk wood. Antwi et al. (2022) found that branch wood has a tangential shrinkage of $(3.8 \pm$

0.7%), a radial shrinkage of $(2.0 \pm 0.5\%)$ and a shrinkage anisotropy of 1.9 ± 0.2 similar to trunk wood, which has a tangential shrinkage of $3.6 \pm 0.6\%$ and a radial shrinkage of $1.9 \pm 0.2\%$ with a shrinkage anisotropy of 1.9 ± 0.4 . *R. heudelotii* heartwood dries rapidly with little or no degradation. Its tangential shrinkage RT ranges from 4.7 to 5.4% and its radial shrinkage RR ranges from 1.9 to 2.4%. Its shrinkage anisotropy ranges from 2.0 to 2.8. The wood is easy to saw and work, and nails without splitting, but turning and planing are difficult (Tchoundjeu and Atangana, 2007). Thus, the wood of the species studied has, overall, a lower propensity to deformation than the wood of *R. heudelotii*, which nevertheless has the best dimensional stability. These species have good potential for sculptural works requiring low dimensional variation in the face of humidity variations.

Conclusion

At the end of this work, it appears that Benin has many species of wood that can be used in sculpture such as *C. patens*, *T. scleroxylon*, *M. excelsa*, *A. leiocarpa*, *D. mespiliformis*, *H. floribunda*, and *T. superba*. Thus to better value some of them, we determined the most important physical, mechanical and acoustic properties of *T. superba*, *C. patens* and *H. floribunda*. Taking into account their velocity of about 4000 m s^{-1} and basic properties, such as density and modulus of elasticity, these three species offer great potential for instrument making. Based on the densities found, *T. superba* and *H. floribunda* are good candidates for guitar case panels. Because of their modulus of elasticity, all three species

can be used for the manufacture of soundboards; *T. superba* and *H. floribunda* for the back and sides of violins and wind instruments. By combining the speed of sound and the coefficient of acoustic radiation of these three species of wood, it is concluded that the woods of these species are potential resonance woods for the manufacture of soundboards. The specific stiffness of each of these three species is greater than 15 GPa and reinforces their nature as resonance woods. Their acoustic impedance suggests their use in strings. With their internal friction, the three species studied are in the range of wood species with good acoustic quality. *T. superba* and *C. patens* have peak ACE responses above $1000 \text{ m}^4 \text{ kg}^{-1} \text{ s}^{-1}$ while all three wood species have a radiation coefficient above $7 \text{ m}^4 \text{ kg}^{-1} \text{ s}^{-1}$. Compared to the properties *Azelia* spp. *H. floribunda* can be a good substitute in the manufacture of musical instruments especially xylophone bars. Their shrinkage and shrinkage anisotropy predestine them to good use in art sculpture.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Seed dispersal of a range restricted and vulnerable species, *Guibourtia copallifera* Benn. in Sierra Leone

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In the early 20th Century, *Guibourtia copallifera* Benn. was extensively exploited as a source of gum copal. Its geographic distribution in Sierra Leone is now restricted to a few square kilometers in a single badly degraded forest reserve. We conducted a series of seed rain experiments to quantify its seed dispersal potential. Transects of seed traps were laid out and a total of 1,164,880 seeds were collected over the two months when *G. copallifera* sheds its" seeds. Seeds from a further 85 species were collected. The majority of species were wind dispersed followed by terrestrial animals, bats and birds. Leguminosae-Caesalpiniaceae had the highest number of species, followed by Euphorbiaceae, Apocynaceae, Rubiaceae and Sterculiaceae. There is no statistically significant difference between the transects ($p>0.05$, $df=79$, $F=0.023$) or the types of traps ($p>0.05$, $df=118$, $t=0.089$). Median dispersal distances of *G. copallifera* seeds was 41.22 m from the forest edge. The reserve still possesses a high natural regeneration potential for *G. copallifera* but increasing human disturbance is opening the canopy allowing pioneering species to dominate and reducing the ability of *G. copallifera* seedlings to establish.

Key words: *Guibourtia copallifera*, seed rain, seed dispersal, forest reserve, Sierra Leone.

INTRODUCTION

Seed production and dispersal are important functional attributes for the maintenance of plant populations, influencing the spatial distribution and composition of the plant community, in addition to affecting gene flow within and between populations and enabling the colonization of new sites and habitat restoration (McConkey et al., 2012; Kroiss and Hillers-Lambers, 2015).

Changes in the composition and abundance of seeds in the seed rain will have strong effects on the vegetation community and seed bank composition (Pearse et al.,

2017; Barnes and Chapman, 2014). Strong disturbances lead to seed bank depletion either by massive germination of the seeds, or by the loss of a large number of seeds (Travlos et al., 2020; Ndor et al., 2012). Since 2002, Sierra Leone has lost 29,500 hectares of humid primary forest, a decrease of 11% (Global Forest Watch, 2020). At present, these resources are confronted with increasing deforestation due to growing population and demand for more agricultural land, timber, charcoal, fuel wood, mining and recurrent bush fires.

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Research addressing community wide and species-specific seed rain patterns of forests in Sierra Leone is lacking. Most approaches to understanding tropical forest dynamics and plant species availability have focused on patterns of vegetation structure, composition, diversity and distribution (Sesay, 2019; Fayiah et al., 2020; Mattia and Sesay, 2020). Kasewe forest reserve has been through series of human exploitations since it was first gazetted in 1919 (Munro and Hiemstra-van der Horst, 2012a; Munro et al., 2017) for resources like gum copal, timber, poles, and other non-timber forest products (NTFPs) and bush meat. During the Ebola crisis of 2014 - 2016 the population within the forest increased as people fled their communities and found the forest as a source of livelihood. The resulting exploitations have expanded and increased over the years to levels which are no longer sustainable. Exploitation rates increased through artificial deforestation the use of power saws offered by affluent merchants in return for charcoal and related products to their benefactors in cities where these products are in high demand.

The most preferred species for charcoal production by the exploiters is *G. copalliofera*, a species of tree that was heavily exploited for gum copal during the colonial period but no longer being done at present. The high quality of charcoal produced from this species and the increased exploitation it currently faces in this unprotected reserve could pre-dispose this limited geographical range species to extirpation.

This study therefore aimed to characterize and evaluate the dynamics of seed dispersal by *G. copalliofera*; specifically, to quantify the seed rain along the different land uses in the forest reserve, as well as analyze for differences in total abundance, species richness, diversity and composition in the seed rain.

MATERIALS AND METHODS

Study site

Kasewe Forest Reserve (8°18'53"N, 12°15'43"W) occupies a small range of steep sided hills of volcanic origin rising up to 500 m above the surrounding plain (Bowden, 1997; Lytwyna et al., 2006). The reserve covers 2,331 hectares, and the dominant vegetation is tropical forest containing a mosaic of moist semi-deciduous forest, evergreen forests and savanna that give way to medium altitude forest on the upper slopes (UNEP, 2008). The reserve is located in Moyamba District in the south-central part of Sierra Leone about 170 km east of the capital city of Freetown. The Bo-Freetown highway is one of the busiest provincial roads in the country, it forms one discernible boundary and allows easy access for exploitation and transport of products such as charcoal and timber from the reserve.

Seedrain sampling

Construction of seed traps and placement

In order to assess the nature of the seed rain and dispersal

capability of *G. copalliofera* in the reserve, seed traps were set up along pre-cut transects in degraded areas adjacent to the forest edge and in the interior of forest patches. Transects were up to 300 m long with equally spaced pairs of sampling units (traps). One trap consisted of a 1.5 m² plastic mesh (1mm mesh) with the corners lifted 1.2 m above ground level on poles, while the other consisted of swept ground demarcated by four wooden stakes driven into the ground but with ends sticking out at about two meters from the mesh trap. Forty-five transects were set out in the forest and five at right angles to the forest edge into the degraded forest. Traps were monitored on a weekly interval for 12 weeks. In the degraded areas, focal trees of *G. copalliofera* were selected from which transects were located with the aim of observing the dispersal dynamics of this species. Transects in the degraded area had traps installed at 10 m intervals out to 300 m from the forest edge.

Seed collection and identification

Traps were emptied on a weekly basis with standard data sheet for each type of trap that was installed. Contents of each trap within the trap line was emptied into a separate labelled bag or container and leaves and twigs removed. Physical states of the fruits and seeds were noted: rotten, germinated, partly eaten, insect infested and animal feeding. Seeds were grouped in two classes namely *G. copalliofera* (Kobo) and others. Trap contents were taken into the laboratory and sorted by hand. All potentially viable *G. copalliofera* seeds (>1.5 mm diameter) were extracted and identified (Cottrell, 2004). Other species were identified by comparison to the seed collections held at the National Herbarium of Sierra Leone in the Department of Biological Sciences, Njala University.

Statistical analyses

Majority of the analyses were done in R version 3.6.2 (R Core 2020). ANOVA single-factor analysis, paired t-tests and linear regression were used to determine the effectiveness of the different types of traps, dispersal in the different environments, changes over time. Variability with seed collected in the different traps was visualized using box plots.

RESULTS

Types of traps and layout of the experiment

Trap types were installed at 45 transect locations to determine if there was a significant difference in capture efficiency. A total of 1,164,880 seeds were collected. Among these 44.94% (523,467) of seeds collected were from stations with both ground and mesh traps; while 22.88% (266,518) from ground traps and 20.97% (244,244) from mesh traps. The effectiveness of the traps types was determined by a t-test. This showed there was no statistical difference between the two types of traps ($p > 0.05$, $df = 118$, $t = 0.089$).

Floristic composition, species richness and diversity of seed rain

A total of 1,164,880 seeds were collected resulting in a final density of 2,107 seeds m² (300 seeds m² week⁻¹). Eighty-five species from forty-one genera were collected; 65 species were identified to species level, 9 to genera, 6

to family and 5 remained unidentified. The large majority of species were dispersed by wind (50 species), followed by animals (20) including birds and bats (15).

Guibourtia copallifera was the most abundant species, followed by *Nesogordonia papaverifera* (A.Chev.), *Hymenocardia lyrata* Tul. and *Memecylon normandii* Jacq. Leguminosae-Caesalpiniaceae had the highest number of species, followed by Euphorbiaceae, Apocynaceae, Rubiaceae and Sterculiaceae. Species belonging to Rhizophoraceae, Chrysobalanaceae, Ixonanthaceae and Ulmaceae were present in very low numbers. Seeds of *G. copallifera* made up 58.43% of all seeds and were slightly more likely to be captured by the mesh traps (61% of seeds) compared to the ground traps (54%). Species richness ranged from 3 to 12 species per trap during the collection period.

Status of seeds in traps

Seed conditions were classified as intact (80 to 85%), germinated (8 to 12%), insect infested (1 to 2%), decayed (rotten) (2 to 3%) or partly eaten (2 to 4%). Intact seeds germinated in 3 to 7 days but the majority of intact seeds failed to germinate.

Seed quality and production are main considerations overseeing the regeneration, structure, also progression of trees in natural forests (Galipeau et al., 1997; Viglas et al., 2013). Harsh and Joshi (1993) have announced 70% harm to *Albizia* seeds because of insects and diseases out of which 40% was because of the insects, *Bruchus bilineatopygus* and *B. sparsemaculatus*. Diminished viability and germination disappointment of these damaged seeds have been accounted for (Ponnuswami et al., 1990). Singh and Bhandari (1986) have detailed scope obliteration of *chilgoza* pine seeds in India at Kalpa, Himachal Pradesh, by *Dioryctria abeitella* Schiff. causing up to 50% seed damage. Essentially, seeds of one more significant species, teak (*Tectona grandis*) are damaged by *Dichocrosis (Conogethes) punctiferalis* (Guenee) (Pyralidae) causing up to 70% seed destruction in storage. Locally, decomposition on forest floor can be constrained by the chemical quality of litter (Krishna and Mohan, 2017; Prescott et al., 2017), microenvironmental conditions (Rodríguez-Paredes et al 2012; Tymen et al., 2012) and soil properties (Li et al., 2018; Kravkaz-Kuscu et al., 2018).

Weekly seed collection

Weekly seed collection was virtually the same for both trap types; median average 1217.4, while that of mesh is 1227.4 (Figure 1).

No significant linear trend was observed for the first 8 weeks the transect traps were set out, but there was then a rapid decline close to zero by week 12. An ANOVA

single-factor analysis was performed to confirm that the results were highly significant ($p < 0.001$). A “broken stick” regression was then conducted to show the effective termination of dispersal at week 8 (Figure 2).

A linear regression of median seed rain per trap per week for weeks 1 to 8 showed an r^2 value of only 0.002, which was not significant ($p > 0.05$, $df=8$, $F=0.017$).

Seed rain by habitat

Total seed rain density was higher in the forest plots. Deposition of seeds in the interior of the forest is 3 times greater than that recorded in the degraded area or forest edge. Variability in patterns of seed deposition was high among sites and transects.

Seed species richness at the forest edge was less in terms of species and numbers. More species were recorded in the interior of the forest than at the edge, with the numbers of *G. copallifera* and other species higher in the interior of the forest and lower at the edges.

Seed numbers are fewer under the parent trees and increases as the distance increases from the trees. In the degraded and forested patches, seeds deposition is observed to be similar. Less of the seeds are found under the parent plant and more seeds recorded at distance. Distances are shorter and reduced in undegraded patches while seeds are carried by wind to long distances in degraded areas. Seeds were successful in dispersing away from the forest edge but that the rate (seeds per trap) was similar (linear regression $r^2 = 0.9702$).

