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Comparative Analysis of Phenotypic and Molecular Characterization of Cassava (*Manihot esculenta Crantz*) Germplasm in Kenya

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Abstract: Cassava (*Manihot esculenta Crantz*) is a crucial staple crop in Kenya, but its genetic diversity has not been fully exploited in breeding programs. This study compares phenotypic and molecular characterisation methods for cassava germplasm to assess its efficacy in reflecting genetic relationships. A total of 131 cassava genotypes were collected and characterised using morphological traits, which grouped the accessions into four major clusters with a similarity index of 0.5. From these, 40 samples were selected for molecular characterisation using the unweighted pair group method with arithmetic means (UPGMA) at a similarity coefficient of 0.35. The study found significant discrepancies between phenotypic and molecular classifications. While phenotypic characterisation categorised accessions into four clusters based on observable traits, molecular analysis revealed two main genetic clusters. Phenotypically distinct accessions from clusters #1, 2, and 4 were grouped together in molecular cluster #1(a), indicating closer genetic relationships. Accessions with similar observable traits, such as "Nyarkogutu-002," were found to be genetically distinct. Additionally, the study found a low correlation (0.0423) between phenotypic and molecular characterisations of cassava germplasm. Therefore, the study recommends using molecular markers to select genetically distant parents, even if they appear phenotypically similar, to enhance allele diversity and heterosis in breeding efforts. Thus, there is a need for a combined approach of phenotypic and molecular characterisation to improve the accuracy of cassava germplasm classification, thus supporting more effective breeding and conservation strategies.

Keywords: Cassava germplasm, Genetic diversity, Molecular characterization, Phenotypic characterization, Kenya

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INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is a staple food crop, particularly in tropical and sub-tropical regions, where it provides a crucial source of carbohydrates for millions of people [8]. Germplasm characterisation is an important aspect of cassava breeding and conservation. It involves figuring out and writing down the genetic diversity of a group of cassava genotypes. This is necessary for creating better varieties and keeping the genetic diversity of crops [2, 13, 14]. Globally, efforts have been made to improve cassava germplasm through phenotypic and molecular characterisation, which are critical for understanding the genetic diversity, adaptability, and breeding potential of cassava cultivars [7].

Phenotypic identification of plants is commonly based on the morphological traits assessed and recorded in the field [11]. Different cultivars can be told apart by phenotypic traits like colour and shape,

branching habit, plant height, stem and petiole colour, root shape and skin colour, maturity date, yield, and the amount of cyanogenic glycosides in the roots [11, 20].

Molecular characterisation is particularly important in cassava due to the crop's vegetative propagation and the presence of morphologically similar but genetically distinct varieties. This makes traditional phenotypic characterisation insufficient for accurate identification and conservation efforts [1]. Moreover, phenotypic characters are highly influenced by environmental factors. Genetic markers help researchers get around the problems that come with phenotypic characterisation by giving them accurate, trustworthy information on genetic variation within and between cassava populations, which makes breeding programs better [10, 18]. Molecular markers, such as microsatellites (SSR), single-nucleotide polymorphisms (SNP), and DNA sequencing technologies, have revolutionised the study of cassava germplasm. These tools make it possible to correctly name varieties, tell the difference between genotypes that are closely related, and figure out how diverse a species' genes are [12, 19]. For instance, using Simple Sequence Repeat (SSR) markers in molecular studies has shown that there is a lot of genetic diversity in cassava germplasm from Africa, Asia, and Latin America. These studies have focused on improving disease resistance and drought tolerance [4, 21].

The integration of phenotypic and molecular data is essential for accelerating cassava breeding efforts and ensuring the sustainability of this vital crop worldwide [19]. Simultaneous studies on phenotypic and genotypic characterisation of cassava germplasm have been carried out in various cassava-growing regions in the world. A study in the United States [15] looked at the genetic diversity of cassava using molecular markers like SSR and paired it with phenotypic traits like root yield and starch content. The study demonstrated significant diversity in cassava germplasm and highlighted the complementarity between molecular and phenotypic characterisation when improving cassava breeding programs.

