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Multiple Sequence Alignment Reveals Diversity among Eight African Bush Mango (*Irvingia gabonensis* Aubry-Lecomte ex O'Rorke) Cultivars

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Authors' contributions

This work was carried out in collaboration between both authors. Author UGA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JOM extensively managed the literature research, critically reviewed and approved the final manuscript. Both authors read and approved the final manuscript.

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Short Communication

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ABSTRACT

Multiple sequence analysis is one of the most widely used model in estimating similarity among genotypes. In a bid to access useful information for the utilization of bush mango genetic resources, nucleotide sequences of eight bush mango (*Irvingia gabonensis*) cultivars were sourced for and retrieved form NCBI data base, and evaluated for diversity and similarity using computational biology approach.

The highest alignment score (26.18), depicting the highest similarity, was between two pairs of sequence combinations; BM07:BM58 and BM12:BM69 respectively, while the least score (19.43) was between BM01: BM13. The phylogenetic tree broadly divided the cultivars into four distinct groups; BM07, BM58 (cluster one), BM01 (cluster 2), BM15, BM13 and BM35 (cluster 3), and BM12, BM69 (cluster 4), while the sequences obtained from the analysis revealed only few fully

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conserved regions, with the single nucleotides A, and T, which were consistent throughout the evolution.
Results obtained from this study indicate that the bush mango cultivars are divergent and can be useful genetic resources for bush mango improvement through breeding.

Keywords: Breeding; genetic diversity; phylogenetic tree; cultivars; genetic variation.

1. INTRODUCTION

Irvingia gabonensis Aubry-Lecomte ex O'Rorke and *Irvingia wombolu* Vermoesen, collectively known as bush mango, are the economically most important species within the family of Irvingiaceae [1,2]. They are priority trees producing non-timber forest products (NTFPs) and widely distributed in the humid lowland forests of West and Central Africa. Ground kernels of both species are used to thicken and flavour soups, while the fruit mesocarp of *Irvingia gabonensis*, sweet bush mango, is appreciated as a snack or fresh fruit and are most valued and fetch high prices in cross-border trade, contributing significantly to the economy of the region as a whole [3].

Despite being of economic value, *I. gabonensis*, has not been widely cultivated in most regions and fruits are still harvested mainly from wild forest trees [3]. Recently, this specie has become the subject of intensive research in Nigeria and all effort is geared towards genetic diversity and characterization for the purpose of enhancing their application in crop improvement and tree domestication [4–6]. However, just like many tree crops, breeding bush mango cultivars is generally laborious, time consuming and expensive as most seedlings could take up to five years to mature and discovery of traits for breeding could take up to eight years of evaluation. However, Genomic studies offers promising methods to reduce evaluation and selection cycles because genome variation plays a major role in determining important traits for breeding. Also, the availability of genomic library and sequence alignment tools, with free accessibility for research use will aid in diversity studies and development of newer breeds.

Multiple sequence alignment (MSA) involving the arrangement of nucleotide sequences so as to identify regions of similarity, has been proven efficient in estimating diversity [7]. A 2017 study in nature reveals MSA to be one of the most widely used modeling methods in biology [8,9]. Since nucleotides are constituents of nucleic acids, which store and transmit genetic information, and the nucleic acids performing the same functions in different organisms are known to exhibit similar sequences, therefore the arrangement of these nucleic acid sequences helps in identifying regions of similarity.

Therefore, the present study was conducted to access the genetic variation and inter-relationship among eight commercially available *Irvingia gabonensis* genotypes using multiple sequence analysis. Results obtained in this study would be a useful guide for the identification of potential parents in *Irvingia* breeding programs.

2. METHODOLOGY

Genomic clones of eight *Irvingia gabonensis* cultivars in Nigeria were retrieved from NCBI nucleotide data base (https://www.ncbi.nlm.nih.gov/nucleotide) in FASTA format using the accession numbers (BM01- AF076787, BM07- AF076788, BM12- AF076789, BM13- AF076790, BM15- AF076791, BM35- AF076792, BM58- AF076793, BM69- AF076794) [10].