Seed dispersal in relation to the forest edge

A one-way ANOVA was used to express the number of seeds caught in relation to distance from the forest edge. No statistically significant difference observed between the 5 transects ($p > 0.05$, $df=79$, $F=0.023$). The number of seeds that lands in the traps varies with the distance from the parent tree. An irregular pattern was observed with several peaks due to rain fall, wind intensity and duration, forest disturbances and canopy cover. Overall median dispersal distance of all species was 41.22 m. But a seed rain of greater than 1 per meter square of *G. copallifera* was observed out to 280 m. At 230 m the number of seeds in the traps fell rapidly. Regarding this species, dispersal distances were narrow and limited within the forest especially in areas where canopy was thick and dense. Whilst the opposite is true for the degraded patches and open areas. Seeds were carried over long distances in days where wind intensity and duration prolonged over a considerable period. Based on this study, wind action and intensity could carry seeds up to 280 m on very windy days but very few were recorded beyond this distance.

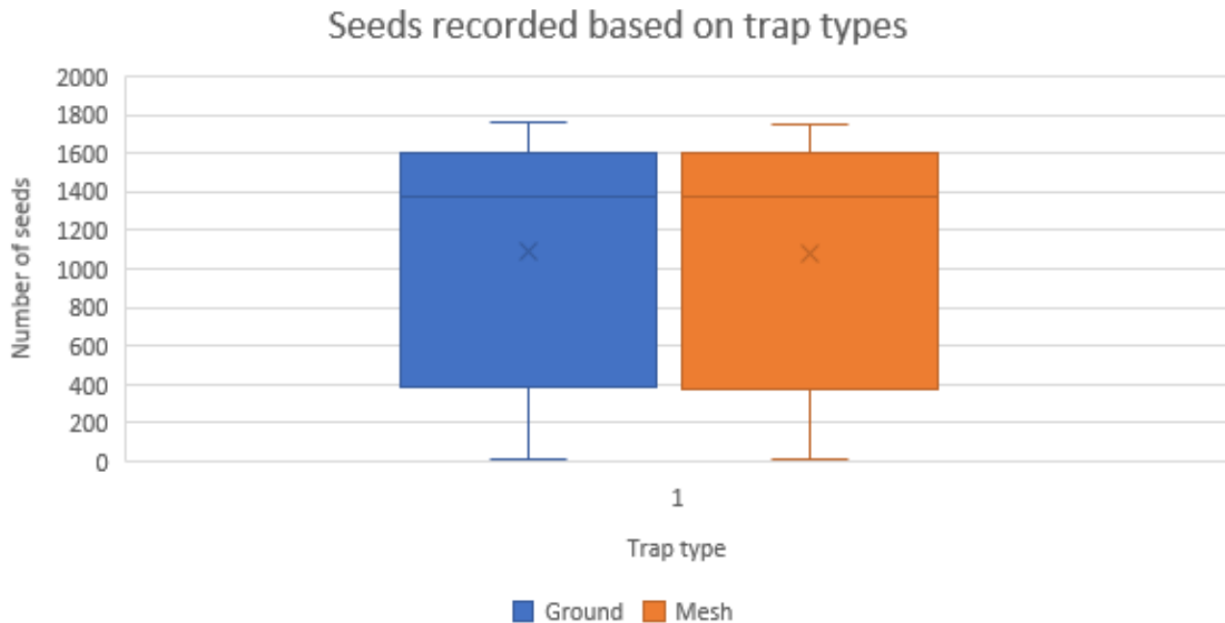


Figure 1. Seed capture by trap type
Source: Authors

Seed dispersal in the forest

The seed rain of the forest was highly variable but with an average of 297 seeds $m^{-2} week^{-1}$, but with considerable spatial and temporal patchiness. More than 60 species were represented in the seed rain. These were dominated by a small number of species producing a large seed crop, with most species represented by few seeds during the period. Total seed rain from wind-dispersed versus animal-dispersed tree species did not match their proportions in the forest. Wind dispersed seeds were relatively common compared to others. Additionally, no previous studies have assessed the proportion of seed rain of this species and this forest patch in general.

DISCUSSION

Types of traps and layout of the experiment

The study shows no statistically significant difference between ground traps and net traps in terms of total seeds collected. Although the total number of seeds is highest in traps with large area, seed density does not vary significantly across traps with different areas which is consistent with Morris et al. (2011). In forest seed rain studies, large seed traps are usually used to capture large seeds of fleshy-fruited and tree species (Vergara-Tabares et al., 2021). However, our values seem consistent with records from other locations such as Carrillo-Arreola et al. (2020) who collected 8,500 seeds

m^{-2} forest matrix. Kitamura et al. (2005) observed 22 species of birds and six species of mammals foraging in 15 different species of fruit-bearing plants in Khao Yai National Park in Thailand.

Floristic composition, species richness and diversity

Results on the species richness and diversity indicate that despite the disturbed nature of the forest there is still a high amount of diversity. *G. copallifera* seeds were the most abundant, followed by *Nesogordonia papaveriefera* (A.Chev.), *Hymenocardia lyrata* Tul. and *Memecylon normandii* Jac.-Fél. The abundance and seed density values in this study (85 species from 41 plant families) are higher than those in several other studies of primary and secondary tropical forests, 16 species (Chalerm Sri et al., 2020), 26 species (Wang et al., 2019), 15 species (César et al., 2017), 40 species (Sritongchuay et al., 2014). Similar levels of diversity are the 65 species recorded in the Serra da Bodoquena National Park (Brachtvogel et al., 2020), 56 species (Dhillon et al., 2003) and 46 species by Villicana-Hernandez et al., (2020) in Yucatan, Mexico.

Seed density was found to be higher in this study relative to two similar studies in semi-deciduous tropical forest for Cameroon ($\cong 297$ seeds. m^{-2} . year $^{-1}$; Hardesty and Parker, 2002 Nuñez et al., 2021;), and the southeast of Brazil ($\cong 442$ seeds. m^{-2} . Year $^{-1}$; Grombone-Guaratini and Rodrigues, 2002). The higher number of seeds found in this study reflect that the large number of mature trees producing a high number of seeds produced by *G.*

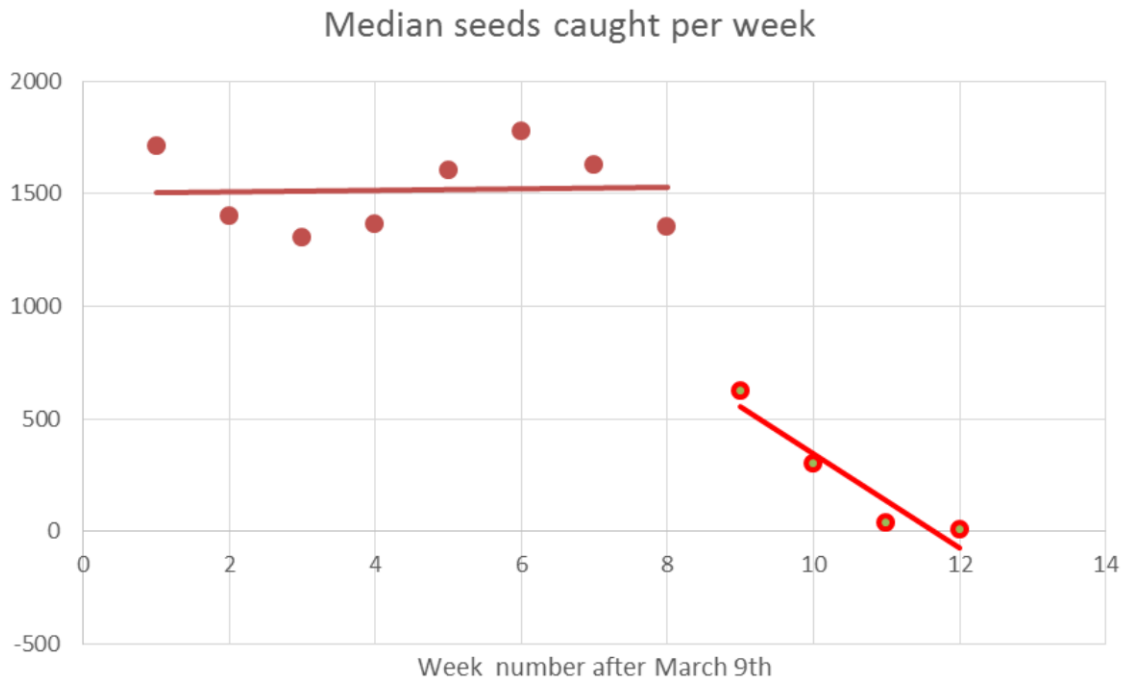


Figure 2. Seedrain by week number.
Source: Authors

copallifera (Peili et al., 2019; Wickert et al., 2017). Peak abundance of tree seeds coincides with the start of the rainy season, while seeds of other herbaceous species peak at other times.

Human disturbances in this forest also strongly affect the plant community structure; more specifically, *G. copallifera* species are gaining dominance in non-degraded areas and losing dominance in degraded areas. The seed dispersal distance for this species was strongly correlated with the plant than with seed traits (Augspurger et al., 2017; Thomson et al., 2011). Indeed, the dispersal of *G. copallifera* seeds in our study was largely due to plant character more than the seed size. The parent plant crown and the height positively influenced the seed dispersal of this species.

Status of seeds in traps

Conditions of collected seeds in traps varied depending on the several prevailing factors like rainfall, wind, humidity, sunlight, germination rate and volume of leaf litters. Damage was less in the mesh traps as a result of the fact that ground trap is being affected by run off, ground animals and insect prey which reduces the evidence on the ground compared to mesh trap which drains water and is more difficult to access by terrestrial seed predators.

Germinated seeds were recorded even in the absence of soil especially for *G. copallifera*. Regarding seed

decay, the principal factors thought to control paces of litter decay include barometrical relative humidity (Gregorich et al., 2017; Beyaert and Voroney, 2011), the herbaceous plant layer (Ossola et al., 2016; Zirbel et al., 2017), and soil temperature and dampness (Cortez 1998; Mao et al. 2018; Sun and Zhao, 2016). Song et al. (2021) observed that decomposition rate of leaves increased under mats of exotic weed *Tradescantia fluminensis* Vell.: This is attributed to a positive microclimate and living space for the decomposers. Studies on seed insect pests, conducted at the Pakistan Forest Institute, Peshawar, revealed that seeds of 30 out of 70 tree species were infested with 24 insect species. Among them 86% were Coleoptera, and 5.6% were Lepidoptera, while 8.4 % was a hymenopteran parasite (Rehman, 1993).

Weekly seed collection

The number of seeds captured weekly on the interior and edges of the forest seem to coincide with the frequency of monthly wind, sun and rain data for that area. More seeds are captured in weeks with more frequent rainy days and less in less windy and raining days in the weeks. When the rate and duration of precipitation increased in September, the data showed a significant decrease in the number of seeds captured particularly for *G. copallifera*, since this was at the end of its fruiting season. The temporal variability of seed rain related to

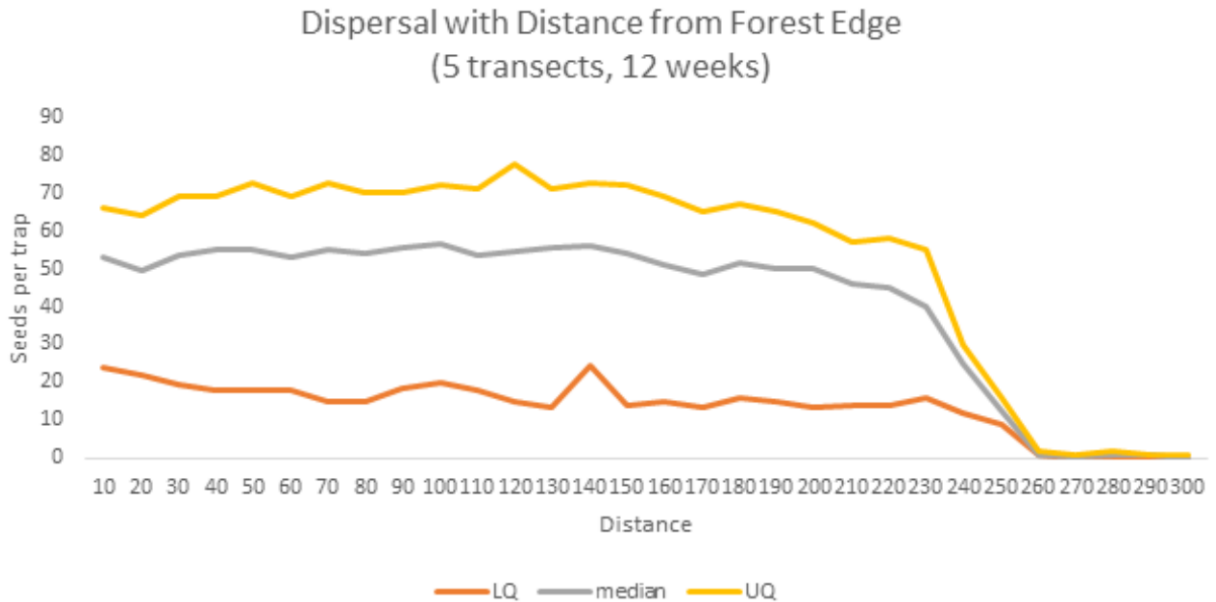


Figure 3. Dispersal away from forest edge
Source: Authors

rainfall has been reported by other studies (Gummadi et al., 2017; Perini et al., 2019).

