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# Molecular Characterization of Edible Aroids Growing in Parts of Rivers State

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**Abstract** **Original Research Article**

Cocoyam (Colocasia esculenta and Xanthosoma sagittifolium) plays a critical role in food security and livelihoods in many tropical regions, yet it remains a neglected and underutilized crop, facing threats from germplasm loss and limited research attention. Understanding the genetic diversity of existing cocoyam germplasm is crucial for effective conservation and breeding programs. This study aimed to assess the genetic diversity and relationships among six cocoyam accessions from three local government areas in Rivers State, Nigeria, using molecular characterization. Field experiments were carried out between June 20, 2021 and January 20, 2022, and also in June 20, 2022 and January 20, 2023, in three local government areas of Rivers State, Nigeria—Obio/Akpor, Emohua, and Ikwerre. Six different cocoyam accessions of the National Root Crop Institute (NRCRI), Umudike, were used for the study: NCe001, NCe002, NCe003, and Xanthosoma sagittifolium (NXs001, NXs002, NXs003). DNA genomic was isolated from the plant samples by Zymo Quick DNA Plant/Seed Mini PrepKit, and DNA purity and concentration were measured by NanoDrop Spectrophotometry. The target region was amplified by OneTaq® Quick-Load® 2X Master Mix and specific primers (RBCL 1 and ATP) in a Polymerase Chain Reaction (PCR). PCR products were enzymatically purified, and sequencing was carried out. Sequence analysis involved visualizing chromatograms with Chromaslite, editing with BioEdit, and performing a Basic Local Alignment Search Tool (BLAST) search against the NCBI database. Comparable sequences were downloaded and aligned using ClustalW. The evolutionary history and phylogenetic relationships among the six accessions were inferred using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) and Neighbor-Joining method in MEGA 7.0.26, based on nucleotide sequence data. The molecular analyses successfully amplified and sequenced target regions. The results show a phylogenetic tree with a distance scale (0–5) indicating genetic similarity among plant varieties. NXS003, NCE003, NCE002, and NCE001 form a close cluster, suggesting high relatedness. NXS003 and NCE003 are the most similar pair, while NXS001 and NXS002 are more distantly related. NXS002 appears to be the most genetically distinct variety in the group. The results from the phylogenetic trees revealed that samples RCBBR_P1, P2, P4, P5, P7, P8, P9, and P10 consistently clustered with Colocasia esculenta accessions such as cultivar lipu (MT447085.1), isolate RR (JN105690.1), and Taimo (LC767269.1). The GenBank similarity scores ranging between 97.93% and 99.05% indicate that these samples are genetically like C. esculenta, suggesting they may represent landraces, cultivars, or locally adapted variants of the species. Interestingly, samples such as RCBBR_P3, P6, P11, and P12 clustered with Xanthosoma sagittifolium, showing similarity values up to 99.47% for P3 and P12, and as low as 96.19% for P6. These findings suggest that while P3 and P12 are closely related to the known X. sagittifolium, sample P6 may represent a genetically divergent or under-characterized variant. Samples such as RCBBR_P6 and RCBBR_P11 displayed long branch lengths and formed isolated clades, indicating substantial genetic divergence.

**Keywords:** Edible Ariods, Cocoyam, Colocasia esculents, Xanthosoma sagittifolium, Root and Tuber Crops, Nigeria, Rivers State, Food Security, Genetic Diversity, Conservation, Molecular Characterization, DNA Extraction, PCR, Sequencing, Genetic Markers, SSR, Phylogenetic Analysis, Germplasm, Cultivars, Genotyping, Neighbor-joining.

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## INTRODUCTION

Xanthosoma and Colocasia are tropically grown for their cultural ceremonial uses and delicious corms, cormels, and leaves; they are members of the Araceae (aroid) family. Colocasia has about 25 species and Xanthosoma has about 50. More than 150 species belonging to 100 genera make up this large family; they include epiphytes, vines, creepers, and

terrestrial plants. It is a lowland crop, most likely because to its high moisture requirements for production and its sensitivity to temperature, which is likely caused by its huge transpiring surfaces.

Edible aroids—particularly Colocasia esculenta (taro) and Xanthosoma sagittifolium (cocoyam)—are culturally and nutritionally significant root and tuber crops widely cultivated in West Africa (Bammite et al., 2021). Despite their importance, they remain underutilized and genetically under-characterized in many Nigerian states, including Rivers State (Bammite et al., 2021).

Traditional morphological identification of aroids is often compromised by high phenotypic plasticity and environmental influences, leading to misclassification and redundancy within germplasm collections (Bammite et al., 2021; Fufa et al., 2022). In contrast, molecular techniques provide precise, reproducible insight into genetic diversity, population structure, and cultivar distinctiveness (Fufa et al., 2022; Fufa et al., 2022).

Recent studies across West Africa have highlighted both molecular and morphological approaches in assessing genetic diversity of aroids: a study in Benin catalogued farmer-recognized varieties of taro and cocoyam using agro-morphological descriptors, while research conducted in Nigeria using DArTSeq-SNP markers revealed substantial genetic diversity and clustering within Colocasia accessions (Bammite et al., 2021; Oladimeji, 2025). For complete genetic information on Nigerian populations of taro, Fufa et al. (2022) employed SNP-based genotyping. This ascertained that their genetic architectures and variances differ, which could be useful for breeding and conservation.

Given the lack of molecular data on Rivers State germplasm, this study aims to:

**Analyse the genetic makeup** of the aroids using techniques like DNA sequencing. This helps in: Identifying and classifying species accurately at the genetic level, understanding the genetic relationships between different aroid varieties and assessing genetic diversity within and between populations.

## MATERIALS AND METHOD

**Study Area:** The study was conducted in three local government areas of Rivers State namely: Obio/Akpor, Emohua and Ikwerre. However, planting of plants, watering, experimental set up and sample collection were done in one community, each, from these local government areas. Station 1 was Aluu in Ikwerre LGA; Station 2 was in Isiodu in Emohua LGA while Station 3 Ozuoba Community in Obio/akpor LGA. The research farm for Omuoko in Aluu lies on longitude 04 56' 01.3N and latitude 006 55'17.2E. That of Isiodu in Emohua lies on longitude 04 55' 25.4N and latitude 006 52'09.5E. Ozuoba in Obio/Akpor lies on longitude 04 52' 20.4N and latitude 006 55'47.8E.

