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Exploring the Population Structure and Genetic Diversity of *Moringa Oleifera* Using DArTSeq Derived SNP Markers.

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# Abstract

Moringa oleifera is a versatile tree native to the foothills of the Himalayas and now naturalized in the tropics. Moringa is nutritionally significant owing to its high nutrients and antioxidant content. The genetic diversity of Moringa across the tropics remains under investigated. 3968 SNP markers derived from DArTSeq technology were filtered to 1913 informative markers and used to investigate the genetic makeup of Moringa oleifera. Unweighted neighbour joining phylogeny and principal coordinate analysis revealed four distinct clusters related to the geographic origin: Caribbean (Haiti/Jamaica) and East African (Kenya/Tanzania) were identified to be a subset of the West African (Mali/Ghana) population while the South African (Malawi) segregated distinctly. Philippines samples clustered separately and farthest as expected. Analysis of molecular variance revealed high gene flow within populations (77%) compared to among populations (23%). Bayesian modelling in structure with best k being two still distinctly segregated the South African population from the other African regions suggesting a distinct introduction. The significant admixture of individuals noted in structure is typical of unnatural introductions possibly through human mediated events. This study highlights the power of SNP markers from DArTSeq technology in elucidating the genetic structure and molecular diversity of Moringa oleifera. The findings contribute to our understanding of the genetic resources of this important plant species and have implications for germplasm collection, improvement, conservation, utilisation strategies and policies. Further research utilising advanced genomic tools will enhance our knowledge of Moringa oleifera and support its sustainable utilisation for various applications.

Keywords: Diversity array technology, genetic structure, germplasm conservation, Moringa oleifera

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## Introduction

*Moringa oleifera*, a fast-growing deciduous tree in the Moringaceae family (Rajalakshmi et al., 2017), It thrives in tropical and subtropical regions across Asia, Africa, South and Central America, and the Caribbean (Saini et al., 2013). Known as "the natural nutrition of the tropics," Moringa is valued for its nutrient-rich leaves, seeds, and pods, which contain higher levels of vitamins, minerals, and protein compared to common foods like carrots, oranges, and milk. This exceptional nutritional profile positions Moringa as a prime candidate for food fortification, particularly in developing countries with prevalent nutrient deficiencies (Shahzad et al., 2013).In agriculture, Moringa serves as nutritious fodder, while its pharmacological applications include treatments for diabetes, hypertension, and anaemia due to its antioxidant properties . Moreover, it shows promise in biotechnology as a source for biodiesel and water purification agents. Moringa's resilience to harsh environments makes it particularly beneficial in arid regions facing food insecurity. Despite its advantages, Moringa remains underutilized and is recognized among the 101 neglected crops by the African Orphaned Crop Consortium (Dawson et al., 2018). Recent advances in genomic research, including the use of DArTSeq technology provides a platform for improved breeding and conservation efforts by enabling high-resolution genetic diversity assessments across the Moringa genome (Kilian et al., 2005). This study focused on the genetic diversity and

population structure of Moringa accessions from various countries, highlighting the potential of modern genotyping technologies for enhancing breeding and conservation strategies.

#### **Material and Methods**

A total of 330 samples, including 95 accessions of *Moringa oleifera* from ICRAF gene bank: 83 from Kenya, 2 from Mali, 4 from Malawi, and 2 from the Philippines and single accessions from Tanzania, Haiti, Jamaica were utilised for DNA isolation by Bioline Isolate II Plant DNA Kit. The DArTSeq sequencing by synthesis process included complexity reduction via restriction enzyme digestion, ligation of adaptors, amplification, and sequencing, resulting in Single Nucleotide Polymorphisms (SNPs) that were scored for reference and alternate alleles. A total of 3,968 SNPs were generated and filtered based on set criteria. Informative SNP markers were identified using the Polymorphism Information Content (PIC) calculation, with adjustments made for false discovery rates. Hardy-Weinberg Equilibrium tests were conducted in Arlequin (Excoffier et al., 2005)and pairwise Nei's chord genetic distances were calculated in microsatellite analyser (Dieringer & Schlötterer, 2003) to construct phylogenetic trees in PHYLIP (Felsenstein, 1989). Genetic structure was assessed with STRUCTURE software (Pritchard & Wen, 2002), while inter- and intra-population variations were analysed using Analysis of Molecular Variance (AMOVA).

#### **Results and Discussion**

#### Genetic diversity and phylogenetic analysis

There were high levels of genetic distinctness among and within Moringa populations across the continents as per comparisons using false discovery rates (FDRs) at alpha levels of 0.05 and expected heterozygosity (He) between 0.05056 and 0.5008, which accounted for both richness and evenness of alleles. Philippines populations had the longest genetic distance from the rest of the populations (0.2692) hence the most diverse. This is possibly because Philippines population had multiple gene pools introduced due to its geographical proximity to India; the Moringa center of origin. Kenyan and Tanzanian had the shortest genetic distance from each other (0.2082 and 0.2092 respectively) hence the least diverse inferring a common source of introduction. The phylogenetic analysis revealed four main clades corresponding to geographic origins. South African population (Malawi) formed a separate clade from all the African populations. This signified that there were two major Moringa introduction events in Africa, South African population having a unique introduction while the rest having a common introduction event. Moringa was possibly directly introduced to Malawi by settlers from a specific region in India and at a unique time point (Prasuna et al., 2009., Olsona, 2017). Ghanaian, Malian, Haitian, and Jamaican populations were monophyletic. Specifically, clustering of these four populations supports the hypothesis that the Pacific slave trade introduced a Malian population to Jamaica and Haiti.



Figure 1a) population and b) individual Neighbor joining trees showing genetic relationships among the 19 provenances and 330 accessions respectively using Nei's chord genetic distance.

### Analysis of Molecular Variance



Figure 2. Pie chart illustrating the proportion of molecular variance among and within populations

From the AMOVA analysis, the diversity structuring was 77% within populations and 23% among populations. According to diversity structuring for outcrossing plant species gene flow is attributed to pollen movement (Sheng et al., 2005), and mating systems in plants contribute highly to patterns of genetic diversity within and among populations (Kithure et al., 2015).

#### Genetic structure

Genetic structuring has important implications in ecological restoration exercises (Muchugi et al., 2008). Structure at K two hypothesize that Moringa was introduced in the African continent via two major events, Malawi population having been introduced by settlers from a specific region in India at a unique time point (Prasuna et al., 2009; Olsona 2017). unique introduction. At K four, west African and Caribbean population grouped together suggesting that the Caribbean and Eastern African populations are a subset of West African population. This supports the hypothesis that the Pacific slave trade introduced a Malian population to Jamaica and Haiti (Muluvi et al., 1999).



**Figure 3:** Population structure used to estimate the best K value using Bayesian-based population genetic structure analysis a) K=2 and B) K=4. Each solid bar represents an accession, with coloured areas corresponding to clusters. Admixed genomes of accessions are represented by bars with multiple colours.

### **Conclusions and Outlook**

These research findings identified Malawi and Philippines as the most unique and diverse populations among African and rest of the populations respectively hence should be the focus of conservation efforts. East African and Caribbean population were identified as subsets of the Western Africa population. A notable admixture of entries not complying with geographical distribution is typical to unnatural mixing conceivably through anthropogenic events. Minimal genetic diversity noted among most of the African populations is presumed to be due to small number of *Moringa* accessions introduced from India to Africa serving as founder population. These findings have implications for *Moringa* germplasm collection, improvement, conservation, utilization strategies and policies.

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