Seed rain was observed to be greater in the interior of the forest than at the edges. Studies on the interior and edges observed that seed rain on both sites was higher along the forest edge, also observed by other studies (Diogo et al., 2015; Dunham et al., 2018; de Melo et al., 2006; Magrach et al., 2014; Razafindratsima et al., 2021). The low abundance and diversity of seed captured in the degraded area shows a limitation on seed dispersal either due to felling of trees, and or increasing human activities. Similar results by other studies for seed dispersal from forests adjacent to pasture found that seeds were mostly anemochory and limited to 10m distance (Aide and Cavelier, 1994), 5 m (Holl,1999), and 4 m (Zimmerman et al., 2000; Cubiña and Aide, 2001) from the forest edge.

The fact that the seed rain dynamics in both forest edge and interior was affected by the surrounding tree heights, number of trees, species and intensity of human activities, shows that matured and taller trees produce more seeds and have a higher chance to disperse more seeds (Thomson et al., 2011).

Seed rain numbers

Over sixty species were shedding seed at the same time as *G. copallifera*, however, it is possible that some species have been missed due to human error (misclassification or identification), low density or being physically restricted to small areas in the forest; additional

species disperse at other times of the year. *G. copallifera* seeds dominated seed rain traps. Numbers of seed dispersed by this species is plentiful on the forest floor.

Relationship between distance and dispersal frequency

The most general and perhaps obvious dispersal pattern found in the study was the fairly uniform seed rain out to about quarter of a kilometer from the seed source (Figure 3). The results illustrate how an analysis of a species seed dynamics can contribute to a better understanding of the general ecological phenomena, including species dispersal and spread. This is an indication to the fact that this species has the ability to establish itself in distant locations in the absence of the parent plant. The proportion of seed that is randomly dispersed over relatively long distances from the source plants of *G. copallifera* ranged from 5 to 50 % of the total seed set. Despite this long-distance dispersal advantage of *G. copallifera* seeds, its' establishment in the new area is opposed by predation, soil conditions, depth of seed in soil / leaflitters and human activities.

Conclusion

G. copallifera has an extremely restricted geographical distribution in Sierra Leone with the majority of the population in a single small and degraded forest reserve. Disturbance to the forest through charcoal burning allows

the pioneer species to be dominant and in some grass patches which form fire-climax vegetation communities are establishing. Our results showed that *G. copallifera* produces a lot of wind dispersed seeds and densities of up to 70 to 100 per sq meters were observed 250 meters from the forest edge. Only 0.8% of the seeds were found to be empty. Dispersal of *G. copallifera* seeds would seem to be sufficient to allow it to maintain its population in the forest but not to disperse to other similar forest patches which are many kilometers away.

The results obtained demonstrate that Kasewe Forest Reserve still has a high natural regeneration potential for *G. copallifera*; nevertheless, there are several causes for concern which will be critical for continued natural regeneration; the on-going disturbances have reduced tree cover, increased the number of clearings and increased the availability of light, thereby facilitating the dominance of pioneer species. Thus, the national and district authorities must be careful for the implementation of the conservation measures and enhance effective co-management and protection program involving the local people to ensure fruitful conservation of this species in its most abundant site. Protection and aided natural regeneration may be another alternative option for effective natural regeneration and conservation of this species especially in degraded habitats within the reserve.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Screening for complete submergence seedling tolerance in Rice (*Oryza sativa* L.) genotypes collected from Sudan and South Sudan

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Eighteen rice genotypes from Sudan and South Sudan and two genotypes (FR13A tolerant and IR42 sensitive checks) from the International Rice Research Institute were screened for complete submergence at seedling stage in a protected house. Most of the genotypes showed zero survival, except genotypes [NBGA (2.08%), Banban (3.92%), Masury1 (5.56%), Pipanfary Red2 (11.11%) and Sommboy (14.58%)]. Sensitive genotypes registered 2 to 3 folds reduction in root length. Mean soluble sugar concentration in roots increased by 41% and decreased by 57.3 and 80.5% in stem and leaves, respectively. Mean starch concentration was reduced by 13.8% in roots and 77.7% in stems; whereas it increased by 115% in leaves for all genotypes. Genotypes with some degree of tolerance exhibited higher soluble sugar and lower starch concentrations. Submergence reduced chlorophyll *a*, chlorophyll *b* and total chlorophyll (*a+b*) by 83.0, 83.3 and 82.7%, respectively. Genotypes that showed survival after submergence maintained higher values of chlorophyll *a*, *b* and (*a+b*) as compared to other genotypes. Genotypes tolerant to submergence such as Pipanfary Red2 and Sommboy had higher soluble sugar and starch concentrations and chlorophyll content and could be developed to be grown under rain-fed lowland flooded areas of Sudan and South Sudan.

Key words: rice; submergence; seedling stage; starch; soluble sugar; chlorophyll.

INTRODUCTION

Rice (*Oryza sativa* L.) production in Sudan has a great potential due to the availability of land, water resources as well as a large diversity in agro-climatic zones. Low land rice has been cultivated as the major activity and a key source of employment for rural population at traditional areas of its production (White Nile state in Sudan and low land swamps in South Sudan). In these areas the crop is cultivated as irrigated and flood ecosystem (Gasim et al., 2018). Low yields of 0.98 to 1.2

t ha⁻¹ were produced. One of the major problems associated with this low yield is crop establishment as farmers use direct seeding, a simplified cultivation mode widely used all over the world (Sun et al., 2019). The seeds and young plants can be damaged by drought conditions before flood arrives in addition to weed competition.

Sudden flooding, as a result of global climate change, where large volume of water enters the field in a short

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time can lead to a high rate of seedling death because they face low oxygen dependent metabolic activity for proper utilization of carbohydrates (Su et al., 2022), and consequently low yield. Moreover, the depth and time of water in which the crop is grown and water conditions such as turbulence (Debarbata and Jijnasa, 2021; Chen et al., 2018), traditional varieties together with low inputs supply, poor drainage, poor maintenance of developed lowland swamps and poor soil management, are among the most important factors for low rice yield (Osman et al., 2012).

Tolerant rice genotypes seedlings normally have more total sugars as compared to seedlings of sensitive genotypes, which are used during submergence to supply energy for growth and maintenance metabolism. Thus initially higher content of soluble and insoluble sugars can be regarded as an advantage for submergence tolerance (Sayani et al., 2017).

Enhanced tolerance of flooding during germination and early seedling growth could help improve crop establishment and promote more widespread adoption of direct seeding. Not much research on submergence screening tolerance has been done on low land rice in Sudan. The objective of this research was to screen some Sudanese rice genotypes in response to complete submergence to develop submergence tolerant high yielding cultivars for sustainable traditional lowland rice production in Sudan.

MATERIALS AND METHODS

Planting material, experiment lay out and screening for complete submergence seedling tolerance

Eighteen Sudanese and South Sudanese rice genotypes seven from Aweil, South Sudan (NBGS1, NBGS2, NBGS3, NBGA, BG400-1, BG90-2, Banban) and eleven from Sudan (Masury 1, Masury 2, Pipanfary, Pipanfary Red 1, Pipanfary Red 2, JaingArri Red, Sommboy, Comarwa, Painjla, Tagmizedo, BactingArri), which were never tested for submergence tolerance, as well as two cultivars from International Rice Research Institute (IRRI), namely FR13A as tolerant cultivar and IR42 intolerant checks, were used. The experiment was conducted in a protected house in a concrete tank at IRRI.

The seeds were first soaked in water then the floating ones were removed, to assure the germination of the whole healthy seeds. They were incubated in the oven at 33°C for 24 h. Water was removed from the seeds and seeds were left for pre-germination at 33°C for 48 h.

The following day, the well pre-germinated seeds were selected and sown 0.5 cm below the soil surface in trays. The soil was mixed with 3 g Solophos fertilizer, 3 g Muriate of Potash and 6 g Ammonium Sulfate before sowing. Three rows per accession and 20 seeds per row were sown based on the experiment layout, using randomized complete block design with three replications, and covered with another layer of sieved garden soil. The pre-germinated seeds were left to grow under normal conditions for 21 days with regular watering using tap water and continuous weeding. After 21 days of normal growth, the number of seedlings of the first row was counted for seedling survival and the second row was used for sampling of chlorophyll (leaves) and carbohydrates

(leaves, stems and roots) for the determination of soluble sugar and starch content. The third row was used for shoot and root length. Thereafter, the entire setup of seedling trays for submergence were submerged in the concrete tank at around 11:00 to 2:00 pm so as to allow the plants to photosynthesize before the treatment and the water depth was 80 cm above the soil surface.

The floodwater temperatures and other floodwater conditions (dissolved O₂, light penetration and pH) were monitored daily in the morning (7:00 to 8:00) and in the afternoon (13:00 to 14:00) at various depths during submergence days using oxygen meter, light meter and pH meter, respectively. The seedlings were submerged for 11 days, and then de-submerged in the 12th day in the afternoon, when light intensity was low, and shoot and root lengths were measured. Samples for chlorophyll and carbohydrates determination were harvested as before submergence, for the submerged seedlings and the control.

Then the seedlings for assessing survival were left for another 21 days growth recovery under normal conditions with normal watering. Thereafter, the survived seedlings per entry were counted and the survival percentage was determined as follows:

$$\text{Percentage seedling survival} = \frac{\text{Number of survived seedlings at 21 days - submergence (Y)}}{\text{Number of seedlings before submergence (X)}} \times 10$$

Analysis of soluble sugar

Subsamples of about 200 mg each of the plant material (roots, stems and leaves) were freeze dried in liquid nitrogen, ground and extracted using 80 % ethanol (v/v). The sugar extract was cooled to room temperature and root sample extracts were diluted to 15 mL because the concentration of sugar in the root is low, while stem and leaf sample extracts were diluted to 25 mL. The residues were dried at 70°C for 24 h prior to starch assay.

For colorimetric determination of soluble sugar, 0.3 mL of the sugar extract was added to the test tube and mixed with 3.0 mL anthrone reagent followed by measurement of absorbance read at 620 nm (Fales, 1951).

Determination of starch

The residue previously dried at 70°C, which remained after soluble sugar extraction, was oven dried and used for starch analysis using the method described by Setter and Laureles (1996). The starch was then boiled for 3 h while vortex was mixed thoroughly every 20 min. The root samples were diluted to 15mL, while stem and leaf samples were diluted to 25mL. The starch hydrolysate (standard) was diluted to 50mL with distilled water. Absorbance was read at 450 nm against a blank sample (reference) after the procedure of Kunst et al. (1988).

Determination of chlorophyll

The samples of one leaf from each accession were first flash-frozen in liquid nitrogen (N₂) immediately after bagging and stored at -80°C. Then, they were fully freeze-dried for five days. Chlorophyll concentration was determined following the method of Mackinney (1941) in acetone extracts. Readings were carried out using spectrophotometer machine (SHIMADZU CORP UV 1800) and absorbance was recorded at four wavelengths of 470, 645, 646 and 663 nm. The concentration of chlorophyll *a*, chlorophyll *b* and total chlorophyll (*a+b*) were then determined based on the equation outlined in Lichtenthaler and Wellburn (1983).

Statistical analysis

Computer program (R-STAR, 2013) was used for statistical analysis of the data. The data were analyzed according to the standard statistical procedure as described by Gomez and Gomez (1984) for complete randomized block design.

RESULTS AND DISCUSSION

Screening for complete submergence seedling tolerance of 20 rice genotypes under control and complete submerged conditions

Submergence is a major environmental constraint to rice production throughout the world resulting in substantial yield losses and approximately one-fourth of the global rice crops are grown in lowlands that are prone to seasonal flooding (Debarbata and Jijnasa, 2021).

In the present study, statistical analysis for complete submergence tolerance of 20 rice genotypes showed highly significant ($P \leq 0.01$) differences between the genotypes in shoot length and root length measured after 21 days and 32 days under control and submerged conditions; while the result for percent seedling survival showed highly significant differences between the genotypes under submerged condition (Table 1). Complete submergence tolerant (FR13A) and sensitive genotypes (IR42 plus the rest of genotypes) had similar seedling survival under control condition; whereas under submerged condition the seedlings survival was greatly reduced by 96.2% for both tolerant and intolerant genotypes; most of the intolerant genotypes approached zero survival, except for genotypes NBGA (2.08%), Banban (3.92%), Masury1 (5.56%), Pipanfary Red2 (11.11%), and Sommboy (14.58%) in comparison to FR13A (42.54%). This suggests their tolerance under rain-fed lowland flooded areas.

Similar results were reported by Su et al. (2022) who found more than 78.1% survival in the rice seeds of strongly submergence-tolerant rice genotype as compared to only 56% survival for submergence-sensitive genotypes when treated by long-term submergence. This high reduction in genotypes survival under complete submergence might be due to the fact that plant survival is affected by depth and extent of submergence (Rachmawati et al., 2019).