**Sources of Materials:** The materials used for this study comprised of plants part (leaves) of the two genera of cocoyam namely: Colocasia esculenta (taro) and Xanthosoma sagittifolium (tannia). Out of these genera, three cultivars each, were obtained from each communities were they were planted and grown. The cultivar was: The Xanthosoma species were NXs001 (Ede ocha) and NXs003 (Okorokoro), whereas the Colocosia species were NCe001 (Coco India), NCe002 (Ede ofe green), and NCe003 (Ede ofe purple).

**Duration of the Study:** This field experiment was conducted in June, 2021 to January, 2022 and repeated in June, 2022 and January, 2023 cropping seasons on fourteen point 10meters by six point five meters (14.10m x 6.5m) plot size.

**Plants Used for the Study:** Taro (colocasia esculanta): coco india - NCE 001, Ede ofe green – NCE 002, Ede ukpong – NCE 003. Xanthosoma sagittifolium: Ede ocha – NXS 001, Ede uhie – NXS 002, Ede okorokoro – NXS 003. All accessions were previously gotten from National Root Crop Institute (NRCRI), Umudike, South East Nigeria. The planting materials which included the sprouted small cocoyam corm of the accession were collected from National Root Crop Institute (NRCRI), Umudike with moderated sized corm of weight 50-100g.

### Molecular Studies

i. **DNA Extraction and Genotyping:** The genomic DNA of the plant species for the study will be extracted at the Regional Centre for Biotechnology, University of Port Harcourt, Nigeria and shipped for Diversity Arrays Technology (DArT) sequencing at the BecA/ILRI, Nairobi-hub.

ii. **Plant DNA Extraction Protocol:** Using the Zymo Quick DNA Plant/Seed Mini PrepKit, the plant DNA was extracted.

### Nano Drop Spectrophotometry

### Determination of DNA Concentration and Purity Using Nanodrop

The DNA's concentration and purity were assessed using Thermo Fisher Scientific's NanoDrop 2000c spectrophotometer. Purity is measured as the ratio of ultraviolet (UV) light absorption at 260 nm to that at 280 nm.

**Gel Electrophoresis:** In order to obtain a transparent solution, 0.75g of agarose powder and 50ml of 1X Tris Boris EDTA (TBE) buffer were combined in measuring flask and microwaved for two minutes. This was done using 1.5% agarose gel.

**Polymerase Chain Reactioin (PCR):** The target region used as amplified was OneTaq® Quick-Load® 2X Master Mix (New England Biolabs, USA, Catalogue No. M0486) in Eppendorf Mastercycler (Nexus Gradient 230, Germany. . The primer sets that was used comprised forward primer RBCL 1 – AACACCAGCTTTRAATCCAA and reverse primer - ATP - ACATCKARTACKGGACCAATAA.

PCR products that were purified using enzymatic method (ExoSAP).

### Data analysis

To determine the evolutionary history of six accessions, sequences were first processed using Chromas Lite

for base calling and the edited with BioEdit. Then, using a compiled matrix of nucleotide sequences, a phylogenetic tree was built using MEGA 7.0.26's Unweighted Pair Group Method with Arithmetic Mean (UPGMA) hierarchical clustering technique. After deleting gaps and missing data, the final data set consisted of 558 sites, from which evolutionary distances were computed using Maximum Composite Likelihood technique.

### Phylogenetic Tree

The evolutionary history of the five nucleotide sequences was determined using the Neighbour-Joining method in MEGA 11. The evolutionary distances, which were determined by the Jukes-Cantor technique and represented number of base substitutions per site, are proportional to it branch lengths of the phylogenetic trees. The reliability of the tree was assessed with a bootstrap test of 500 replicates and all ambiguous positions were removed from the final dataset of 668 total positions.

## 4.3 RESULTS AND DISCUSSIONS

**Table 1:** NanoDrop spectrometry characteristics of the genomic DNA from the plant varieties

| S/N | Isolate code | A260 | A280 | Purity ($\frac{A260}{A280}$) | DNA Concentration (ng/µl) |
|---|---|---|---|---|---|
| 1 | RCBBR_P1 | 2.3565 | 1.2935 | 1.82 | 117.85 |
| 2 | RCBBR_P2 | 1.8385 | 0.988 | 1.86 | 91.95 |
| 3 | RCBBR_P3 | 2.0555 | 1.1015 | 1.87 | 102.75 |
| 4 | RCBBR_P4 | 1.448 | 0.762 | 1.9 | 72.4 |
| 5 | RCBBR_P5 | 2.7595 | 1.4965 | 1.85 | 137.95 |
| 6 | RCBBR_P6 | 2.0755 | 1.125 | 1.85 | 103.75 |
| 7 | RCBBR_P7 | 2.3015 | 1.248 | 1.85 | 115.1 |
| 8 | RCBBR_P8 | 1.486 | 0.793 | 1.88 | 74.25 |
| 9 | RCBBR_P9 | 2.928 | 1.7275 | 1.7 | 146.4 |
| 10 | RCBBR_P10 | 0.769 | 0.3985 | 1.93 | 38.45 |
| 11 | RCBBR_P11 | 1.239 | 0.6955 | 1.78 | 62 |
| 12 | RCBBR_P12 | 0.9695 | 0.501 | 1.94 | 48.5 |

The result of the quantification and purity of the DNA sample of the varieties studied are shown in table 1 above. The concentration of the isolated genomic DNA of the varieties studied ranged between 48.5 -11785. The standard DNA concentration ideal for sequencing is 50ng/µl, however the genomic DNA of some of the sample leaves was very high since it was above the standard DNA concentration. A concentration of 117 ng/µL is relatively high and may be because of using a larger amount of starting material (e.g., plant tissue, cells, or blood) or minimal DNA loss during elusion steps or washes. The purity as measured by the absorbance ratio A260/280nm (DNA/RNA) ranged between 0.501nm- 1.4965nm and the A260/230nm ranged between 0.769nm – 2.928nm as the secondary measure of nucleic acid purity for the presence or absence of co-purified contaminants such as proteins before sequencing. In terms of purity, however, all the samples fall under range for DNA purity. Making them good for sequencing.