Similarly, in Asia, a study [23] in India utilised both morphological descriptors and molecular tools like Random Amplified Polymorphic DNA (RAPD) markers to characterise local cassava varieties. The study found that molecular data helped properly figure out the genetic connections between cassava accessions, which could not be done completely with phenotypic traits alone. [16] carried out studies on the morphological characteristics of popular cassava cultivars grown in Kenya. They identified four cluster groups. They then characterised the same germplasm using genetic markers [17]. The second study also identified four cluster groups. Therefore, the objective of the current study was to carry out a comparative analysis of *the phenotypic and molecular characterisation of cassava (Manihot esculenta Crantz) germplasm in Kenya.*

MATERIALS AND METHODS

Phenotypic characterisation

From the sample collection of 131 cassava accessions, 40 were selected for phenotypic characterisation (Table 1). The plants harvested from each accession were cut into pieces, with each having 4-5 nodes. Each accession was planted in three rows, with each row planted with 5 plants. The spacing was 1 m between rows and 1 m between plants. Normal agronomic practices were carried out during the experimental period. No fertiliser or pesticides were applied on the crop.

Data Collection

Phenotypic data was collected on the plants in the middle row of each accession. Phenotypic characterisation was done using the selected morphological and agronomic descriptors for the characterisation of cassava as described by [11]. The observations were made at 3, 6, 9, and 12 months after planting (MAP). The phenotypic traits that were looked at were the shape of the central leaflet, the edges of the lobes, the colour of the stem's epidermis and exterior, the colour of the leaf, the orientation of the petiole, the length of the root puduncle, the colour of the root cortex, the shape of the root, its taste, and its thickness.

Data Analysis

The phenotypic variation among the studied accessions was explored using a multivariate analysis technique [12]. Multivariate analysis of the 40 data matrix, comprising principal component analysis (PCA), was processed using IBM SPSS statistics software version 25. In the PCA, eigenvalues and load coefficient values were generated from the data set. The structure of morphological changeability was visualised using ascending hierarchical clustering (AHC) based on data and Ward’s Method to plot a dendrogram [12]. The principal component analysis and correlation matrices were used to determine the relationships among the traits.

Table 1: Selected cassava accessions analyzed for phenotypic and genotypic characters

Accession No.	Variety	Location collected	County
114	Nyakanyamkago	Sigiria	Migori
104	Nyar-ICIPE	Sigiria	Migori
73	Busia-004	Busia	Busia
112	Agriculture-020	Ranen	Migori
113	Agriculture-021	Maram	Homa bay
105	Obar dak-002	Nyamarere	Migori
18	Katune	Kiboko	Makueni
20	Kazanzwara	Kiboko	Makueni
25	Nyaeta	Kehancha	Migori
40	Nyagire	Maram	Homa bay
44	Unknown variety-003	Maram	Homa bay
61	Machoberi	Kegonga;Kehancha	Migori
74	Nyarkogutu-002	Ngothe	Migori
75	Nyarkadera	Kadera	Migori
87	Nyatanga-004	Rongo	Migori
88	F-19	Mtwapa	Kilifi
99	MM96/0067	Mtwapa	Kilifi
10	Agriculture-001	Ranen	Migori
6	Mbale-002	Mida Creek	Kilifi
9	Mary Kaluorore	Ranen	Migori
96	Agriculture-017	Mtwapa	Kilifi
7	Agriculture-001	Ranen	Migori
14	Kamgundho	Rapogi	Migori
16	KBK-4	Kiboko	Makueni
62	AdhiamboLera	Awendo	Migori
89	Mtwapa-009	Mtwapa	Kilifi
98	MM96/0067	Mtwapa	Kilifi
4	Mbale-001	Miida Creek	Kilifi
15	Kasukali	Kiboko	Makueni
117	Toji	Kendu bay	Homa bay
58	Agriculture-010	Kegonga, Kehancha	Migori
68	Madam	Opapo	Migori
63	Unknown variety-003	Rakwaro	Migori
109	Selele-007	Sigiria	Migori
125	Selele-009	Ranen	Migori
24	Selele-002	Rongo	Migori
35	Selele-004	Rakwaro	Migori
36	Nyatanga-002	Uriri	Migori
39	Selele-005	Maram	Migori
64	Selele-006	Busia	Busia

Molecular characterization

The same 40 cassava samples presented in Table 1 were analysed for genetic variations. Molecular characterisation was carried out as described by [17]. The study involved genomic DNA extraction from 40 cassava leaf samples, collected two months post-planting and preserved at -86°C. The DNA was isolated using the CTAB method, involving incubation with a pre-heated buffer, chloroform: isoamyl alcohol purification, isopropanol precipitation, and ethanol washing. The resulting DNA pellets were dried, dissolved in nuclease-free water, and treated with RNase to remove RNA contaminants. Agarose gel electrophoresis confirmed the quality and quantity of the DNA. The final concentration was set at 50 ng/µL so that it could be stored at -20°C.