Under control condition, the highest (59.50cm) shoot length measured 21 days after seeding was revealed by the genotype Masury1, which was greater than that of FR13A (51.91cm); whereas the least (38.67 cm) shoot length was obtained for the genotype BG400-1 which was more or less the same as that of IR42 (37.80cm). Moreover, the highest (94.25cm) shoot length measured 32 days after seedling growth under control condition was revealed by the genotype Tagmizedo, and was greater than that of FR13A (90.49cm) and the lowest (61.06 cm) shoot length was exhibited by the genotype BG400-1,

which was greater than that of IR42 (59.42cm). The highest (14.62cm) root length measured 32 days after seedling growth in control condition was attained by the genotype Pipanfary Red1 longer than FR13A (10.33cm) and the lowest (5.53cm) root length was obtained for Masury1 less than that of IR42 (8.17cm) (Table 1).

Under the submerged condition, shoot length measured after 21 days seedling growth was increased by 3.06%. The highest (55.92 cm) shoot length was exhibited by the genotype Masury1, which was more or less the same (55.22cm) as FR13A and the lowest (44.92 cm) shoot length was registered by the genotype BG400, which was less (40.04cm) than that of IR42 (Table 1). These results are confirmed by Pramanik et al. (2016) who found that most of the rice genotypes showed enhanced shoot elongation in response to submergence. Also Nurrahma et al. (2021a) reported significant increase in shoot length by more than 34.3% after 6 days of submergence than the control and 40.9% by the end of the 6-day recovery period and explained that shoot elongation of these genotypes represents transient submergence intolerance. Studying the emergence rate of plants, Su et al. (2022) concluded that submergence-tolerant rice will rapidly elongate in a submerged environment and by that it becomes exposed to oxygen outside the water where the supply of oxygen and the production of carbohydrates can be maintained to improve plant survival. That is why escape strategy in rice is shown by rapid underwater shoot elongation especially in deep water lowland rice where vigorous stem extension prevents total submergence with increased depth of flood water and keep the upper leaves above water surface for normal gas exchange, and thereby survive submergence (Wu et al., 2018; Kuanar et al., 2019).

On the other hand, the shoot length measured after 11 days of complete submergence was reduced by 13.7%. Tolerant check FR13A as well as genotypes that showed some survival degree such as NBGA, Sommboy and Pipanfary Red2 reduced their shoot elongation as tolerant genotypes, were found to conserve energy during flooding for maintenance and survival processes as strategy in response to short term inundation (Pramanik et al., 2016). Likewise, the highest (10.98cm and 10.54cm) root lengths measured after 11 days of complete submergence was achieved by the genotypes Pipanfary Red2 and Masury2, respectively, which were higher (7.46cm) than that of FR13A and the lowest (3.45 cm) root length was obtained by Sommboy lower (7.46 cm) than that obtained for IR42 (Table 1). From the results, it is clear that root elongation followed an opposite pattern to that of shoots, whereby the accessions that showed tolerance registered higher root lengths as compared to intolerant ones. It is interesting to note that all genotypes that showed zero percent survival under submergence, such as Tagmezido, Pipanfary and Pipanfary Red1 registered a 2 to 3 folds reduction in root length under submerged conditions. This significant

Table 1. Percentage of seedling survival, shoot length and root length (cm) of 20 rice genotypes after 11 days of complete submergence under control and submerged conditions.

Genotype	Control condition				Submerged condition			
	S %	SL(21D)2D)	SL(32D)21)	RL(32D)21D)	S %	SL(21D) 21D)	SL(32D) 21D)	RL(32D) 21D)
NBGS1	100.00	47.72	76.71	12.03	0.00	50.40	81.84	9.80
NBGS2	94.44	51.11	76.48	7.36	0.00	50.45	73.67	9.68
NBGS3	91.88	49.00	70.32	13.95	0.00	50.25	59.17	6.69
NBGA	86.62	49.89	85.34	7.31	2.08	52.46	57.54	7.52
BG400-1	97.37	38.67	61.06	5.94	0.00	44.92	63.58	7.74
BG90-2	82.50	46.29	69.65	6.31	0.00	49.72	70.18	6.76
Banban	96.43	52.55	79.38	8.59	3.92	47.32	86.05	8.27
Masury1	97.06	59.50	83.13	5.53	5.56	55.92	81.28	9.95
Pipanfary Red1	89.72	50.39	82.66	14.62	0.00	57.54	65.30	3.65
JaingArri Red	92.50	48.75	83.59	8.80	0.00	48.55	54.97	3.78
Sommboy	94.44	50.50	88.44	10.52	14.58	53.16	79.83	3.45
Comarwa	100.00	40.63	76.11	9.13	0.00	45.89	49.07	8.15
Painjla	65.63	47.21	81.69	9.61	0.00	52.53	58.39	8.38
Pipanfary	94.44	47.05	81.84	11.86	0.00	45.05	54.76	4.54
Tagmizedo	100.00	55.41	94.25	12.08	0.00	55.04	71.76	4.58
Pipanfary Red2	100.00	53.22	93.16	10.72	11.11	53.45	92.31	10.98
BactingArri	92.11	51.35	85.45	12.13	0.00	47.46	67.83	7.91
Masury2	77.06	51.09	89.69	13.52	0.00	54.56	90.72	10.54
FR13A	87.50	51.91	90.49	10.33	42.54	55.22	75.46	10.46
IR42	100.00	37.80	59.42	8.17	0.00	40.04	55.29	7.46
Mean	91.98	49.00	80.44	9.93	6.20	50.50	69.45	7.35
CV (%)	24.3	22.21	42.48	53.21	53.21	44.92	35.47	41.36
SE±	1.07	0.57	0.94	0.12	0.12	0.59	0.82	0.09
LSD(P≤0.01)	3.05	1.63	2.67	0.35	0.35	1.67	2.33	0.26

S %= survival percent, SL (21D)= shoot length 21 days, SL(32D)= shoot length 32 days, RL (32D)= root length 32 days.

Source: Authors

reduction in root length of most genotypes may be due to complete submergence, which greatly reduces the amount of oxygen that reaches roots via aerenchyma thereby limiting root development.

On the other hand the tolerant check FR13A and genotypes with some degree of survival (e.g.,

Pipanfary Red2, Banban, Masury1 and NBGA) either had similar root lengths under both controlled and submerged conditions or increased their root length values when submerged. Increased root length with plant survival after submergence might explain the ability of the

tolerant genotypes to maintain carbohydrate supply and aeration to support growth and functioning of the roots. Under submerged conditions root system elongation support the upright rooting of seedlings and the higher root volume forms a larger root surface area, thereby

Table 2. Variation in 20 rice genotypes in soluble sugar concentrations in roots stems and leaves after 11 days of complete submergence under control and submerged conditions.

Genotype	21 days after sowing			32 days after sowing					
	Control			Control	Submerged	Control	Submerged	Control	Submerged
	Roots	Stem	Leaves	Roots	Stem	Leaves	Roots	Stem	Leaves
NBGS1	1.37	3.65	5.09	0.77	1.27	4.28	2.28	6.18	1.16
NBGS2	2.59	2.90	2.77	0.68	1.23	3.11	2.31	5.81	0.96
NBGS3	2.20	2.80	12.0	1.33	1.90	3.13	2.01	4.23	1.17
NBGA	1.65	2.36	11.6	2.67	3.97	4.03	0.85	7.77	0.97
BG400-1	2.12	4.04	8.56	1.39	1.77	5.97	1.49	8.52	1.04
BG90-2	1.97	4.32	7.28	0.97	1.67	2.61	1.42	5.35	1.43
Banban	1.29	2.74	6.76	1.22	1.19	3.37	0.77	5.70	1.27
Masury1	2.20	4.44	6.34	0.84	2.52	2.17	2.59	4.49	1.75
Pipanfary Red1	2.16	3.97	4.91	1.61	1.68	6.59	0.78	8.21	0.93
JaingArri Red	1.01	3.87	4.63	1.77	1.90	5.40	1.92	8.29	1.45
Somboy	1.55	4.33	6.50	1.53	4.95	4.21	0.93	5.23	1.27
Comarwa	1.51	3.47	5.20	2.15	2.88	4.36	2.00	4.99	1.85
Painjla	0.90	2.41	4.76	0.81	1.84	2.38	1.61	5.32	1.08
Pipanfary	2.12	4.71	7.05	1.07	2.39	1.76	1.68	5.71	0.91
Tagmizedo	1.94	5.23	7.70	0.89	2.48	4.34	0.84	7.60	1.48
Pipanfary Red2	1.69	4.59	14.3	1.06	1.87	3.06	2.47	6.66	1.37
BactingArri	1.99	5.27	5.59	2.95	2.00	4.79	1.29	6.75	1.15
Masury2	1.64	4.17	4.70	1.39	1.51	2.94	1.66	6.05	1.15
FR13A	1.85	4.30	5.12	3.53	1.29	6.38	3.24	7.35	1.47
IR42	1.52	4.07	4.98	0.91	1.61	3.29	1.17	7.73	1.17
Mean	1.77	3.88	6.79	1.48	2.09	3.91	1.67	6.40	1.25
CV (%)	24.33	22.21	42.4	53.21	44.92	35.47	41.36	20.6	20.86
SE±	0.02	0.05	0.08	0.02	0.03	0.05	0.02	0.08	0.01
LSD (p≤ 0.01)	0.06	0.13	0.24	0.05	0.08	0.14	0.06	0.22	0.04

Source: Authors

increasing the amount of absorbed oxygen and nutrients underwater (Su et al., 2022).

Variation in carbohydrate concentration in seedlings of 20 rice genotypes under control and complete submerged conditions

Statistical analysis, under both control and submerged conditions, showed highly significant ($P \leq 0.01$) differences between the genotypes for soluble sugar and starch concentration in roots, stems and leaves at all sampling dates (Tables 2 and 3). Mean soluble sugar concentrations in roots, and leaves measured after 32 days of seedling growth under control conditions were lower by 16.6, and 5.8%, respectively, than those measured 21 days of seedling growth, while that of stems was more or less the same. This reduction observed among the genotypes indicates that soluble sugar decreases with increasing time of seedling growth (Table 2). On the other hand, mean starch in stems and in leaves measured after 32 days seedling growth was

reduced by 77 and 22%, respectively, than that measured 21 days seedling growth, with exception of some genotypes, whereas mean starch concentration in roots was increased by 3.6%. Highest increase was exhibited by some genotypes such as Pipanfary Red2, FR13A (Table 2).

Under complete submergence, mean soluble sugar concentration in roots was increased by 41%. Regarding individual genotypes, BactingArri showed 32.2% decrease in mean soluble sugar concentration, while Somboy registered the highest (69%) increase in mean soluble sugar among genotypes followed by Masury1 (66.3%) and the tolerant check FR13A (63.5%). Mean soluble sugar in stems was reduced under complete submergence by 57.3%.

However, Masury1 achieved an increase by (16.1%). The greatest (88.2%) reduction in stem soluble sugar concentration was registered for Pipanfary Red1 and the least (4.5%) was recorded for Pipanfary. Mean soluble sugar in leaves was reduced by 80.5%, highest (88.7%) reduction in mean soluble sugar concentration was registered for Pipanfary Red1 and least (61.0%) reduction

Table 3. Variation in 20 rice genotypes in starch concentrations in roots stems and leaves after 11 days of complete submergence under control and submerged conditions.

Genotypes	21 days after sowing			32 days after sowing					
	Control			Control	Submerged	Control	Submerged	Control	Submerged
	Roots	Stem	Leaves	Roots		Stem		Leaves	
NBGS1	0.32	0.95	0.29	0.28	0.25	1.31	0.39	0.38	0.61
NBGS2	0.38	0.98	0.36	0.24	0.27	0.98	0.45	0.31	0.58
NBGS3	0.37	2.04	0.61	0.18	0.28	1.05	0.44	0.36	0.79
NBGA	0.31	0.94	0.57	0.19	0.32	1.15	0.37	0.34	0.70
BG400-1	0.29	1.19	0.36	0.77	0.24	2.38	0.36	0.48	0.80
BG90-2	0.31	1.08	0.34	0.30	0.24	0.72	0.42	0.28	0.82
Banban	0.29	0.64	0.74	0.16	0.26	1.00	0.40	0.34	0.87
Masury1	0.33	0.91	0.32	0.17	0.26	0.61	0.37	0.27	0.85
Pipanfary Red1	0.28	1.33	0.60	0.18	0.23	1.29	0.33	0.43	1.11
JaingArri Red	0.2	0.66	0.29	0.16	0.23	0.91	0.37	0.32	0.59
Sommboy	0.25	1.71	0.37	0.18	0.27	1.02	0.45	0.38	0.78
Comarwa	0.24	0.61	0.26	0.27	0.26	0.78	0.36	0.22	0.62
Painjla	0.19	0.60	0.26	0.25	0.26	0.69	0.43	0.30	0.74
Pipanfary	0.29	0.75	0.28	0.19	0.23	0.60	0.63	0.37	0.61
Tagmizedo	0.3	0.98	0.33	0.19	0.15	0.99	0.50	0.36	0.70
Pipanfary Red2	0.26	0.90	0.47	0.18	0.26	1.09	0.36	0.32	0.62
BactingArri	0.21	1.35	0.92	0.34	0.28	1.10	0.48	0.33	1.11
Masury2	0.21	1.14	0.41	0.21	0.29	0.84	0.36	0.35	0.61
FR13A	0.32	1.80	0.61	0.67	0.23	3.15	0.75	0.32	0.54
IR42	0.28	1.27	0.49	0.65	0.21	2.2	0.41	0.38	0.60
Mean	0.28	1.09	0.44	0.29	0.25	1.93	0.43	0.34	0.73
CV (%)	19.10	36.84	40.60	64.06	14.03	54.45	23.60	16.88	22.30
SE±	0.00	0.01	0.01	0.00	0.00	0.02	0.01	0.00	0.01
LSD (P≤0.01).	0.01	0.04	0.02	0.01	0.01	0.04	0.01	0.01	0.02

Source: Authors

was observed for BactingArri (Table 2). The results suggest the effects of flooding on conversion of soluble sugars into starch. These findings are in agreement with those of Samanta et al. (2020) who reported on the notable reduction in the soluble sugar content in plants under submerged condition in comparison to those under control conditions and that tolerant genotypes showed less reduction in their sugar content than sensitive ones.