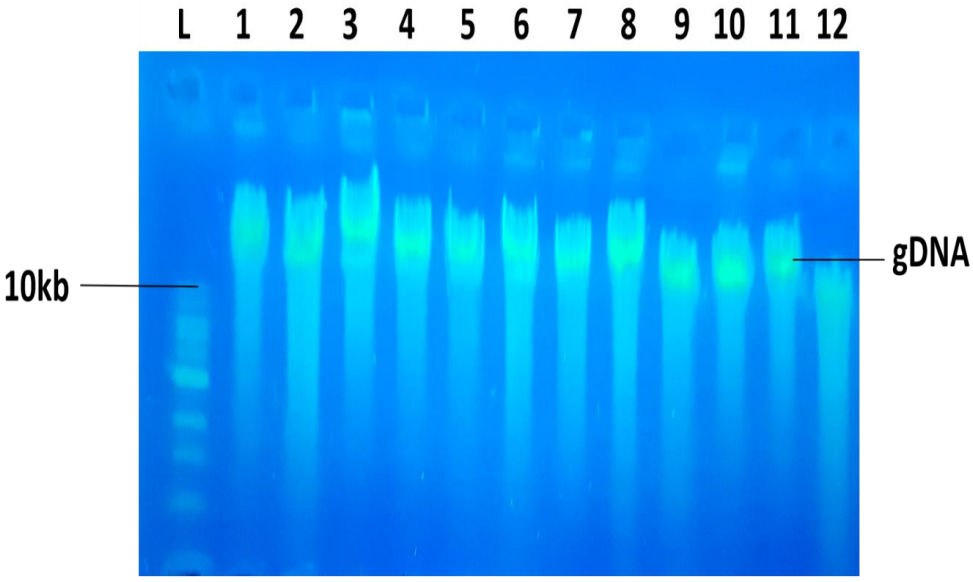


**Plate 1:** Gel electrophoresis image of the genomic DNA from the plants (lane 1= DNA ladder; Lane 2-13 = genomic DNA of the plants in ascending order: RCBBR_P1=RCBBR_P12).

The result from plate 1 shows the agarose gel electrophoresis image of the genomic DNA from plant varieties. The DNA band result indicate that good quality and intact genomic DNA were obtained on 1% Agarose gel electrophoresis with ethidium bromide as staining reagent.

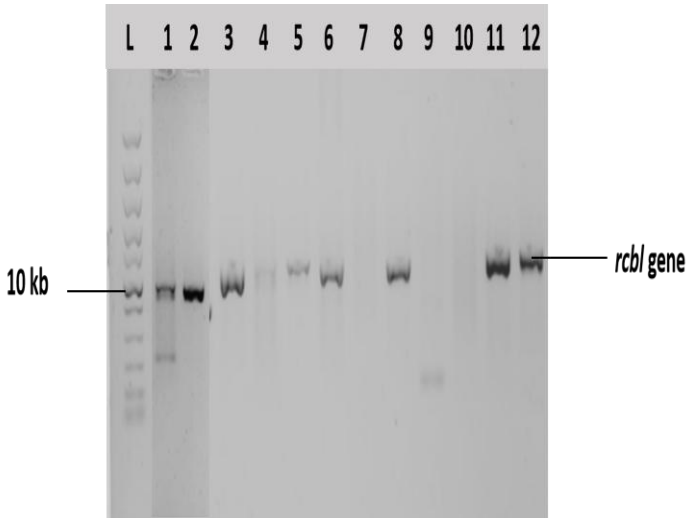


**Plate 2:** Agarose gel electrophoresis of the *rbcl* gene amplicons from the plants

The result of amplified DNA after polymerase chain reaction and gel electrophoresis is shown in Plate 2. From the results, all samples showed clear amplifications of the genomic DNA. The result indicated that the DNA of the varieties studied were successfully amplified. It was also observed that the sequences had band sizes of about 10kb.

The format of the sequence data when there was converted to FASTA. FASTA format displays text-based results which represent nucleotide sequences in base pairs by single letter code. These sequences were then sent to the NBCBI database for phylogenetic analysis.

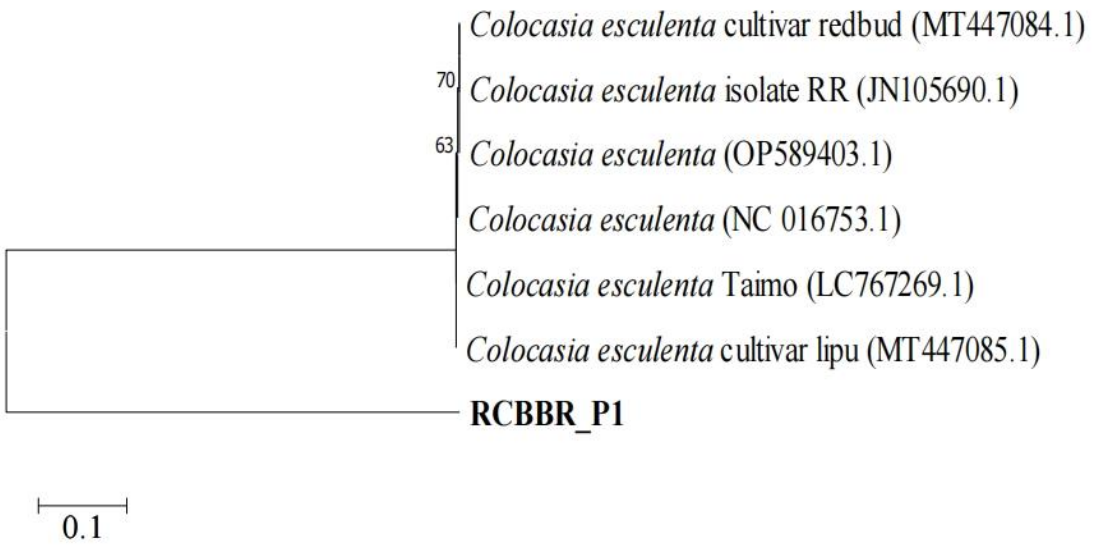


**Figure 1:** Neighbor-joining evolutionary relationship of the plant variety

Figure 1 presents a dendrogram showing the evolutionary relationships between Colocasia esculenta samples and reference sample RCBBR_P1. The C. esculenta samples cluster closely, indicating a shared ancestry, with bootstrap values (70 and 63) providing moderate support. RCBBR_P1 forms a distinct branch, suggesting it is genetically divergent and may represent a separate lineage.