For genetic diversity analysis, start codon-targeted primer amplification (SCoT-PCR) was performed with 15 primers that consistently produced clear and reproducible bands. The PCR reactions were optimised and carried out in a total volume of 25 µL under specific thermocycling conditions, including 35 cycles of denaturation, annealing, and extension. PCR products were resolved on agarose gels, stained with ethidium bromide, and visualised using gel imaging systems.

Genetic diversity and polymorphism among cassava accessions were analysed through binary scoring of

PCR bands. Software tools such as Popgene and PowerMarker were employed for calculating genetic distances and polymorphism information content (PIC). Molecular variance analysis was done with GENALEX 6.5, and relationships between accessions were found using UPGMA methods and cluster analysis.

RESULTS

Comparison of the phenotypic and genotypic characterisations of the sampled cassava varieties in Kenya

Molecular characterisation separated the 40 cassava accessions into two distinct clusters (Table 2). Cluster #1 had 20 accessions, while Cluster #2 had 20. In each of these two clusters, the varieties had been collected from different counties and locations.

Phenotypic characterisation grouped the 40 accessions into four clusters (Table 2). Cluster #1 had 20 accessions, cluster #2 had 8 accessions, and cluster #3 had 4 accessions. The last cluster #4 had 8 accessions (Table 2). The correlation between genotypic and phenotypic characterisation was 0.04225771.

Table 2: Derived clusters of the 40 cassava accessions based on phenotypic (agro-morphological) and genotypic (molecular) characterization

Accession no.	Variety	Location collected	County	Genotype cluster no.	Phenotype cluster no.
104	Nyar-ICIPE	Sigiria	Migori	1	1
64	Selele-006	Busia	Busia	1	4
125	Selele-009	Ranen	Migori	1	4
96	Agriculture-017	Mtwapa	Kilifi	1	2
105	Obar dak-002	Nyamarere	Migori	1	1
99	MM96/0067	Mtwapa	Kilifi	1	1
89	Mtwapa-009	Mtwapa	Kilifi	1	2
63	Unknown variety-003	Rakwaro	Migori	1	4
73	Busia-004	Busia	Busia	1	1
68	Madam	Opapo	Migori	1	3
117	Toji	Kendu bay	Homa bay	1	3
114	Nyakanyamkago	Sigiria	Migori	1	1
88	F-19	Mtwapa	Kilifi	1	1
98	MM96/0067	Mtwapa	Kilifi	1	2
87	Nyatanga-004	Rongo	Migori	1	1
113	Agriculture-021	Maram	Homa bay	1	1
112	Agriculture-020	Ranen	Migori	1	1
109	Selele-007	Sigiria	Migori	1	4
75	Nyarkadera	Kadera	Migori	1	1
74	Nyarkogutu-002	Ngothe	Migori	1	1
10	Agriculture-001	Ranen	Migori	2	1
62	AdhiamboLera	Awendo	Migori	2	2
61	Machoberi	Kegonga;Kehancha	Migori	2	1
58	Agriculture-010	Kegonga, Kehancha	Migori	2	3
44	Unknown variety-003	Maram	Homa bay	2	1
36	Nyatanga-002	Uriri	Migori	2	4
35	Selele-004	Rakwaro	Migori	2	4
24	Selele-002	Rongo	Migori	2	4
20	Kazanzwara	Kiboko	Makueni	2	1
18	Katune	Kiboko	Makueni	2	1
25	Nyaeta	Kehancha	Migori	2	1
16	KBK-4	Kiboko	Makueni	2	2
15	Kasukali	Kiboko	Makueni	2	3
14	Kamgundho	Rapogi	Migori	2	2
9	Mary Kaluorore	Ranen	Migori	2	1
7	Agriculture-001	Ranen	Migori	2	2
40	Nyagire	Maram	Homa bay	2	1
6	Mbale-002	Mida Creek	Kilifi	2	1
4	Mbale-001	Miida Creek	Kilifi	2	2
39	Selele-005	Maram	Migori	2	4

DISCUSSION

The findings from this study affirm the importance of integrating phenotypic and molecular characterisations in understanding cassava germplasm diversity in Kenya. While phenotypic characterisation provides valuable insights into regional adaptations and observable traits, it is often limited by inconsistencies, such as farmer-assigned naming conventions and environmental influences. The same cassava variety may have different names across regions, leading to misclassification and variability [5]. Also, morphological traits like plant height and root shape can change depending on the environment and stage of development, which makes them less reliable for showing genetic relationships accurately [22]. Consequently, morphological characterisation is best suited as a preliminary exploratory tool to guide deeper molecular investigations [9]. The Kenyan study's low correlation (0.0423) between molecular and agromorphological characterisations echoes findings by [2], who highlighted that phenotypic traits often fail to reflect underlying genetic diversity.