Mean starch concentration in roots was reduced by 13.8% under complete submerged condition than under controlled condition. It is clear from these results that soluble sugar concentrations were higher than starch concentrations in roots, stems and leaves under controlled and submerged conditions as well as the higher increase in soluble sugar might indicate higher rates of solubilization of structural carbohydrates supply during submergence (Samanta et al., 2020).

Mean starch concentration in stems was reduced by 77.7%. However, Pipanfary, registered an increase of 5%, the highest (81.4%) reduction was recorded for IR42 and least (37.7) reduction was by Painjla. Mean starch concentration in leaves was increased by 115% for all

genotypes with Pipanfary Red2 registering the highest (236%) increase and IR42 the least (57.9%) increase (Table 3). High rates of soluble sugars are necessary for elongation during submergence period and insoluble sugar is used for maintenance of structural build-up during recovery period, thus initially higher content of soluble and insoluble sugars can be regarded as an advantage for submergence tolerance (Sayani et al., 2017).

Under submerged condition, the increase in mean soluble sugar concentration in roots and reduction in mean starch concentration, suggest that the genotypes used some stored starch for the growth of roots under complete submerged condition, in which tolerant genotypes, revealed lower soluble sugar and starch concentrations.

The reduction in mean soluble sugar concentration and starch concentration in the stems indicate that tolerant genotypes obtained higher soluble sugar and starch concentrations than the intolerant ones as the reduction of photosynthetic function caused by changes in plant photosynthetic pigments under adverse conditions was

Table 4. Variation in 20 rice genotypes in chlorophyll concentrations after 11 days of complete submergence under control and submerged conditions.

Genotype	21 days after sowing			32 days after sowing						
	Co		a + b	Co	Sub	Co		Sub	Co	Sub
	a	b		a	b	a	b	a + b		
BGS1	1.02	0.35	1.38	1.13	0.19	0.61	0.07	1.74	0.26	
NBGS2	1.13	0.39	1.52	0.89	0.29	0.63	0.08	1.52	0.37	
NBGS3	0.91	0.32	1.23	1.49	0.18	0.58	0.08	2.07	0.26	
NBGA	0.97	0.38	1.35	1.57	0.17	0.61	0.08	2.18	0.25	
BG400-1	1.12	0.39	1.51	1.39	0.18	0.52	0.09	1.91	0.27	
BG90-2	1.31	0.46	1.77	1.64	0.23	0.60	0.10	2.23	0.33	
Banban	1.09	0.37	1.46	1.34	0.21	0.48	0.05	1.82	0.26	
Masury1	1.26	0.47	1.73	1.10	0.36	0.38	0.09	1.48	0.45	
Pipanfary Red1	1.11	0.40	1.51	1.13	0.21	0.38	0.08	1.51	0.29	
JaingArri Red	1.27	0.44	1.71	1.48	0.20	0.53	0.10	2.02	0.30	
Sommboy	1.08	0.36	1.45	1.27	0.17	0.43	0.04	1.70	0.21	
Comarwa	0.96	0.31	1.28	1.45	0.16	0.53	0.04	1.97	0.20	
Painjla	0.77	0.28	1.05	1.20	0.16	0.42	0.06	1.62	0.21	
Pipanfary	1.12	0.34	1.46	1.13	0.27	0.39	0.10	1.52	0.37	
Tagmizedo	0.68	0.23	0.91	1.27	0.12	0.46	0.05	1.73	0.18	
Pipanfary Red2	1.10	0.36	1.47	1.39	0.22	0.49	0.13	1.88	0.35	
BactingArri	1.28	0.48	1.76	1.09	0.19	0.37	0.08	1.46	0.27	
Masury2	1.25	0.45	1.69	1.09	0.11	0.42	0.05	1.74	0.16	
FR13A	1.06	0.34	1.40	0.80	0.41	0.31	0.19	1.11	0.60	
IR42	0.90	0.35	1.25	1.06	0.23	0.41	0.12	1.48	0.35	
Mean	1.07	0.37	1.44	1.24	0.21	0.48	0.08	1.73	0.30	
CV (%)	15.85	15.94	19.62	17.81	34.07	19.62	42.66	16.61	34.35	
SE±	0.01	0.01	0.02	0.01	0.00	0.01	0.00	0.02	0.00	
LSD	0.04	0.02	0.05	0.04	0.01	0.02	0.00	0.06	0.01	

Co= control, Sub= submerged.

Source: Authors

found to affect the production of carbohydrates and plants survival (Su et al., 2022).

In leaves, the high reduction in mean soluble sugar concentration and high increase in mean starch concentrations for all genotypes, might indicate that the genotypes conserved their starch to be used by the plant for recovery after de-submergence. The higher reserved carbohydrate in the tolerant varieties might assist in a faster restart of their normal growth after desubmergence (Pramanik et al., 2016).

Data in Table 4 show highly significant ($P \leq 0.01$) differences between the genotypes for chlorophyll a, b and total chlorophyll (a+b) content under control and submerged conditions. These results are in contrast with those of Panda and Sarkar (2014) who reported non-significant effects of chlorophyll concentrations under control condition.

Under control condition, chlorophyll concentrations in plant leaves were increased with time among the genotypes. Chlorophyll a, chlorophyll b and total chlorophyll (a+b) concentrations measured after 32 days

of seedling growth were higher by 15.8, 29.7 and 20.1%, respectively, than those measured after 21 days of seedling growth, with exception of some genotypes (Table 4). The highest chlorophyll a and total chlorophyll (a+b) concentrations were observed for genotype BG90-2, whereas the highest chlorophyll b concentration was registered by genotype NBGS2 and the lowest chlorophyll a, chlorophyll b and total chlorophyll (a+b) concentrations, were obtained for the tolerant cultivar FR13A.

Submergence reduced chlorophyll a, chlorophyll b and total chlorophyll (a+b) concentrations among the genotypes by 83, 83.3 and 82.7% respectively. This reduction had most effect on intolerant genotypes as compared to the tolerant ones. Similar findings were reported by Nurrahma et al. (2021b) who found that submergence lowered concentrations of chlorophyll by 72.4% versus control in a sensitive genotype as compared to only 14.4% reduction in a tolerant genotype. Tolerant genotypes are able to maintain vital processes, principally photosynthesis, minimizing the detrimental

effects of submergence stress (Nurrahma et al., 2021b).

In this study, the highest chlorophyll *a*, chlorophyll *b* and total chlorophyll (*a+b*) concentrations, were achieved by the tolerant check FR13A and the lowest chlorophyll *a* was shown by Masury2.

While, the lowest chlorophyll *b* was obtained for accessions Sommboy and Comarwa, and lowest total chlorophyll concentration (*a+b*), was registered by accession Masury2 (Table 4).

Leaf photosynthesis is one of the earliest plant responses to submergence, which resulted in significant reduction of chlorophyll *a*, chlorophyll *b* and total chlorophyll (*a+b*) after 11 days submergence in both sensitive and tolerant genotypes, in which the intolerant genotypes were the most affected ones. On the other hand, the tolerant genotype FR13A and the Sudanese genotypes (Masury 1, Pipanfary Red1) which showed survival after submergence for 11 days, maintained significantly higher values of chlorophyll *a*, chlorophyll *b* and total chlorophyll (*a+b*). This shows that genotypes with higher chlorophyll concentrations during and after submergence have the highest survival. Su et al. (2022) reported that chlorophyll is the medium for absorption, transformation and light energy transmission in plants, therefore under submergence plants with higher chlorophyll content have better chances of carbohydrates production and plants survival and concluded that the level of chlorophyll content can be used to evaluate the potential of dry matter production and plant survival.

Conclusion

From the results of this study, it could be concluded that submergence tolerant genotypes such as Pipanfary Red2 and Sommboy had higher soluble sugar and starch concentrations and chlorophyll content, required for plant survival under stress and recovery afterwards. These genotypes could be developed to be grown under rain-fed lowland flooded areas of Sudan and South Sudan.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Molecular characterization of banana genotypes by SSR markers

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Banana and plantain are monocotyledonous herbs in the genus *Musa* (*Musa* spp.) and grown globally due to their diverse uses as export income, food and fiber. Banana is also grown widely in Ethiopia; however, the crop attracted very limited research attention and has little genetic information available. Therefore, the main objective of this study was to assess the genetic diversity and population structure of banana genotypes using 14 SSR markers. The genetic diversity of 96 banana genotypes obtained from Melkasa Agricultural Research center was analyzed using 14 SSR markers. A total of 187 alleles were identified, and the number of alleles per marker (N_a) ranged from 6 to 21 with an average of 13.36. The range of polymorphic information content per marker was 0.52 to 0.93, with an average of 0.82. The results of phylogenetic analysis, principal coordinate analysis, and structure analysis showed an admixture of the populations indicating that the genotype grouping pattern did not exactly correspond to the genotypes' breeding history and genome composition. However, Clusters I to III from the phylogenetic analysis and $K=3$ from the population structure analysis confirmed the existence of 3 major groups among the genotypes as a whole. Analysis of molecular variance (AMOVA) revealed the presence of higher genetic variation within the population than between the population. Generally, genetic diversity and population structure obtained from this study provide inputs for the improvement of the crop.

Key words: Alleles, banana, genetic diversity, *Musa* spp., polymorphic information content, SSR markers.

INTRODUCTION

Banana and plantains are monocotyledonous herbs in the genus *Musa* (*Musa* spp.) which originated in Southeast Asia and the western Pacific (Langhe et al., 2009). They are year-round, perennial fruit crops with a rapid rate of growth that are commonly grown throughout

the world's tropics and subtropics. After cassava, maize, and yams, bananas and plantains are rated the fourth most important crop in sub-Saharan Africa (SSA) (Igwe et al., 2022). The crop makes a considerable contribution to the export revenue and food security of these regions,

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and other banana plant parts are used locally for food and fiber. The annual global banana production was 116 million tons in 2017 to 2019, with an estimated value of USD 31 billion (FAO, 2020).

Different genotypes, including diploids (AA, AB and BB), triploids (AAA, AAB and ABB), and tetraploids (AAAA, AAAB, AABB and ABBB), were generated from *Musa acuminata* (AA) and *Musa balbisiana* (BB). The different ploidy levels and chromosome numbers of bananas have led to complexity in taxonomy and are associated with parthenocarpy, leading to female sterility, seedless fruit, and non-viable seeds (De Jesus et al., 2013). The majority of edible genotypes grown by farmers worldwide are triploids ($2n = 3x = 33$ chromosomes), and they are categorized into three main groups: AAA (Cavendish or dessert bananas), ABB (cooking bananas), and AAB (plantain). The plantain subgroup (AAB), is primarily cultivated by small farmers for local use and is crucial to the agriculture of tropical humid forest regions in Africa, Central and South America, and Asia (Noyer et al., 2005; Okeh et al., 2022).

Ethiopia, which is located in the tropics, has great potential for banana production (Gebre et al., 2022). The most widely grown and consumed fruit in the country is the Cavendish or dessert banana. It has a significant socio-economic role in the general well-being of rural communities, including food security and income generation, especially in the south and southwest of the country (Gebre et al., 2022). Banana production contributes around 47.83% of producers' consumption, specifically, about 49.19% for income generation, 0.47% for animal feed and 2.52% for other purposes (CSA, 2015; Gebre et al., 2022). It covers about 59.64% (53,956.16 ha) of the total fruit area, about 68.00% (478,251.04 tones) of the total fruits produced, and about 38.30% (2,574,035) of the total fruit-producing farmers in the country (CSA, 2015; Gebre et al., 2022). However, the production of this crop has become susceptible to both abiotic and biotic factors depending on their genomic constitutions. It has been discovered that genotypes with the "B" genome (specifically the ABB type) are more resistant to abiotic and biotic stress than those with only the "A" genome (Igwe et al., 2022). This imposes the need for genotype testing and crop improvement supported by the conservation of characterized banana genotypes. The global population is rapidly increasing, with over 9 billion people predicted by 2050 (Ehrlich and Harte, 2015). Feeding this excessively growing population is generating a lot of pressure on agricultural crop production. Therefore, knowing the plant's genetic background is crucial for developing high-yielding and pest resistance genotypes. Assessing variability among available genotypes is a crucial task for identifying variation with useful traits for crop improvement and conservation programs. These characterizations can be achieved using conventional methods based on morphological traits, and the use of

molecular markers. However, molecular methods provide precise genetic information based on the advancement and use of technologies used to identify genetic variability present in the genotypes, offering information about the variability found at the DNA level and enabling the genetic differentiation among individuals (Christelová et al., 2017). Due to their multi-allelic nature, high reproducibility, and co-dominant inheritance, microsatellites or simple sequence repeats (SSRs) are among the most useful PCR-based DNA markers in population genetics and germplasm characterization studies (Powell et al., 1996).