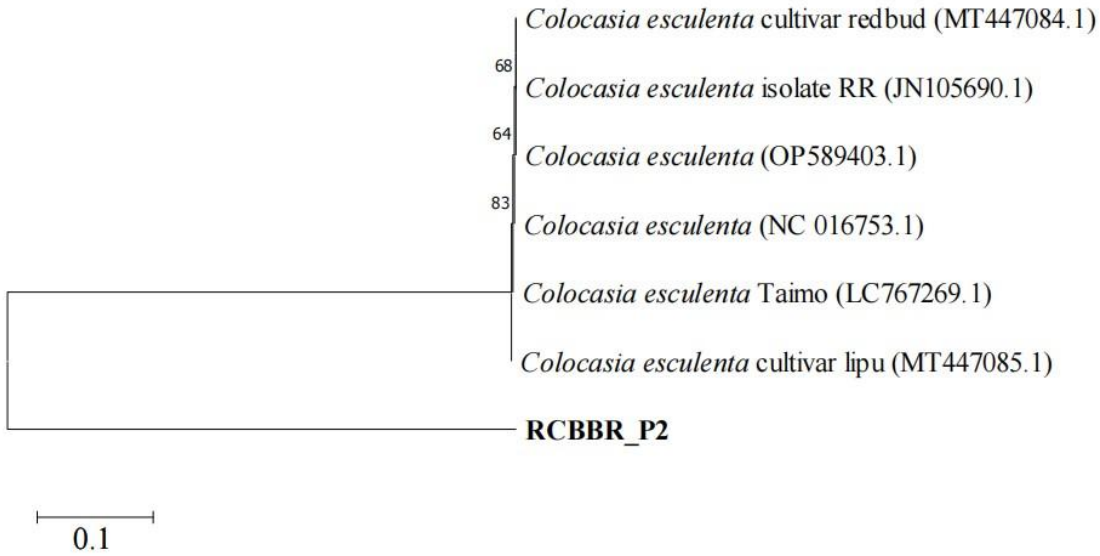


**Figure 2:** Neighbor-joining evolutionary relationship of the plant variety

## Colocasia esculenta samples and RCBBR_P2.

The C. esculenta samples form a tight cluster, indicating close genetic relatedness and a shared recent ancestor. RCBBR_P2 branches separately, suggesting it is a genetically distinct or divergent lineage. Bootstrap values (64-83) provide moderate to strong support for the groupings and the scale bar indicates 10% genetic divergence at a value of 0.1.

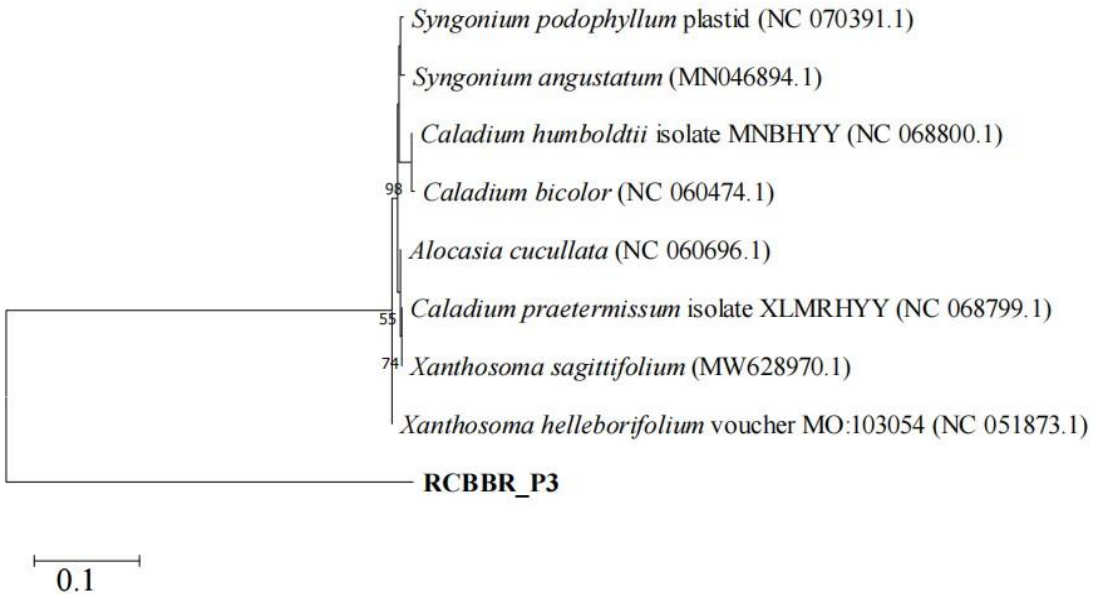


**Figure 3:** Neighbor-joining evolutionary relationship of the plant variety

Figure 3 presents a phylogenetic tree showing relationships among Araceae species, including Syngonium, Caladium, Alocasia, and Xanthosoma, with RCBBR_P3 as an additional sample. RCBBR_P3 forms a separate, distant branch, indicating it is genetically distinct and may represent a novel or unclassified lineage. Strong to moderate bootstrap values (98, 74, 55) support the internal clustering of related genera. The tree highlights RCBBR_P3 as an evolutionary outlier within the Araceae family.

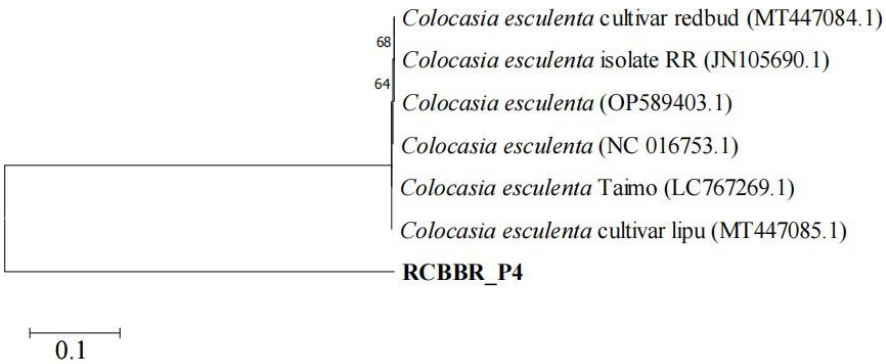


**Figure 4:** Neighbor-joining evolutionary relationship of the plant variety

Figure 4 presents a phylogenetic tree highlighting relationships among Colocasia esculenta samples and the distinct sample RCBBR_P4. All samples except RCBBR_P4 cluster closely, indicating strong genetic similarity and a shared lineage within C. esculenta. RCBBR_P4 branches independently, suggesting it is genetically divergent and may represent a separate species or lineage. Bootstrap values (68, 64) and the 0.1 scale bar support the confidence and degree of divergence in the tree structure.

