MARKER-ASSISTED SELECTION FOR HIGH-YIELDING AND DISEASE-RESISTANT TRAITS AMONG SELECTED NIGERIAN COCONUT (*Cocos nucifera* L.) ACCESSIONS

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: April 16, 2024 Accepted: June 28, 2024

Abstract: Agricultural growth in Nigeria is increasingly recognized to be central to sustained improvement in economic development, food security and poverty alleviation. However, climate change and its adverse effects such as drought have taken their tolls on the growth of plants and crop yield. Despite these climate conditions in Nigeria, underutilized crops like Coconut may still provide farmers with promising alternatives for enhancing nutrition, food security and income generation. This study utilized the marker assisted selection technique to select for high-yielding and disease resistant accessions among coconut (*Cocos nucifera* L.) germplasm from Nigeria Institute for Oil Palm Research (NIFOR). Ten Simple Sequence Repeat (SSR) markers, comprising of five high-yield and five disease-resistant associated markers. The study revealed a diverse combination of marker amplifications among sampled accession. However, only 10 of the coconut accessions showed all marker combinations, except for CNKGDEST123 which showed polymorphic amplifications among samples. One accession, 753 (NUR), showed an amplified band closest to the expected band size for CNKGDEST123 marker. Hence, this sample is recommended for breeding programmes that involves coconut high yield and disease resistant improvement.

Keywords: Accessions, Coconut, MAS, NIFOR, Polymorphic

Introduction

Coconut (*Cocos nucifera* L.) is a monocotyledonous Arecaceae (Palmaceae) family member and Arecoideae subfamily member (Wang et al., 2021). Coconut is often regarded as a tree of life due to its numerous purposes as a source of healthful drink, fibre, construction materials, charcoal, and oil (for cooking, medical use, industrial applications, and biofuel generation) (Yang et al., 2021). Almost every component of the coconut tree is used to produce cash or to provide sustenance for rural populations. Coconut provides solid assurance as a food supply in subsistence and semi-subsistence agricultural systems, even when other crops fail. It may be grown in sandy coastal locations because it grows well in places with little rainfall, adequate soil drainage, or a plentiful supply of groundwater, and it can endure more soil salt than many other crops (Teulat *et al*., 2000).

Coconut is a tropical evergreen perennial monocotyledon that thrives in humid and sub-humid coastal conditions. Its importance in supporting the existence of coconut producers and its diverse economic applications is continually bearing fruit. It is a significant tropical oil and fruit crop that is extensively dispersed over 93 tropical nations (Dasanayaka *et al*., 2009; Wang *et al*., 2021), with 12.2 million hectares of planted land, 85% of which is located in the Asia-Pacific region. Global coconut oil output in 2014 was 3.1 million tonnes, according to Yang *et al*., (2021).

There is a global demand for coconut oil (Punzalan *et al*., 2019), putting pressure on coconut-producing nations like Nigeria. To accommodate this demand, coconut fruit output must be greatly boosted. As a result, among the huge germplasm, high-yielding climate-smart germplasms that are pest and disease resistant or tolerant must be produced or selected for production. Traditional selection or breeding approaches based on morphological markers are extremely restricted, difficult, and time-consuming, especially when dealing with a perennial crop like coconut. As a result, typical improvement or selection approaches for prospective germplasms are inadequate. Given the time-consuming nature of high-yield coconut breeding and selection, new molecular technologies can greatly aid and speed up these breeding and selection programmes (Bandaranayake & Kearsey, 2022)

Molecular tools have been known to play important roles in modern breeding and selection for crop development. The use of molecular tools can significantly shorten the overall duration of breeding programmes for coconut improvement (Kesawat & Kumar, 2009; Sindhumole & Nair, 2011), where the selection of high-quality planting material is critical for the successful cultivation of perennial crops such as coconut, and yield is realised only after a long period (Sudha *et al*., 2022) Simple Sequence Repeat (SSR) markers, a molecular tool, are widely used in molecular breeding and genetic selection. SSRs are short tandem repeats with di-, tri-, tetra-, and pentanucleotide repeating units (Caro *et al*., 2021, 2022). They are plentiful and widely dispersed across the genome, and repeat units can differ between genotypes/individuals, making it an extremely helpful tool in fingerprinting, genotyping, and genetic diversity investigations (Sharma *et al*. 2008). SSR markers have been used to determine genetic diversity, hybrid confirmation, and identify somaclonal variation in tissue-cultured coconut plants, to name a few applications (Sudha *et al*., 2022). Hence, a good molecular tool employed for marker-assisted selection and/or marker-

assisted breeding in several crops. This study, therefore, seeks to identify coconut varieties with high-yield and disease-resistant genes among the coconut varieties in Nigeria, using associated SSR markers.

Materials and methods

Collection of Samples

Samples were obtained for this investigation at the Nigeria Institute for Oil Palm Research (NIFOR), Ikoga Sub-station, Badagry, Lagos State, Nigeria. Coconut enhancement is one of NIFOR's missions, and the organisation has over a thousand coconut stands. This institute specialised in coconut research and has coconut germplasm collections from both national and foreign sources (e.g., Cote D'Ivoire, Philippines, India, and so on). In this investigation, one hundred coconut accessions were employed. This contained thirteen (13) accessions with high-yielding capacity (as determined by stock records), while other samples were obtained from nurseries and included both local and exotic collections with high-yielding potential.