So far, several scientific research have been conducted on genetic diversity and genotype selections of bananas in various countries (Opara et al., 2010; De Jesus et al., 2013; Christelová et al., 2017; Paofa et al., 2018; Marian et al., 2018; Biswas et al., 2020). However, in Ethiopia, the crop attracted very limited research attention and there is little genetic information available. Therefore, the purpose of this study was to assess the genetic diversity and population structure analysis of banana genotypes using SSR markers and identify diverse genotypes potentially useful for future breeding programs.

MATERIALS AND METHODS

Plant materials and DNA extraction

A total of 96 genotypes obtained from Melkasa Agricultural Research Center (MARC) including improved variety, advanced clones, and landrace were used in this experiment (Table 1). Young leaves were taken from vegetatively propagated plants of each 96 individuals maintained at MARC. The collected fresh leaves were placed in 2 ml autoclaved and labeled Eppendorf tubes and frozen for 24 h at -20°C . After 24 h the leaves were further dried in a freeze dryer (John Morris group) for 24 h and then grounded using Geno Grinder (MM-200, Retsch) for 3 min. Genomic DNA was extracted in two replications using plant DNA extraction protocol based on the method of Diversity Array Technology (DArT, 2019) with some minor modifications. The qualification and quantification of genomic DNA were done using a Nanodrop spectrophotometer (ND-8000, Thermo Scientific). The level of quality was further assessed by running the genomic DNA on 1% agarose gel in TAE buffer using a standard lambda DNA as a reference band. A gel documentation system (Bio Doc-IT Imaging system) was used to visualize the DNA bands under UV light (Cambridge, UK). Samples with high band intensity, purity, and lesser smear were selected and normalized to 50 ng/ μl for further PCR analysis.

Primer selection and PCR optimization

A total of 20 lyophilized primer pairs were reconstituted using nuclease-free water to obtain 100 μM stock solutions. All primers were diluted to a working concentration of 10 μM and finally stored at 20°C . Applicability of each primer was optimized using the "gradient pcr" methodology. PCR based on reliability, polymorphism, and specificity to the target region, 14 SSR markers were selected (Table 2) for final analysis out of the 20 tested primers.

Table 1. List of 96 banana genotypes used in this study.

No.	Genotype	Level of advancement	Genome group	Banana type
1	Americani	Improved variety	AAA	Dessert banana
2	Dinke-2	Landrace accession	AAA	Dessert banana
3	Dwarf Cavendish	Improved variety	AAA	Dessert banana
4	Green Red	Improved variety	AAA	Dessert banana
5	Poyo	Improved variety	AAA	Dessert banana
6	Williams-1	Improved variety	AAA	Dessert banana
7	Ducasse Hybrid	Improved variety	ABB	Cooking banana
8	Butuza	Improved variety	AAA	Dessert banana
9	Robusta	Improved variety	AAA	Dessert banana
10	Grande Naine	Improved variety	AAA	Dessert banana
11	Giant Cavendish	Improved variety	AAA	Dessert banana
12	Red	Improved variety	AAA	Dessert banana
13	Silk	Improved variety	AAB	Plantain banana
14	Wondo genet-2	Landrace accession	AAA	Dessert banana
15	Matoke	Improved variety	AAA	Dessert banana
16	Wondo genet-1	Landrace accession	AAA	Dessert banana
17	Kitawira	Improved variety	AAA	Dessert banana
18	Kibungo-1	Improved variety	----	Unknown
19	Cardaba	Improved variety	ABB	Cooking banana
20	Saba	Improved variety	ABB	Cooking banana
21	Bluggoe	Improved variety	ABB	Cooking banana
22	Pelipita	Improved variety	ABB	Cooking banana
23	Prata	Improved variety	AAB	Plantain banana
24	Cubian Red	Advanced clones	AAA	Dessert banana
25	Gros Michel	Advanced clones	AAA	Dessert banana
26	Giant Parfitt (ITC1246)	Advanced clones	-----	Unknown
27	NamO ITC1303	Advanced clones	-----	Unknown
28	NtebwaO ITC1461	Advanced clones	-----	Unknown
29	Chinese Cavendish ITC0547	Advanced clones	AAA	Dessert banana
30	Ice cream	Advanced clones	ABB	Cooking banana
31	Thai (Aka kluay Khay)	Advanced clones	-----	Unknown
32	Pisan Umbuk ITC30686	Advanced clones	-----	Unknown
33	FHIA-25 ITC1418	Advanced clones	AAB	Plantain banana
34	SuuO ITC1462	Advanced clones	----	Unknown
35	Nante ITC1353	Advanced clones	----	Unknown
36	Veimama ITC0576	Advanced clones	----	Unknown
37	FHIA#18 hybrids	Advanced clones	AAAB	Unknown
38	Lakika	Advanced clones	----	Unknown
39	Ibwi ITC1465	Advanced clones	----	Unknown
40	Plantain or Cooking made size	Advanced clones	AAB	Plantain banana
41	FHIA-23 ITC1265	Advanced clones	AAAA	Unknown
42	Fai palagi ITC1059	Advanced clones	----	Unknown
43	Naine de China ITC0178	Advanced clones	----	Unknown
44	Suce (French for Sugar)	Advanced clones	AA	Unknown
45	Champa Nasik	Improved variety	----	Unknown
46	Meraro	Improved variety	AAA	Dessert banana
47	Ikimaga	Improved variety	AAA	Dessert banana
48	Borocemsa	Improved variety	ABB	Cooking banana
49	Ginir-1	Landrace accession	AAA	Dessert banana
50	Nijuru	Improved variety	AAA	Dessert banana
51	Kitawira	Improved variety	AAA	Dessert banana

Table 1. Contd.

52	Chibul Angombe	Improved variety	AAA	Dessert banana
53	Matoke	Improved variety	AAA	Dessert banana
54	Ghana Cooking	Advanced clones	AAB	Plantain banana
55	Pisang Raja	Improved variety	AAB	Plantain banana
56	Ambowoha Selle-1	Landrace accession	----	Unknown
57	Giant Cavendish	Improved variety	AAA	Dessert banana
58	Grande Naine	Improved variety	AAA	Dessert banana
59	Robusta	Improved variety	AAA	Dessert banana
60	Ambowoha Selle-2	Landrace accession	AAA	Dessert banana
61	Williams-2	Improved variety	AAA	Dessert banana
62	Dinke-1	Improved variety	AAA	Dessert banana
63	Poyo	Improved variety	AAA	Dessert banana
64	Kamara Masenge	Improved variety	AAB	Plantain banana
65	Dwarf Cavendish	Improved variety	AAA	Dessert banana
66	Chinese Dwarf	Improved variety	AAA	Dessert banana
67	Horn	Improved variety	AAB	Plantain banana
68	Ambo-3	Landrace accession	AAA	Dessert banana
69	Lady Finger	Improved variety	AAB	Plantain banana
70	Paracido Alrey	Improved variety	----	Unknown
71	Ambo-2	Landrace accession	AAA	Dessert banana
72	Ambowoha Selle-3	Landrace accession	AAA	Dessert banana
73	Williams-1	Improved variety	AAA	Dessert banana
74	Ducasse Hybrid	Improved variety	ABB	Cooking banana
75	Butuza	Improved variety	AAA	Dessert banana
76	Williams Hybrid	Improved variety	AAA	Dessert banana
77	Giner-2	Landrace accession	AAA	Dessert banana
78	Lacatan	Improved variety	AAA	Dessert banana
79	Uganda Red	Improved variety	----	Unknown
80	Pisang sri	Improved variety	----	Unknown
81	Gittity	Improved variety	----	Unknown
82	Wondo gent-3	Landrace accession	ABB	Cooking banana
83	Wondo gent-4	Landrace accession	AAA	Dessert banana
84	Nijuru	Improved variety	AAA	Dessert banana
85	Imbogo	Improved variety	----	Unknown
86	Cardaba	Improved variety	ABB	Cooking banana
87	Kenya-1	Landrace accession	AAA	Dessert banana
88	Cachaco	Improved variety	ABB	Dessert banana
89	Bodles Altafort	Improved variety	AAAA	Unknown
90	Suce Sugar	Advanced clones	AA	Unknown
91	Dwarf parfitt ITC0548	Advanced clones	----	Unknown
92	FHIA-17 ITC1264	Advanced clones	AAAA	Unknown
93	Ntindii ITC1464	Advanced clones	-----	Unknown
94	Cuban Yellow	Advanced clones	-----	Unknown
95	Cocos ITC0451	Advanced clones	-----	Unknown
96	Kitarasa ITC1451	Advanced clones	-----	Unknown

Source: Authors

Genotyping (whole sample amplification)

The GeneAmp®PCR System 9700 thermal cycler was used to conduct the PCR reaction in a total of 12.5 µl volume. Briefly, the recipe contains 6.25 µl one Taq 2x Master Mix (M04821) Biolabs

England, with standard buffer (which contains all PCR reaction components, MgCl₂, PCR buffer, dNTPs and Taq DNA polymerase), 0.5 µl forward primer, 0.5 µl reverse primer, 0.25 µl DMSO, 3 µl nuclease-free water and 2 µl genomic DNA. The PCR was programmed with an initial denaturation phase of 3 min at

Table 2. List of SSR markers, primer sequences, Repeat motives and their annealing temperatures.

Marker	Primer's sequence (5' - 3')	Repeat motives	Annealing temp. (°C)	Expected size	Reference
AGMI101/102	F: TGCAGTTGACAAACCCACACA R: TTGGGAAGGAAAATAAGAAGATAGA	(GA)3	54.6	190-250*	Kaemmer et al., 1997
Ma1/24	F: GAGCCCATTAAGCTGAACA R: CCGACAGTCAACATAACAATACA	(CT)13	56.7	170-250*	Crouch et al., 1998
Ma1/27	F: TGAATCCCAAGTTTGGTCAAG R: CAAAACACTGTCCCCATCTC	(GA)9	51.7	120-185*	Crouch et al., 1998
Ma3/103	F: TCGCCTCTCTTTAGCTCTG R: TGTTGGAGGATCTGAGATTG	(CT)10	56.7	135-165*	Crouch et al., 1998
AGMI103/104	F: ACAGAATCGCTAACCCCTAATCCTCA R: CCCTTTGCGTGCCCCCTAA	(GAGAAA)3GATGA(GAA)2	57.9	150-260	Kaemmer et al., 1997
AGMI95/96	F: ACTTATTCCTCCGCACTCAA R: ACTCTCGCCCATCTTCATCC	(TC)6N24 (TC)7	58.8	263-275	Kaemmer et al., 1997
AGMI93/94	F: AACAACTAGGATGGTAATGTGTGGAA R: GATCTGAGGATGGTTCTGTTGGAGTG	(GA)13	58	152-176	Kaemmer et al., 1997
AGMI187/188	F: GCAACTTTGGCAGCATTTT R: TGATGGACTCATGTGTACCTACTAT	(CT)12	50.8	303-306	Kaemmer et al., 1997
AGMI105/108	F: TCCCAACCCCTGCAACCACT R: ATGACCTGTGCAACATCCTTT	(GA)16N76	54.6	285-299	Kaemmer et al., 1997
Ma1/17	F: AGGCGGGGAATCGGTAGA R: GGC GGGAGACAGATGGAGT	(GA)14	58	280-375*	Crouch et al., 1998
MaOCEN13	F: GCTGCTATTTTGTCTTGGTG R: CTTGATGCTGGGATTCTGG	(TC)16	50.2	141-200	Creste et al., 2006
MaOCEN1	F: TCTCAGGAAGGGCAACAATC R: GGACCAAAGGGAAAGAAACC	(CT)17	58	210-250	Creste et al., 2006
MaOCEN3	F: GGAGGAAATGGAGGTCAACA R: TTCGGGATAGGAGGAGGAG	(GA)10	60	180-250	Creste et al., 2006
AGMI125/126	F: TTAAAGGTGGGTTAGCATTAGG R: TTTGATGTCACAATGGTGTCC	(GA)20	57.2	360-372	Kaemmer et al., 1997

*Those fragment size information are obtained from the present study while the rest are from literature sources.

Source: Authors

94°C, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 50.2-60°C depending on the primers for 1 min, and elongation at 72°C for 2 min, with a final

elongation at 72°C for 10 min, and a holding step at 4°C.

PCR products were loaded on a 3% percent agarose gel (w/v) using a 6x loading dye that contained gel red.

Electrophoresis was carried out in 1 TAE buffer, for three h at 100 constant volts. The gel was stained with gel red and a BioDoc-it imaging device (Cambridge, UK) was used to

Table 3. Summary of various diversity parameters for 96 banana genotypes using 14 SSR markers.