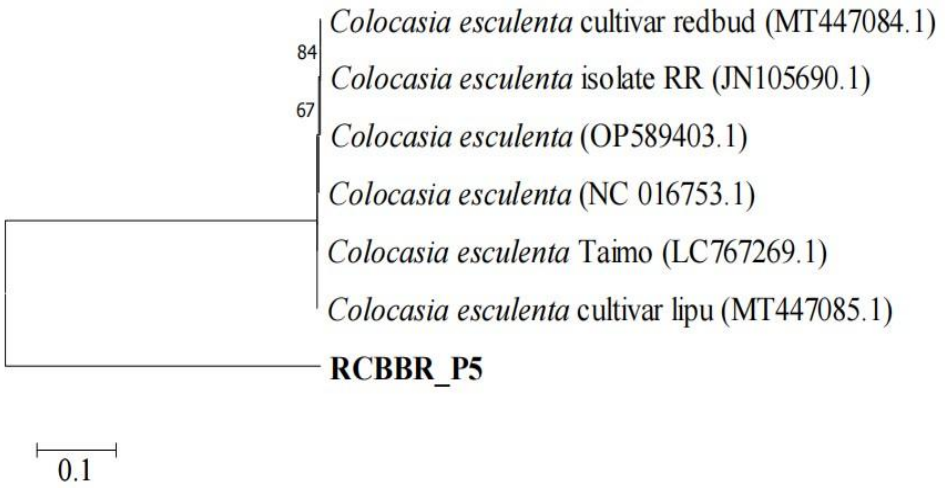


**Figure 5:** Neighbor-joining evolutionary relationship of the plant variety

Figure 5 presents a neighbor-joining phylogenetic tree showing genetic relationships among Colocasia esculenta accessions and RCBBR_P5. While the C. esculenta samples form a close cluster with strong-to-moderate bootstrap support (84, 67), RCBBR_P5 branches off independently. Its long branch length indicates significant genetic divergence from the others. This suggests RCBBR_P5 may represent a different species, a unique variant, or an unclassified subgroup within Colocasia.

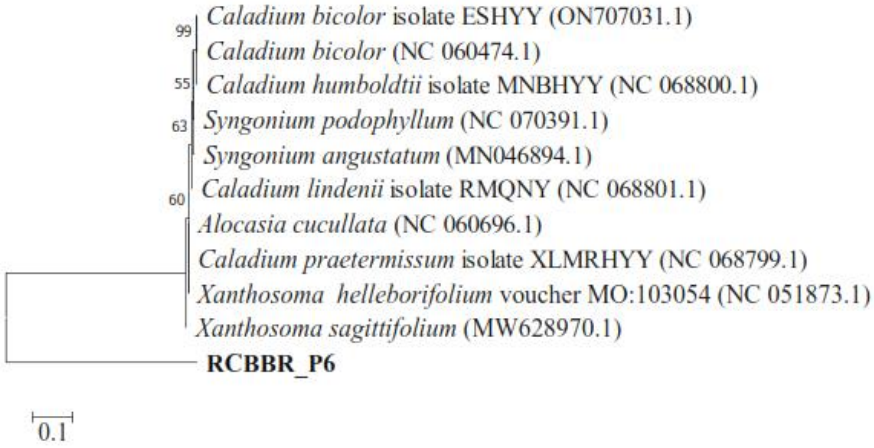


**Figure 6:** Neighbor-joining evolutionary relationship of the plant variety

Figure 6 shows a neighbor-joining phylogenetic tree of various Araceae species, highlighting evolutionary relationships among genera like Caladium, Syngonium, Alocasia, and Xanthosoma. RCBBR_P6 branches separately, indicating it is genetically distinct and possibly a novel or uncharacterized lineage. Bootstrap values (99–55) provide varying confidence levels for specific clades, with strong support for Caladium bicolor isolates. The tree underscores RCBBR_P6 as a genetic outlier, suggesting further investigation into its taxonomic identity.

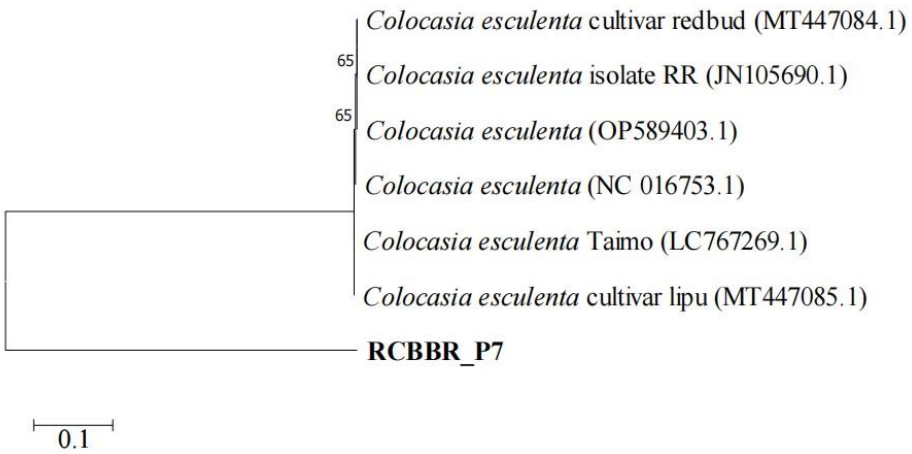


**Figure 7:** Neighbor-joining evolutionary relationship of the plant variety

Figure 7 presents a phylogenetic tree showing evolutionary relationships among Colocasia esculenta samples and RCBBR_P7. While C. esculenta cultivars cluster closely with moderate bootstrap support (65), RCBBR_P7 branches off distinctly, indicating significant genetic divergence. Its long branch suggests it may represent a divergent lineage, subspecies, or a closely related species. Further molecular analysis is needed to clarify RCBBR_P7's taxonomic identity.