Extraction of Genomic DNA

gDNA was extracted from young leaves of samples using the Quick DNA Plant/Seed Miniprep Kit (Zymo Research Corp., USA) according to the manufacturer's instructions. The extracted DNA was quantified and tested for integrity using a NanoDrop Spectrophotometer and 0.8% agarose electrophoresis stained with ethidium bromide.

PCR analysis

The study included ten SSR markers previously identified by Punzalan *et al*., (2019), including five high-yield linked markers and five disease-resistant linked markers (Table 1). PCR reactions were optimised to 12.5 µL reaction, using 1 µL of 50 ng DNA template, 0.25 µL of 10 nM forward primer, 0.25 µL of 10 nM reverse primer, 6.25 µL of One Taq® Quick-Load® 2x Master Mix with standard buffer (BioLabs Inc., New England), and 4.75 µL nuclease-free water. The combination was run at 95 °C for 5 minutes, then 35 cycles of 95 °C for 30 seconds, 53 °C for 1 minute, and 68 °C for 1 minute for 30 seconds. A 5-minute extension run at 68 °C was completed.

Analysis of data

Amplicons were run on 2% agarose electrophoresis stained with ethidium bromide (5 mg/mL) for 1 h 30 min on 70 V. Gels were viewed under UV light gel documentation system. Amplicon sizes were verified using GelAnalyzer 19.1.

Table 1. SSR markers used in the study

Primer ID	Forward primer	Reverse primer	bp
Disease-Resistance Markers			
CLUSTER 63256	GGTTGCCCATGTTGAGAGAT	GGTGAGGGAGCAGAGTGAAG	380
CLUSTER 33.1	AAATGGTTGGTGGACGAAAA	GGTCGATCTACAGGCTGCAT	199
CNKGDEST123	GCAGTTTGACTGCTGCACTTTGCC	ACACACACACACACACACACACACA	150
CLUSTER 182	GGAGCTGTGAGATCGAGTCC	GCTAAAAGGCATTGCTGAGG	182
CLUSTER 48979	TCTGGTGCAGACAGTGGAAG	ATCACGGGGCCATATAAACA	489
High Yield Markers			
CLUSTER-69820	TGTGTTCTTACCACGGCAAA	GGAGGATGCCAAAGTGTTGT	214
CLUSTER-9737	TGTGGGACACTATGGCTGAA	TCGTCCTTGTCAATGCTCTG	147
CLUSTER 1142	GCAGCCACTTGTTCCTTCTC	AATACCACGAGGTGGTCAGC	364
CLUSTER 57477	TCATGGACCGTGATGAAGAA	GCGCAGAAAAGAGAACAACC	105
CLUSTER 24844	TTCTCCCCATCACTTCAAGG	CAATTGGAGCCCACGTAGTT	309

Results and Discussions

Evaluation of marker performance

A total of 9 markers (5 high-yield markers and 4 markers) produced amplicons among the sampled coconut accessions, except CLUSTER 48979 which did not produce any amplification. The success of amplifications also varied among the markers used. Among the high-yield markers, CLUSTER 57477 gave a 67% success rate while CLUSTER-9737 gave the least amplification success of 54%. Similarly, CLUSTER 63526 gave a success of 67% while the least success was observed with CLUSTER 182 marker among the evaluated markers used in the study (Figure 1).

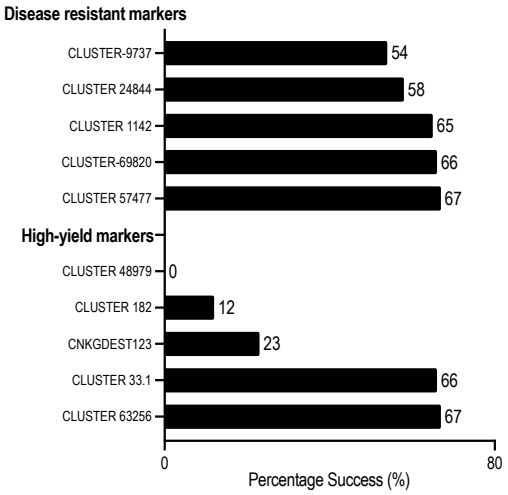

Figure 1. Marker performance among sample coconuts evaluated for the study

Marker-assisted selection for high-yield and disease resistant

It was also discovered that all amplified markers had single band sizes predicted, except for CNKGDEST123, which had two amplicons from the samples amplified. These amplicons varied from 176 to 518 bp (Table 1). Although 93 of the accessions showed at least one marker amplification, of which 44 accessions had diverse combinations of the five high-yield markers, and 18 genotypes combined four disease-resistant markers. However, only 10 accessions of accessions evaluated were able to show amplification for the 9 markers evaluated. Of these, only one (753 (NUR)) showed a close band (176 bp) while the other 9 accessions had band sizes relatively distant from the targeted band size when compared to the expected band (150 bp) from CNKGDEST123 (Table 2).