Marker	MAF	GD	PIC	Na	I	Ne	Ho	He	PHWE	F
AGMI101/102	0.37	0.77	0.74	6.00	1.471	4.003	0.000	0.749	0.000***	1.000
Ma1/24	0.30	0.86	0.85	14.00	1.841	5.622	0.022	0.791	0.000***	0.970
Ma1/27	0.27	0.84	0.82	11.00	1.722	4.926	0.067	0.783	0.000***	0.919
Ma3/Ma3	0.23	0.83	0.80	7.00	1.453	3.811	0.000	0.727	0.000***	1.000
AGMI103/104	0.55	0.59	0.52	6.00	0.995	2.304	0.006	0.561	0.000***	0.990
AGMI95/96	0.15	0.91	0.90	15.00	2.202	8.053	0.000	0.872	0.000***	1.000
AGMI93/94	0.16	0.89	0.88	13.00	1.995	6.607	0.000	0.837	0.000***	1.000
AGMI187/188	0.21	0.91	0.90	19.00	2.237	8.134	0.000	0.870	0.000***	1.000
AGMI105/108	0.33	0.84	0.83	17.00	2.022	5.906	0.000	0.826	0.000***	1.000
Ma1/17	0.35	0.79	0.77	9.00	1.563	4.255	0.000	0.739	0.000***	1.000
MaOCEN13	0.36	0.81	0.80	13.00	1.844	5.144	0.000	0.800	0.000***	1.000
MaOCEN1F	0.19	0.91	0.90	19.00	2.161	7.392	0.000	0.856	0.000***	1.000
MaOCEN3	0.34	0.84	0.82	17.00	1.843	5.122	0.035	0.801	0.000***	0.956
AGMI125/126	0.12	0.93	0.93	21.00	2.341	9.236	0.035	0.887	0.000***	0.960
Mean	0.28	0.84	0.82	13.36	1.835	5.751	0.012	0.793	-	0.985

Where MAF = Major allele frequency, GD= Gene diversity, PIC = Polymorphic information content, Na= Number of alleles, I = Shannon's Information Index, Ne = Effective number of alleles, Ho = Observed heterozygosity, He = Expected heterozygosity, F = Fixation Index, PHWE = *P*-value for deviation from Hardy Weinberg Equilibrium, ns = not significant, *** = *P* < 0.0001 and hence highly significant.

Source: Authors

view the gel image under UV light. A 100 base pair SMO BIO DNA ladder was used as a molecular ruler to compare DNA bands and estimate the sizes of the DNA fragments.

Data scoring and analysis

The PyElph 1.4 image analyzer software tool was used to score the molecular weights of clearly resolved bands amplified by the SSR markers (Pavel and Vasile, 2012). The software power marker ver. 3.5 was used to calculate the number of alleles (Na), major allele frequency (MAF), and the polymorphic information content (PIC) (Liu and Muse, 2005). The number of effective alleles (Ne), observed (Ho), and expected (He) heterozygosity, Shannon's information index (I), gene flow (Nm), Wright's fixation index (FST, FIS, FIT) were computed with GenAlEx ver. 6.502 software (White and Peakall, 2015). Cluster analysis was performed using DARwin software version 6.0 (Perrier and Jacquemoud-Collet, 2006).

A dendrogram was constructed using the dissimilarity matrix as input data to visualize the pattern of a cluster both within and between genotypes.

Patterns of genotype clustering were examined using the Unweighted Neighbor-Joining (NJ) method (Saitou and Nei, 1987). Principal coordinate analysis (PCoA) was carried out using DARwin software version 6.0 to examine the distribution of variance among samples and determine the power of ordination. (Perrier and Jean-Claude Collet, 2006)

The population genetic structure was computed using the Bayesian statistical model calculated using the Structure software version 2.3.4 (Pritchard et al., 2000). A burn-in period of 100,000 and a run length of 200,000 for the Monte Carlo Markov chain (MCMC) with 20 iterations for each K were used to calculate the best likely number of population clusters (K-value). The web-based structure harvester version 0.6.92 (Earl and Von Holdt, 2012) was used to identify best probable K value according to Evanno et al. (2005). The labeling of each population cluster based on K value was determined using Clumpak beta (Kopelman et al., 2015). GenAlEx version 6.503 software (Peakall and Smouse, 2006) was

used to calculate the analysis of molecular variance (AMOVA) after the result of population structure analysis based on the value of K obtained.

RESULTS AND DISCUSSION

Gene diversity indices and marker polymorphism

The 14 SSR markers used to assess the genetic diversity of banana genotypes detected a total of 187 alleles with a mean of 13.36 alleles per locus (Table 3). The number of alleles per marker ranged from 6 to 21 with an average of 13.36 alleles. The lowest and the highest numbers of effective alleles were observed for markers AGMI103/104 (2.304) and AGMI125/126 (9.236) in the same order. The lowest value of Shannon's information (I) index was recorded for marker AGMI103/104 (0.99) while AGMI125/126 exhibited the highest value (2.341). The mean expected heterozygosity was 0.793 with a minimum of 0.561 (AGMI103/104) and a maximum of 0.887 (AGMI125/126). The observed heterozygosity has a range of 0.0 to 0.067, with a mean of 0.012. The current study found more alleles than the report by Jesus et al. (2013), which found 182 alleles in total, ranging in number from 7 to 15. On the other hand, Christelová et al. (2017) reported a higher mean number of alleles (21.5) for 695 Musa accessions using 19 SSR markers. A relatively higher number of alleles were observed in the present study compared to the previous reports indicating the existence of high genetic variation among the genotypes.

The markers in the current study showed higher

polymorphic information content (PIC), with values ranging from 0.52 (AGMI103/104) to 0.93 (AGMI125/126) and an average value of 0.82. The 14 SSR markers used in this study revealed an average PIC value greater than 0.5 indicating a high discriminating power of the analyzed loci. According to Botstein et al. (1980), a PIC value greater than 0.5 implies a locus with a high degree of polymorphism, whereas a value less than 0.25 indicates a locus with a lower degree of polymorphism. Christelová et al. (2017) found a similar result for 695 accessions using 19 SSR markers, with a PIC value ranging from 0.561 to 0.933 and a mean value of 0.789. Similarly, Changadeya et al. (2012), reported an average PIC value of 0.74 which is also lower than the result obtained in this study. The mean expected and observed heterozygosity, and effective alleles obtained in this study differed from the previous reports using SSR markers on bananas (Changadeya et al., 2012; De Jesus et al., 2013; Marian et al., 2018).

The difference in the number and type of genotypes used as well as the number and type of markers may be contributing factors to the variation in the level of allele number and heterozygosity. The study's findings regarding the number of alleles, the effective number of alleles, polymorphic information content (PIC), and expected heterozygosity showed that there was significant genetic diversity present as well as the high potential of the markers to be used in analyzing the genetic diversity of banana genotypes. For all markers, the observed heterozygosity showed low values to the expected heterozygosity, indicating a high level of homozygosity. The observed lower heterozygosity in banana genotypes might possibly be a result of minimal outcrossing.

The average gene diversity (0.84) and expected heterozygosity (0.79) detected among the 96 banana genotypes revealed high levels of variation within the studied banana genotypes.

Phylogenetic relationship

Phylogenetic analysis based on Unweighted Neighbor-joining (NJ) methods grouped the 96 banana genotypes into three major groups, by forming different hierarchical sub-groups (Figure 1). Clusters I, II, and III each included 54% (52 genotypes), 24% (23 genotypes), and 22% (21 genotypes) respectively, out of the total genotypes. The majority of genotypes from improved variety were found in the first cluster (37 improved out of 52 genotypes). In Cluster II, majority of the genotypes were from advanced clones. Cluster III mainly consisted of advanced clones and landrace accessions. The current study revealed that genotype grouping patterns are unrelated to breeding history (improved variety, advanced clones, and landrace). The genotypes were mixed up in all the clusters except for a few cases where clusters were

dominated by genotypes of a particular genome composition. The lack of clear clustering among banana genotypes based on breeding history could be attributable to a similar genetic basis that unites all genotypes. A high level of genetic similarity is expected among genotypes from the same sub-cluster because of common ancestry or mutations (Creste et al., 2003). Variations within each cluster in vegetatively propagated crops such as bananas are mainly determined by genotype and genome change caused by mutations whose frequency is determined by how many times a clone has been multiplied and planted (Changadeya et al., 2012).

Further analysis of the 96 genotypes of bananas resolved them into various distinct coordinates based on dessert, cooking, and plantains bananas. Most dessert bananas (AAA genome composition), almost all cooking bananas (ABB genome composition), and almost all plantain bananas (AAB genome composition) formed distinct sub-clusters based on genomic composition within the main cluster (Figure 2). Eight of the 9 cooking type genotypes (cardaba, Saba, Bluggoe, pelipita, Ice cream, Borocemsa, Wondo gent-3, and Cachaco), co-clustered together based on their genomic constitutions, while Ducasse Hybrid fell in a separate sub-cluster with other genotypes of AAA genome. Similar sub-clustering was noted in plantain genotypes such as silk, FHI A-25 ITC1418, plantain or cooking made size, Ghana cooking, Pisang Raja, and Horn that have AAB genomes. Three plantain banana genotypes such as Parata, Kamara Masenge and Lady Finger which have AAB genomes did not cluster with other known AAB genotypes. Similarly, most dessert banana genotypes in which the AAA genome is grouped based on their genomic constitutions. Some genotypes with unknown genomic constitutions grouped closely with the AAA genome, implying that they are members of the AAA genomic group. Furthermore, some genomic groups were successfully resolved, while others, such as those with mixed ploidy groups, were clustered together based on their genetic closeness to their progenitors, *M. acumminata* (A genome), and *M. balbisiana* (B genome). The ABB genomes for example Parata, Kamara Masenge, and Lady Finger were closely grouped, with the same relatedness found between AAA genome groups. The current findings agrees with those of Okeh et al. (2022) who used ISSR and SCoT markers, and Igwe et al. (2021) who used CDDP markers and found genotype clustering mainly based on the genomic constitution.

Principal coordinate analysis (PCoA)

The principal coordinate analysis (PCoA) generated by the genetic dissimilarity matrix explained 22.23% of the total genetic variation. The first, second, and third axis explained 8.08, 7.61 and 6.54% of the genetic variation, respectively. The two-dimensional plot of PCoA analysis

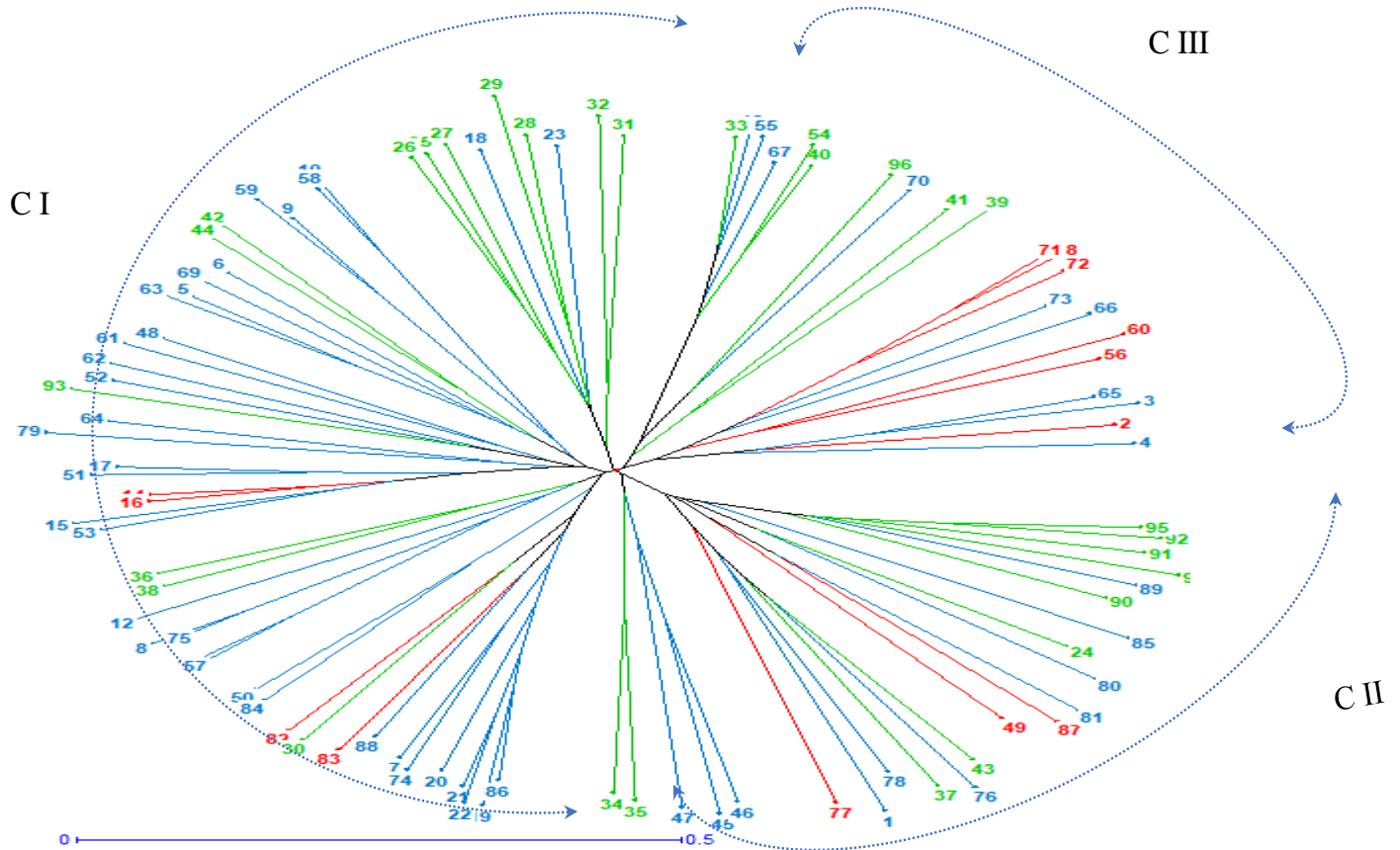


Figure 1. Genetic relationship of 96 banana genotypes by 14 SSR markers as resulted from unweighted neighbor-joining (NJ) analysis (Blue= Improved variety, Green= Advanced clones, and Red= Landrace accessions). Source: Authors

displayed in Figure 3 showed that the genotypes spread all over the plot in such a way that none of the populations (improved variety, advanced clones, and landraces) formed a distinct group. The overall PCoA analysis grouping pattern is consistent with the NJ cluster. In both analyses, there was no unique clustering of populations from priory information with the grouping patterns obtained in the results (Figures 1 to 3) confirming an admixed population. Hence pattern of genetic relationship and variation among the genotypes is not based on the anticipated priory information rather it is seems the result of actual difference in the genetic background of the genotypes studied.