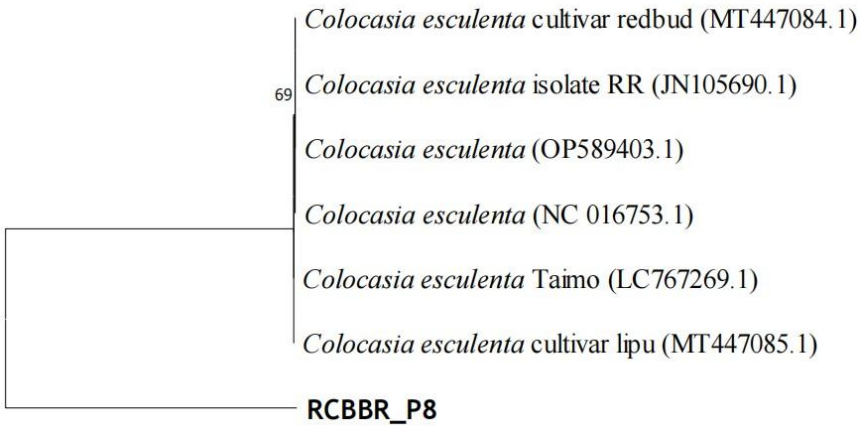


**Figure 8:** Neighbor-joining evolutionary relationship of the plant variety

Figure 8 shows a phylogenetic tree comparing Colocasia esculenta samples with RCBBR_P8. While the C. esculenta sequences cluster tightly with moderate bootstrap support (69), RCBBR_P8 forms a separate, distant branch. This suggests RCBBR_P8 may represent a divergent lineage, distinct species, or hybrid within the Colocasia genus. Further genetic analysis is needed to confirm its identity and evolutionary placement.

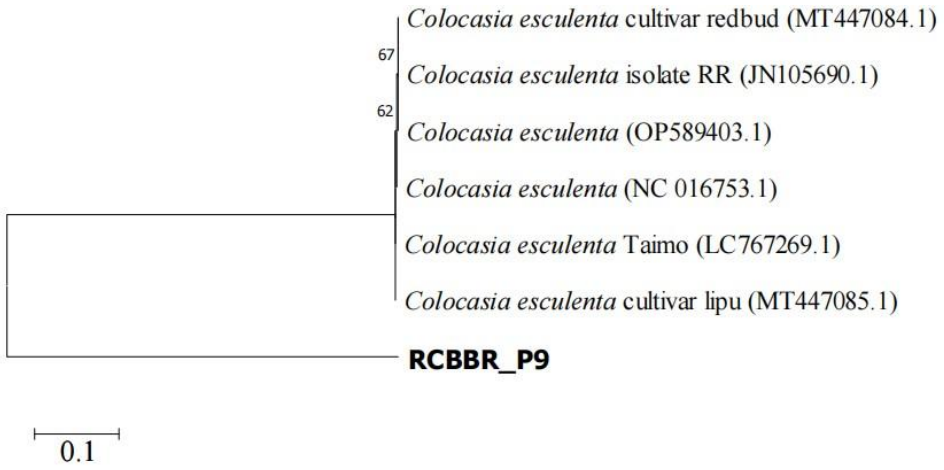


**Figure 9:** Neighbor-joining evolutionary relationship of the plant variety

Figure 9 displays a phylogenetic tree comparing Colocasia esculenta accessions with RCBBR_P9. All C. esculenta samples cluster together, while RCBBR_P9 forms a distinct branch, indicating notable genetic divergence. Bootstrap values (67, 62) provide moderate support for internal relationships. The Long Branch leading to RCBBR_P9 suggests it may be a unique variant, different species, or a highly divergent lineage within the Colocasia genus.

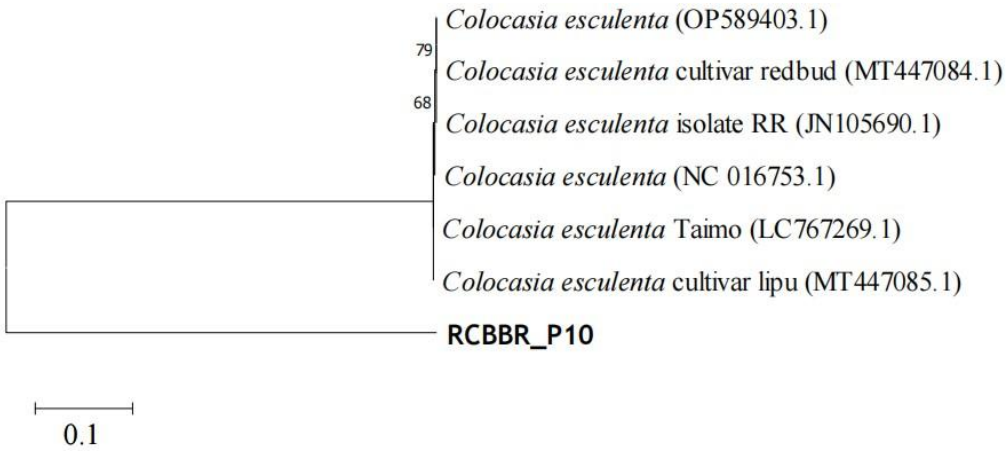


**Figure 10:** Neighbor-joining evolutionary relationship of the plant variety

Figure 10 presents a phylogenetic tree comparing Colocasia esculenta accessions with RCBBR_P10. RCBBR_P10 appears on a distinct, long branch, indicating significant genetic divergence from the C. esculenta group. Bootstrap values of 79 and 68 offer moderate to strong support for the clustering of other accessions. This suggests RCBBR_P10 may represent a unique variant, a different species, or a highly divergent lineage within the Colocasia genus.