Multiple sequence alignment was performed using a multiple sequence alignment program called Cluster W. The alignment and phylogenetic reconstructions were done using the function "build" of ETE3 v3.1.1 [11] as implemented on the genome net (www://.genome.jp/tools/ete/), and ML tree was inferred using PhyML v20160115, ran with model GTR and parameters: --nclasses 4 -o tlr --alpha e --bootstrap -2 -f m --pinv e [12]. Branch supports are the Chi2-based parametric values return by the approximate likelihood ratio test.

3. RESULTS

The alignment score for the eight *Irvingia gabonensis* genotypes are presented as 29 paired sequence combinations in Table 1. In general, low alignment score was recorded for all the genotypes. The highest alignment score (26.18), which depicts the highest similarity, was between two pairs of sequence combinations; BM07:BM58 and BM12:BM69 respectively, while

the least score (19.43) was between BM01: BM13, followed by BM01: BM58 (20.54) and BM01: BM35 (20.74).

To confirm these findings, a phylogenetic tree representing the eight *Irvingia gabonesis* varieties was obtained (Fig. 2). The phylogenetic tree broadly placed the eight genotypes into four distinct clusters; BM07:BM58 (cluster one), BM01 (cluster 2), BM15, BM13 and BM35 (cluster 3), and BM12:BM69 (cluster 4). It can be observed that BM01 and BM13, which had the least alignment score, falls into different clusters, while BM07, BM58 and BM1, BM69, which had the highest alignment score, respectively, falls into the same cluster (Fig. 1).

The sequence obtained from CLUSTAL W shows that there were only few fully conserved regions with the single nucleotides A, and T which did not changed throughout the evolution (Fig. 2).

4. DISCUSSION

Sequence alignment score is very useful in estimating the genetic similarity among genotypes. As a rule of thumb, the higher the sequence alignment score between two biological organisms, the closer the relationship between them [13]. The present study unveiled a very low alignment score between two of the cultivars, BM01 and BM13, moreover, two other pairs (BM01: BM58, and BM01: BM35) also had low sequence alignment scores, indicating a very high diversity among them. This suggests that apparent genetic diversity inherent in these genotypes would remain unknown, except they are evaluated. The discovery of their genetic potentials could enhance and transform specific breeding program. To confirm this findings, the phylogenetic tree broadly placed these cultivars with lowest alignment scores into different clusters, while those with highest score (BM07, BM58 and BM12, BM69), respectively, were clustered together.

Wider crosses in bush mango has been advocated by [14], such according to them could lead to heterosis in hybrids and the production of new recombinants for desired trait. Genotypes belonging to different clusters are genetically

Table 1. Sequences and alignment score of the eight *Irvingia gabonensis* genotypes

Sequences	Aligned score
BM01:BM07	24.6124
BM01:BM12	21.1706
BM01:BM13	19.4271
BM01:BM15	21.6182
BM01:BM35	20.7407
BM01:BM58	20.5479
BM01:BM69	22.2868
BM07:BM12	21.4623
BM07:BM13	25.2358
BM07:BM15	24.2925
BM07:BM35	24.2925
BM07:BM58	26.1792
BM07:BM69	22.1698
BM12:BM13	20.8955
BM12:BM15	22.5032
BM12:BM35	23.5185
BM12:BM58	22.4277
BM12:BM69	26.1792
BM13:BM15	24.7788
BM13:BM35	23.5185
BM13:BM58	21.7341
BM13:BM69	22.8682
BM15:BM35	22.5926
BM15:BM58	22.6296
BM15:BM69	22.4806
BM35:BM58	22.7778
BM35:BM69	23.4496
BM58:BM69	22.093