Molecular characterisation, by contrast, offers a robust and stable assessment of genetic diversity that is unaffected by environmental or developmental variables [5]. Molecular markers in this study revealed hidden genetic relationships. For example, Kazanzwara-Kiboko and Katune-Kiboko were put in genotypic Cluster #2 even though they were put in phenotypic Cluster #1. These discrepancies highlight the limitations of phenotypic characterisation and the superior precision of molecular techniques, as corroborated by [6] in Uganda and [24] in Burundi. Furthermore, the Kenyan findings resonate with the work of [3], which demonstrated the value of molecular markers in uncovering genetic diversity critical for traits like pest resistance and yield improvement. The Selele varieties in phenotypic Cluster #4 exemplify this disconnect, as they were genetically diverse when analysed molecularly.

Integrating both phenotypic and genotypic approaches is essential for comprehensive cassava breeding strategies and germplasm management. Molecular markers find genetic differences that help breeders make decisions and keep genetic bottlenecks from happening [3]. Phenotypic traits show how plants and animals have adapted to their environment and how well they perform in the wild. This dual approach aligns with that of [9], who emphasised that combining these methods captures a holistic understanding of genetic resources, aiding in accurate identification, conservation, and crop improvement efforts. The differences in clustering seen between phenotypic and molecular assessments show that both methods need to be used together, since phenotypic analysis by itself can't reliably find genetic diversity. Using all of these methods together not only helps with breeding decisions, but it also protects cassava genetic resources, which is a key step towards making crops more resilient and productive.

CONCLUSION

The integration of phenotypic and molecular characterisations is crucial for understanding cassava germplasm diversity. While phenotypic characterisation provides insights into observable traits and regional adaptations, it is limited by environmental influences and inconsistencies, such as variability in naming conventions and developmental stage effects. Molecular characterisation, on the other hand, offers a more stable and accurate assessment of genetic diversity that is unaffected by such variables.

Environmental and developmental factors can change phenotypic traits, like plant height and root shape. This makes them less reliable for figuring out genetic relationships. Consequently, phenotypic analysis should primarily serve as a preliminary tool to guide deeper molecular investigations. The low correlation (0.0423) observed between phenotypic and molecular characterisations in the Kenyan study highlights these limitations.

Molecular markers give us a lot of information about genetic diversity and reveal genetic connections that phenotypic traits can't always find. The discrepancies observed in the clustering of accessions, such as Kazanzwara-Kiboko and Katune-Kiboko, emphasise the precision of molecular techniques in classifying genetic diversity. This finding is supported by similar studies in Uganda and Burundi.

A combined phenotypic and genotypic approach is essential for effective breeding strategies and germplasm management. Molecular markers make sure that accurate identification of genetic variations is possible for traits like pest resistance and yield improvement. Phenotypic traits give useful information about how well something grows in a certain area. This synergy enhances breeding decisions, prevents genetic bottlenecks, and conserves genetic diversity.

The differences between phenotypic and molecular clustering show that we need to use two methods together to fully understand cassava genetic resources. Integrating these techniques aids in breeding programs aimed at improving crop resilience and productivity while preserving the genetic diversity necessary for sustainable agriculture. This holistic strategy aligns with global recommendations for crop improvement and conservation efforts.

RECOMMENDATIONS

Based on the study's findings, cassava breeding

programs should prioritise the integration of molecular characterisations alongside traditional phenotypic methods. Molecular data is needed to choose genetically different parents because morphological traits alone don't always show genetic diversity. This can improve allele diversity and heterosis in breeding efforts. This will enable the development of more resilient and productive cassava cultivars that are better suited to diverse environmental conditions and agricultural demands.

Additionally, cassava breeders should be cautious when relying solely on observable traits, as accessions with similar phenotypic characteristics may have significant genetic differences. Molecular tools, like DNA markers, should be used to accurately figure out genetic relationships and make sure that genetically compatible accessions are chosen, even if they come from different parts of the world. This approach will help avoid the unintended selection of genetically similar plants, which may limit genetic progress in breeding programs.

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Conflict of Interest

"The author(s) declare(s) that there is no conflict of interest."

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