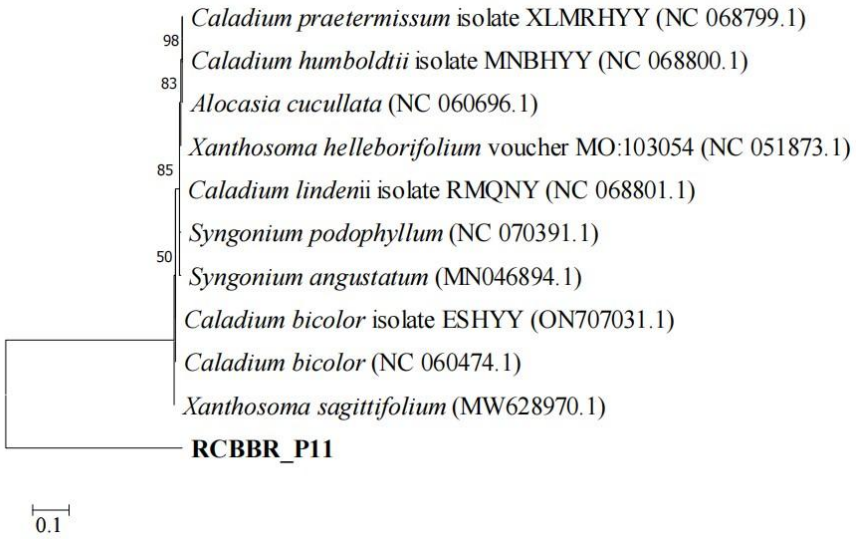


**Figure 11:** Neighbor-joining evolutionary relationship of the plant variety

Figure 11 illustrates a phylogenetic tree comparing RCBBR_P11 with various Araceae species, including Caladium, Alocasia, Xanthosoma, and Syngonium. RCBBR_P11 forms a long, distinct branch, indicating significant genetic divergence from the other taxa. Bootstrap values (98, 85, 83, 50) provide varying levels of confidence in the tree's groupings. The placement of RCBBR_P11 suggests it may represent a separate species or a highly divergent lineage within the Araceae family.

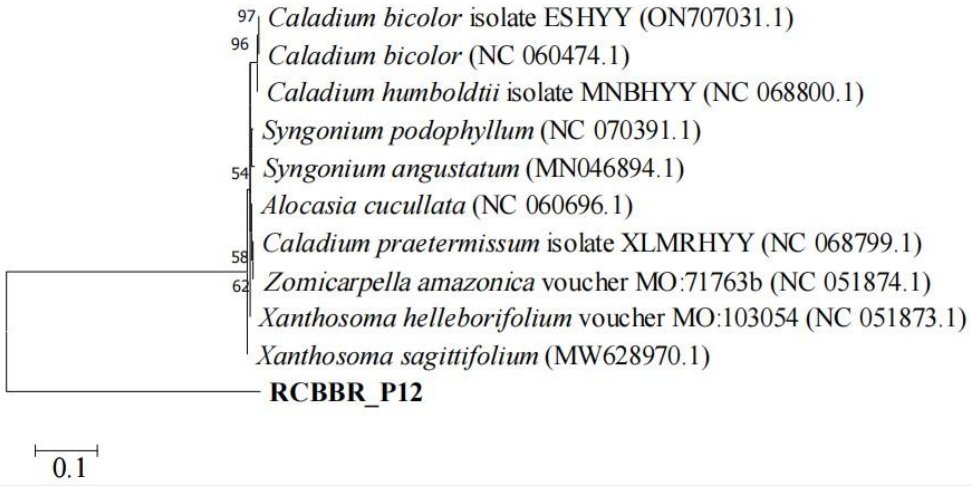


**Figure 12:** Neighbor-joining evolutionary relationship of the plant variety

Figure 12 displays a phylogenetic tree comparing various Araceae species with the sample RCBBR_P12. RCBBR_P12 forms a long, distinct branch, indicating significant genetic divergence from all other taxa. Strong bootstrap values (97, 96) support close clustering among Caladium and Syngonium species, while lower values (54, 58) suggest moderate confidence in other groupings. The position of RCBBR_P12 suggests it may be a highly divergent variant or an uncharacterized lineage within the Araceae family.

**Table 2:** GenBank closest matches and percentage similarity of the different cocoyam varieties**.**

| S/N | Strain | Organism | Closest GenBank Match | Similarity (%) |
|---|---|---|---|---|
| 1 | RCBBR_P1 | *Colocasia esculenta* | *Colocasia esculenta* cultivar lipu | 98.68 |
| 2 | RCBBR_P2 | *Colocasia esculenta* | *Colocasia esculenta* cultivar lipu | 98.10 |
| 3 | RCBBR_P3 | *Xanthosoma sagittifolium* | *Xanthosoma sagittifolium* | 99.47 |
| 4 | RCBBR_P4 | *Colocasia esculenta* | *Colocasia esculenta* cultivar lipu | 97.96 |
| 5 | RCBBR_P5 | *Colocasia esculenta* | *Colocasia esculenta* cultivar lipu | 98.70 |
| 6 | RCBBR_P6 | *Xanthosoma sagittifolium* | *Xanthosoma sagittifolium* | 96.19 |
| 7 | RCBBR_P7 | *Colocasia esculenta* | *Colocasia esculenta* cultivar lipu | 97.93 |
| 8 | RCBBR_P8 | *Colocasia esculenta* | *Colocasia esculenta* cultivar lipu | 99.05 |
| 9 | RCBBR_P9 | *Colocasia esculenta* | *Colocasia esculenta* cultivar lipu | 98.18 |
| 10 | RCBBR_P10 | *Colocasia esculenta* | *Colocasia esculenta* cultivar lipu | 97.97 |
| 11 | RCBBR_P11 | *Xanthosoma sagittifolium* | *Xanthosoma sagittifolium* | 97.05 |
| 12 | RCBBR_P12 | *Xanthosoma sagittifolium* | *Xanthosoma sagittifolium* | 99.47 |

The results in table 2 shows the genebank data from the NCBI data base indicating percentage similarity of the reference samples with samples sequence from the database. From the result, RCBBR_P3, RCBBR_P12 (Xanthosoma sagittifolium) and Xanthosoma sagittifolium from the data base show the highest percentage similarity of 99.47. While RCBBR_P6 (Xanthosoma sagittifolium) and Xanthosoma sagittifolium from the data base has the percentage similarity score of 96.19 which is the lowest. The two basic species with the identified. X. sagittifolium and C. esculenta.