Table 2. Accessions with all 9 marker amplifications in the study

Sample ID	CLUSTER 63256	CLUSTER 33.1	CNKGDEST123	CLUSTER 182	CLUSTER 69820	CLUSTER 9737	CLUSTER 1142	CLUSTER 57477	CLUSTER 24844
	Target band size								
	380 bp	199 bp	150 bp	182 bp	214 bp	147 bp	364 bp	105 bp	309 bp
753 (NUR)	+	+	212, 176	+	+	+	+	+	+
GRN Tall (FDIII) 1708	+	+	233, 213	+	+	+	+	+	+
Green Dwarf 1710 (FDIII)	+	+	298, 265	+	+	+	+	+	+
Atanaiye Ikoga Zebbe Malayian	+	+	270, 227	+	+	+	+	+	+
1061 (Seedling)	+	+	321, 273	+	+	+	+	+	+
233 FD04 Kenya (EXO)	+	+	261	+	+	+	+	+	+
FD06 01	+	+	363, 308	+	+	+	+	+	+
FD07 026 INDI-DIS SUSC	+	+	296, 241	+	+	+	+	+	+
FD 06 O2	+	+	199	+	+	+	+	+	+
FD04 Exotic (Kenya) 178	+	+	294, 219	+	+	+	+	+	+

+ successful amplification of targeted band size

Several factors impede coconut breeding for desired qualities, including a protracted juvenile phase, a long gap between generations, heterozygous nature, a huge region and a long period for testing, a poor multiplication rate, inadequate clonal propagation, and so on (Sudha *et al*., 2022). As a result, marker-assisted breeding is critical for crop development programmes in coconut. The use of molecular markers for selection in breeding and crop improvement, with molecular markers linked to particular traits, allows for the selection of promising genotypes at the early seedling stage itself via marker-assisted selection (Sindhumole & Nair, 2011). This was demonstrated by the works of Azevedo *et al*., (2018) who used molecular markers associated with plant height to select dwarf coconut cultivars which ease the harvesting of mature fruits from trees (Azevedo *et al*., 2018).

Perera, (2014) noted that in the presence of challenges to coconut improvement, breeding is confined to mass selection of phenotypically superior parent palms, and to inter and intra-varietal hybridization. Hence, the notable observation of the 13 genotypes being used in NIFOR as the major coconut stands for nut yield production. These genotype stands have been propagated and maintained overtime on the field where they were collected. However, the problem of genetic erosion of these species might set in if new genotypes with high potentials are not introduced into the population over time. Hence, a need for a deliberate introduction of other cultivars with high-yield and disease-resistant potentials from time to time to avoid the founder's effect on the genetic constitution of the next generations of coconut germplasm.

None of the potential genotypes from the nursery showed all marker combinations, hence may be lacking in important genes combination for high-yield and disease resistance, accordingly. However, since they have not been tried in the field, this assumption may not hold if they possess other unique genes for high productivity and disease resistance. Studies have shown that quantitative traits like yield and resistance to diseases are polygenic. Hence, other potential genes not captured might be revealed if these genotypes are tried on the field.

However, the presence of the different combinations of genes for high-yield and disease resistance observed among the 13 high-yielding genotypes recommended is an indication of a level of diversity among these major genotypes used by the institute. These diverse genetic constitutions of relatively important genes for productivity are a plus within the coconut plantation used in NIFOR as the more diversity the better the population can stand the test of time, especially amid climate change. As a result, early identification of high-yielding and disease-resistant genotypes in the coconut nursery based on marker-assisted selection as demonstrated in this work for genetic improvement will assure the planting of high-quality coconut planting materials (Baudouin *et al*., 2005; Ruane & Sonnino, 2007; Sudha *et al*., 2022)

Conclusion

The successful utilization of molecular markers to screen for germplasm with high-yield and disease-resistant potentials among NIFOR coconut germplasm as seen in current study has revealed the potential application of MAS for the selection of better-producing coconut genotypes which can be applied in other coconut institutes in Nigeria managing or researching in coconut improvements. The study revealed accession 753 (NUR), with most of the marker combinations, which can be selected for a parent in a yield-improvement and disease-resistant breeding program. Other coconut genotypes among the other nine identified genotypes can also be selected to increase variability within the germplasm for distribution to farmers, researchers, and breeders to plant for higher coconut production and resistance to the vast majority of coconut-related diseases.

Acknowledgements

We are grateful immensely to the management and staff of Nigerian institute for Oil Palm Research **(**NIFOR), Ikoga Sub-Station, Badagry for giving us unrestricted but guided access to their coconut germplasm where samples were collected for this study. Worthy of mentioning, also, is Dr. Joshua Sesan Odewale, a retired staff of NIFOR, who assisted us greatly in collection and identification of collected accessions. We also thank the General Manager of Lagos State Coconut Development Authority (LASCODA), for providing us an in-depth insight into the status of coconut in Lagos State.

Funding

The research was supported by the Lagos State Science Research and Innovation Council (LASRIC) grant 2020.

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