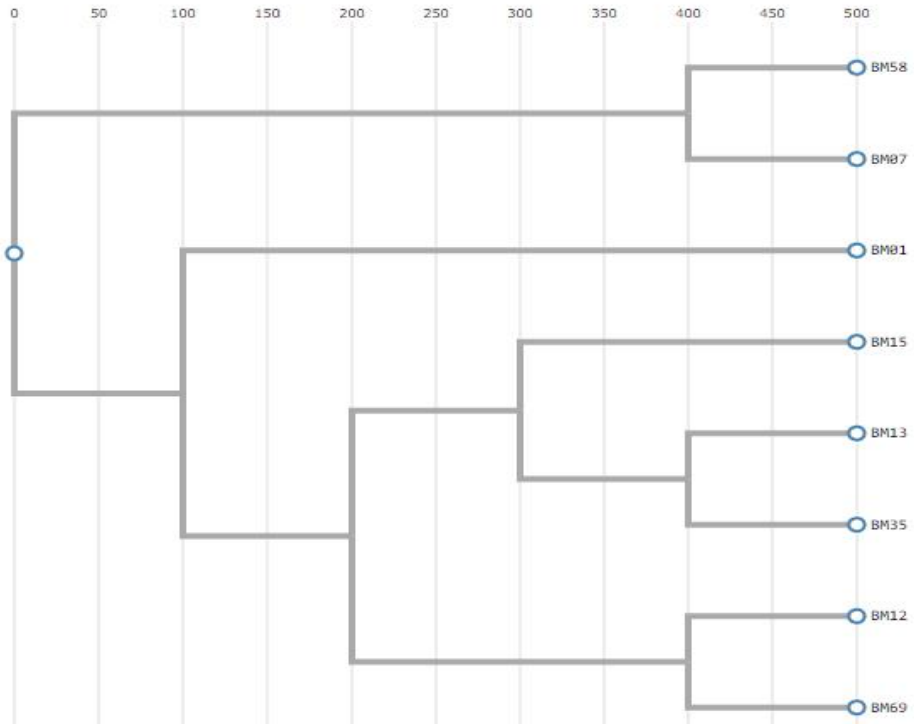

Fig. 1. Phylogenic construction of the eight *Irvingia gabonensis* genotypes

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BM01        AAAAGGATGTTCTT---GG-CTTGAG-GGAGGGCATTGGAGAAAAACAATTTTCGCAAAG
BM07        TG--TGATCATATG---GA-ATCAAA-GAATTACGAAGAACAACATCAACACCTTCATAT
BM15        ATGGCTAGAAAATG---AA-TTTCCC-AAAGTTAACAGGACTGAAACACCATGGCTTATT
BM13        AATCTTCCCATATT---CA-ACATCA-CAAATTGGTCATATCATTTCATAGTTTCACACC
BM35        TTGCAGAAACCACT---AG-ATATGGTGGAATCGGCCTAAGTAACTCTTTGACAGAGACG
BM12        GAATCTCTGCTGTC---AA-GAAACAACTAATCAAAAAGATTAAGTTGACATGGATCATA
BM69        GCTATTTTGATATT---GATGTTTCGTCAGTTAAAGAAGATGATGTTTAGGAGTCTTCTG
BM58        TGTCACATCTTTTTTGTAATGTGTGTTTTGCTGTTGAAGATAGAGTGACTTTGATAGCAG
                                                    *

BM01        AACAACAACTTCACTGGTA-GATGAGTCTGGTGTTTGTGGGA-RGGATGCTGATAAGGAA
BM07        GTCCAAA----CACCTAGA-ACTGTGTCTCCTTTGAGT-----------CCAATAACAAA
BM15        GTTCTTA--TGCATTGTCC-ATTGTATAGCAGTTATGCACATCATTATATGGAAGGGGAA
BM13        ACTATTGCTCATATCTTGATATTACTTATATGTTCCATATCAAGCTTGCCAAGGAAAGCA
BM35        ACAACTAATGGTATTGGAA--TTGTAGATTTTCTTCAA-----------GGAAAGGTGT
BM12        ACTTAACACTGGAAAGCCC-TTTGGGCTTGGGCTCTGAAGCA-----TGGCACTGGCCCA
BM69        GCTTACTTTTTTCTGTTACGACTTAGTCAAATGGATGATCCA-AGATTAGCAATAGGGTC
BM58        GTCCTCAGGGGAAACTCTATTATGAGTGTACTATAGAAAAATTGCAGGAGTAATAAAATA
                                  *