Population structure

The model-based Bayesian algorithm allowed three clusters ($k = 3$) to be identified as the best fit for capturing the major structure in the entire data set. Based on the K value, the clumpak population structure bar plot revealed no clear grouping of populations based on breeding history and genomic background. The observed high

admixture structuring also agreed with the pattern of clustering in PCoA and phylogenetic analysis as far as the priory population information is concerned. This could be possible because of two speculative reasons: one is if genotypes in different populations are derived from the same parents, or if genotypes in a single population evolved from a different line of ancestry.

Analysis of molecular variance

Analysis of molecular variance (AMOVA) was conducted on the basis of the most likely number of grouping ($K=3$) as resulted from population structure analysis. The results revealed that variation within a cluster was accounting for higher variation (97%) than the variation among clusters (2%) (Figure 4) (Table 4), signifying variability of the genotypes within each groups. On the other hand, variability within individuals contributed a smaller proportion (1%) to the total variation both at priory population information level and after the result of population structure analysis ($K= 3$).

The study also revealed that there was little genetic

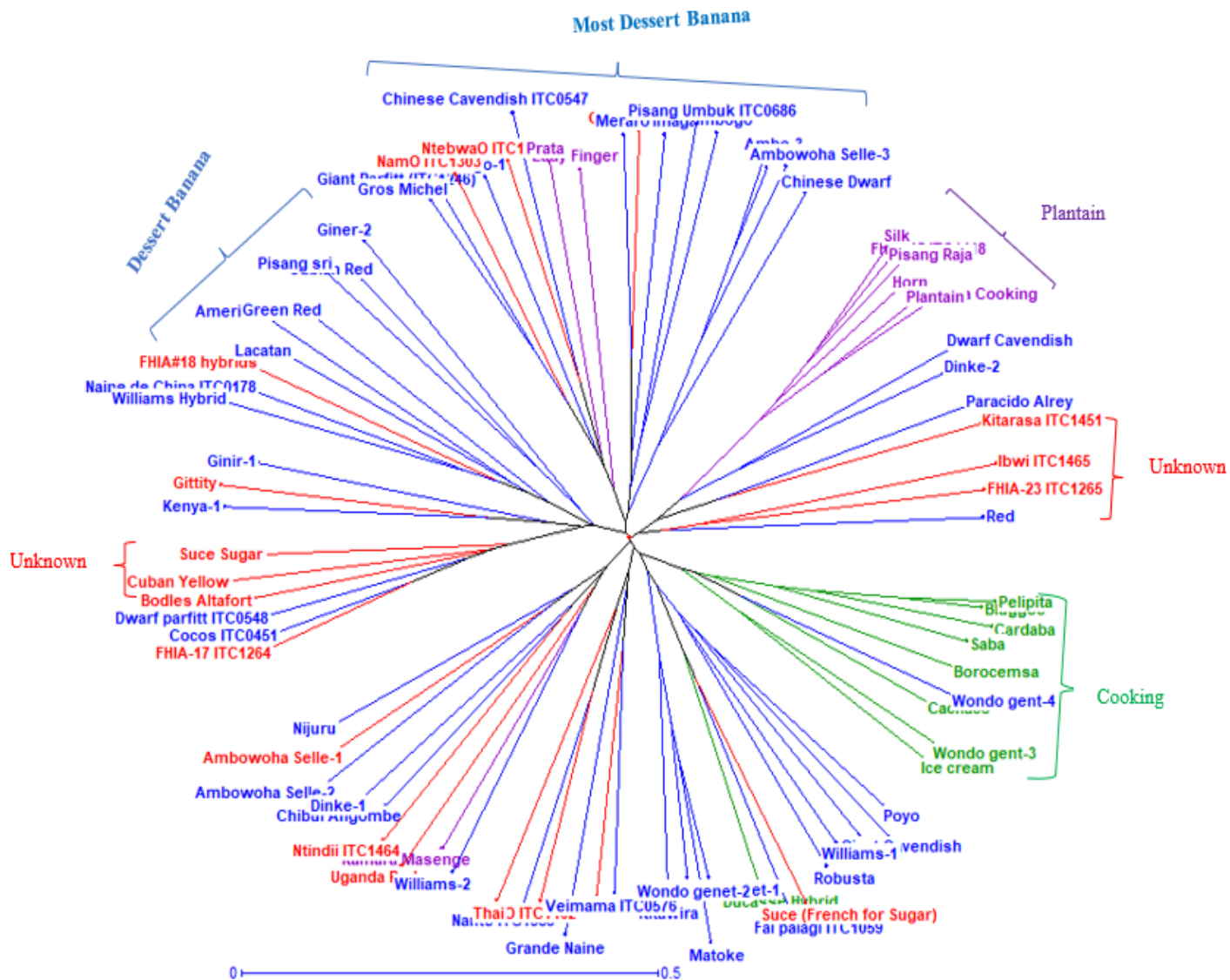


Figure 2. Genetic relationships of 96 banana genotypes by 14 SSR markers as resulted from Unweighted Neighbor-Joining (NJ) analysis and patterns grouping based on use type (Blue=Dessert banana; Green=Cooking banana; pink =plantain). Source: Authors

variation among populations of bananas ($F_{ST} = 0.023$). The magnitude among and within population differentiation was quantified using F-statistics (Fit, Fis and Fst) (Wright, 1951). Population differentiation due to genetic structure is quantified by the fixation index (Fst). Wright (1951) defined the Fst value range as follows: 0 to 0.05 = low, 0.05 to 0.15 = moderate, 0.15 to 0.25 = high, and greater than 0.25 = very large genetic differentiations.

Conclusion

Knowledge of distribution of genetic diversity, and relatedness among genotypes contributes significantly

towards crop improvement. In this study, the genetic diversity of 96 banana genotypes was assessed using 14 SSR markers. The various diversity indices generated showed that the SSR markers utilized in the present study were effective and informative for the banana diversity study. A high level of genetic variation was observed within genotypes, implying that the genotypes within clusters have huge usefulness in the improvement program. Furthermore, the limited sub-grouping patterns observed in relation to the various use types (Dessert, Cooking and Plantain) also confirms the association of the genome based classification with use type grouping. It is crucial to conduct more genetic variability studies using high-resolution markers and using a wider range of

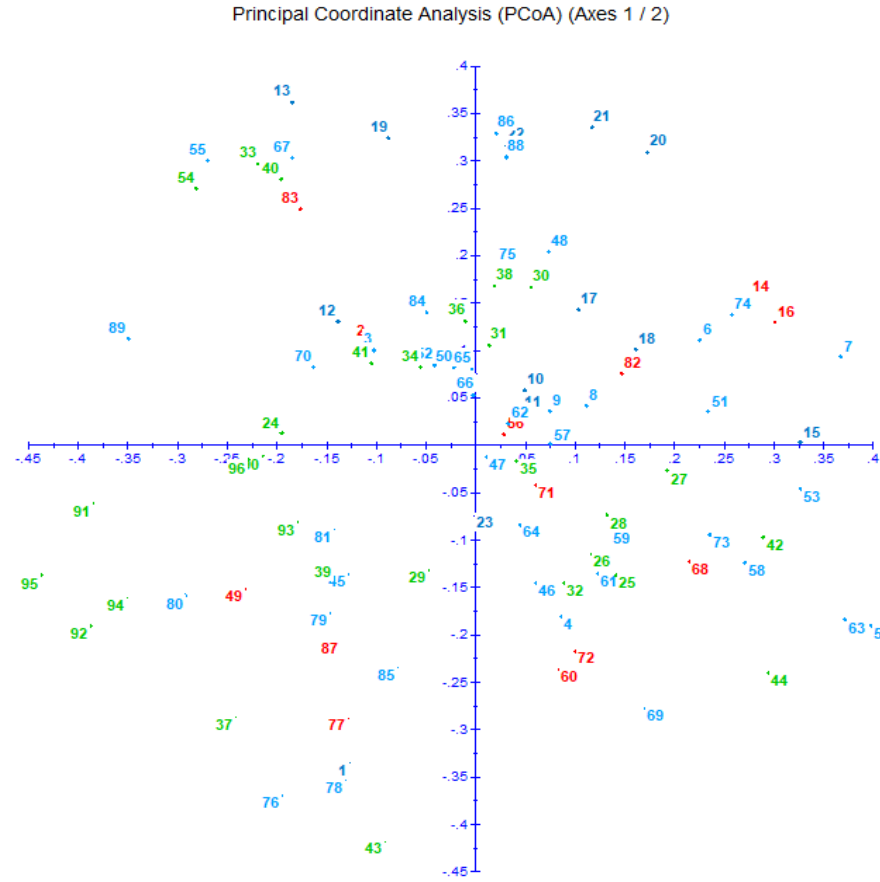


Figure 3. Principal coordinates analysis (PCoA) bi-plot showing the grouping pattern of 96 banana genotypes from three populations (Light blue = Improved varieties; Green=Advanced Clones; Red=Landraces).
Source: Authors

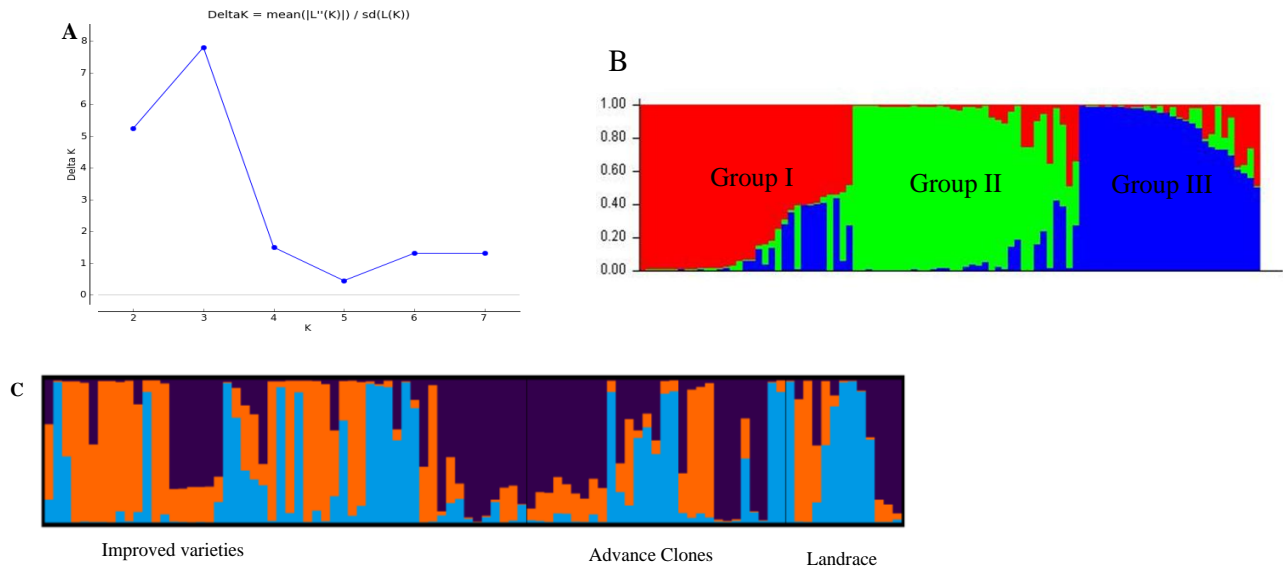


Figure 4. Population structure of 96 banana genotypes. (A) The highest peak at K=3 based on Evano et al. (2005); (B) The three major clusters of 96 genotypes; (C) Estimated population structure for K = 3 based on improved variety, advanced clone, and landrace.
Source: Authors

Table 4. Analysis of molecular variance of 96 banana genotypes after the result of population structure analysis (k= 3).

Source	DF	SS	MS	Est. Var.	% of variation	F-statistics	P value
Among population	2	40.597	20.298	0.140	2	0.023	0.001
Among individuals	93	1099.674	11.824	5.876	97	0.988	0.001
Within individuals	96	7.000	0.073	0.073	1	0.988	0.001
Total	191	1147.271		6.089	100		

DF = degree of freedom, SS=sum of squares, MS=mean squares.

Source: Authors

genotypes in the future to screen further variability and reveal any significant co-clustering patterns with some traits.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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