## Phylogenetic Tree of the Plant Varieties Studied

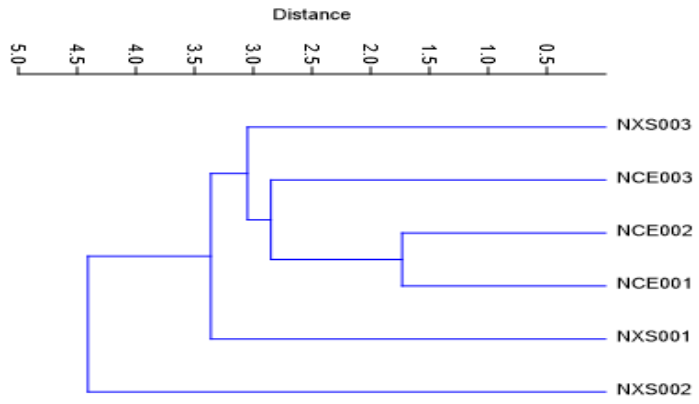


**Figure 13**

Figure 13 results show a phylogenetic tree with a distance scale (0–5) indicating genetic similarity among plant varieties. NXS003, NCE003, NCE002, and NCE001 form a close cluster, suggesting high relatedness. NXS003 and NCE003 are the most similar pair, while NXS001 and NXS002 are more distantly related. NXS002 appears to be the most genetically distinct variety in the group.

**Phylogenetic Tree of the Varieties Studied Which Depict Relationships Between Items Based On Similarity or Evolutionary Distances.**

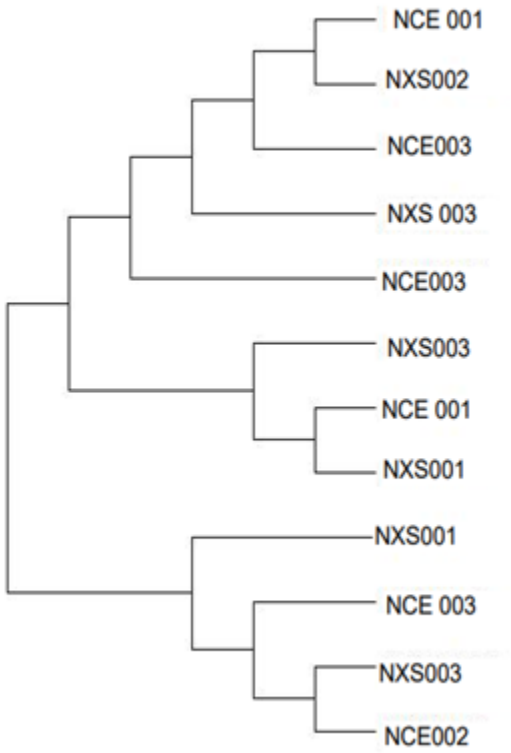


**Figure 14**

Figure 14 results present a phylogenetic tree illustrating evolutionary relationships among studied varieties based on similarity. NCE001 and NXS002 are more closely related to each other than to NCE003. Repeated appearances of NCE003 and NXS003 suggest multiple replicates or analytical splits. The clustering reflects varying degrees of genetic or evolutionary similarity among the varieties.

## DISCUSSION

The phylogenetic analysis of the RCBBR samples using the Neighbor-Joining (NJ) method has provided significant insight into the genetic relationships of these accessions within the Araceae family, particularly in relation to Colocasia esculenta and Xanthosoma sagittifolium. The placement of samples in the phylogenetic tree, supported by bootstrap values and sequence similarity from GenBank, allows for meaningful interpretations regarding their evolutionary lineage and potential novelty. The results from the phylogenetic trees revealed that samples RCBBR_P1, P2, P4, P5, P7, P8, P9, and P10 consistently clustered with Colocasia esculenta accessions such as cultivar lipu (MT447085.1), isolate RR (JN105690.1), and Taimo (LC767269.1). The GenBank similarity scores ranging between **97.93% and 99.05%** indicate that these samples are genetically like C. esculenta, suggesting they may represent landraces, cultivars, or locally adapted variants of the species. This is consistent with previous molecular studies that have reported low genetic divergence among cultivated varieties of C. esculenta due to clonal propagation and limited sexual recombination (Muasya et al., 2021). Interestingly, samples such as RCBBR_P3, P6, P11, and P12 clustered with Xanthosoma sagittifolium, showing similarity values up to **99.47%** for P3 and P12, and as low as **96.19%** for P6. These findings suggest that while P3 and P12 are closely related to the known X. sagittifolium, sample P6 may represent a **genetically divergent or under-characterized variant**. This aligns with findings by Scarcelli et al. (2006), who highlighted the significant genetic diversity within Xanthosoma populations due to their broad geographical distribution and possible hybridization events. Samples such as RCBBR_P6 and RCBBR_P11 displayed long branch lengths and formed isolated clades, indicating substantial genetic divergence. In phylogenetics, long branches typically reflect either accelerated evolution, sequencing artifacts, or truly distinct taxa (Tamura et al., 2011). As a result, the samples may be indicative of novel lineages or species that are poorly characterized on public databases. In an attempt to

better understand their taxonomic status, additional studies through whole-genome sequencing or multilocus methods are needed. Interestingly, bootstrap values were used to validate the phylogenetic assignments. High values ( 98 for RCBBR_P3 and X. sagittifolium) indicate strong support for their evolutionary relationship. Moderate bootstrap support ( 63–70) for internal groupings of C. esculenta is typical in analyses involving closely related cultivars, where genetic divergence is minimal (Lebot et al., 2004). That Colocasia esculenta samples always yielded close clades, indicating degrees of genetic closeness, is yet another strong feature. Their shared history of domestication and tropical Asian and African origins most likely account for their high degree of similarity (Matthews, 2004). Their evolutionary divergence of the Araceae genera, Xanthosoma, Alocasia, Syngonium, and Caladium, is seen in their wider clade. This is because of the extremely varied physical and genetic makeup of Araceae, as previously documented by Mayo et al. (1997).

## CONCLUSION

In summary, the results confirm that most RCBBR samples are genetically aligned with C. esculenta and X. sagittifolium, with a few showing notable divergence. The phylogenetic trees show that within these taxa there may be new lineages or distinct local genotypes. The findings are useful to understand the evolutionary history of tropical root and tuber crops, biodiversity conservation, and improving agriculture practice. The research needs further molecular and morphological characterisation for its validation and identification of possible agronomic use.

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