BM01        GCCATAATAAAGATGGTGCTGCCATCTGAATCTGATGAAG---------CAAG--TTGTA
BM07        CCAAGACCAGCAGCAGCAATATCCATGGAACTTGA--ATC---------CCAA--CTGCT
BM15        GGCATGAGAGTGACT-TATGAGGAATGGTTTGTGG-AATA---------CAAA--GTGGA
BM13        TTTATATCATTGCAGAAGATGATAAATGTTACTCACAATA---------TCATTCCTGAA
BM35        TCTTTATCACTGGCGCAACTGGGTTTT-TGGCCAAGGGTA---------CCACATACATA
BM12        ACTTCCTTAGTGCGCAAACTTTAATTTGTTTTTAAATAAA---------TAAGGGGTACA
BM69        CCATAGTGGTTGATATTCCTGAGGATGGACTTGGAGGATGAT-----TTTAATTAAAATT
BM58        AAATTTTTTAAAAAAAAACTAAAAGAGGTTGCACACATCGTCACTACTCCCAACAGTTCC
                                                          *

BM01        AAAAGCTA-GTTGTGATACCCA----TCGTGGGTATGGGTGGGATTGGTAAAACCACAAT
BM07        CCCAGACA-CGTGGAGTTCCTG----TCTCACCCATTTTCGGAGCTCTTCAACTCGTTTC
BM15        TGTTGTCT-TTGCCGGCCATGT----TCACGCCTATGAAAGATCTGTAAGTGCTCNNTAT
BM13        ATCATACT-CCTCTAGGATTGGGTAATTTAGAGCATTTGGTAAACACCTCTGTTTCACAT
BM35        GATTTCCT-TTCATATGTTATATGTATTATATATACACATTCCTGATTGATGTTCTAAAG
BM12        AGGATNGAACCTGTGAACATTTTGTTATGATGGCTCTATTATCATGTCAAAAAACTCATC
BM69        TGTCGGTATTTTTTAATTTTCTCCTTCCTTTTCAATCAAAGATCTATTCAGATCTGGCTC
BM58        TGCATCCCATTTGTAGCAAAGCTCAATCTCGT-TGTCTACAATGTTGTTCAGATAAACAC
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Fig. 2. Multiple sequence alignment of the eight *Irvingia gabonensis* genotypes

more diverse [14-16]. The high diversity among these cultivars was further justified by the sequences obtained from CLUSTAL W, which showed only few conserved regions, having single nucleotides A, and T which were consistent throughout the evolution.

The result presented in this study would also be a key to specific primer design and further research on other tree crops. Genetic diversity data are scarce for Central/West African tropical tree species [17], and therefore, these results may provide insights into the extent and partitioning of genetic variation expected for species with a similar life history and habitat range.

The distance relation identified among the studied cultivars could be linked to differential genetic identity, and this gives a hint on the presence of divergent genetic resources among *Irvingia* species. Availability of wide genetic resources is necessary for increase in species germplasm (16). Moreover, selection of genetic materials for advance and improvement, a primary process in plant breeding programs, solely depend on genetic stock availability.

5. CONCLUSION

The results from this study indicates that the eight *Irvingia gabonensis* cultivars differed from each other, and they had only few conserved regions. Among them, cultivars BM01: BM13, BM01: BM58 and BM01: BM35 are the most diverse. With this result, trait-based evaluation and selection is possible, and genetic advances in plant vigour and yield may be achieved through a concerted crop improvement program.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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