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Research Paper

Diversity Analysis and Identification of Duplicates of Conserved Sweet Potato (*Ipomoea batatas* (L.) Lam.) Accessions by Morphological Characterization

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Abstract

The conservation of sweet potato germplasm will aid in sustaining biodiversity and ensuring food security. The Plant Genetic Resources Centre (PGRC) has conserved 120 sweet potato accessions which have not been fully characterized yet, and in doing so, identifying duplicates will be needed for the efficient conservation and management of genetic resources. The main objective of this study was to access the genetic diversity of PGRC conserved sweet potato germplasm and identify the presence of duplicates. The data for 09 quantitative traits and 33 qualitative traits of 100 accessions were characterized during the 2019 *Yala* and 2019/20 *Maha* seasons using the international standardized descriptor for sweet potato. The variations were analyzed using cluster analysis and principal component analysis (PCA) with the support of Minitab 17. The results indicated four distinct clusters at a distance of 24.07, and highlighting that there are no duplicates within the collection. The cluster 4 represents 72 accessions and cluster 3 comprises 25 accessions. Cluster 1and 2 represents 2 and 1 accessions, respectively. The results of PCA illustrated that at least 10 PCs are required to explain 70.3%of the character variations among the 100 accessions. The four key characters; petiole pigmentation, primary vine color, predominant flesh color, and secondary skin color, are the significant contributors in high diversity. The findings of this study will be useful for systemic germplasm conservation and utilizing prominent traits in crop varietal improvement programmes.

Keywords: Cluster analysis, Genetic diversity, Duplicates, Morphological characterization, Principal component analysis.

Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) is a vital food crop and income source for millions worldwide. It is extensively cultivated in Africa, Asia, and Latin America, serving as a significant source of carbohydrates, minerals, vitamins, and dietary fiber (Alam, 2021). Additionally, sweet potato contains bioactive compounds

like anthocyanins, carotenoids, and phenolic compounds, which have health-promoting properties (Kaur *et al*., 2022).

The conservation of sweet potato genetic resources is crucial for breeding programs aimed at developing new cultivars with desirable traits, such as disease resistance and high yields. The Plant Genetic Resources Centre (PGRC) is the national institute responsible for preserving and distributing the genetic resources of crop plants and their wild relatives in Sri Lanka (Jayasuriya, *et al*., 1999). PGRC plays a vital role in conserving root and tuber crops such as sweet potato, *Dioscorea*, potato, and country potato accessions in Sri Lanka. PGRC has conserved over 120 accessions of sweet potato, including all cultivated varieties prevalent in Sri Lanka as well as accessions from the International Potato Center (CIP) in Peru.

However, maintaining a large collection of accessions encounters challenges. Identifying duplicates is essential to avoid redundancy and to conduct diversity analysis for effective conservation. Duplicate accessions waste resources and can lead to the loss of unique genetic traits. They can also skew diversity analysis and affect the accuracy of genetic diversity estimates (Valerie, *et al.*, 2004). Therefore, identifying duplicates is crucial for the efficient management of genetic resources.

Morphological characterization is a reliable method for identifying duplicates and analyzing the diversity of sweet potato accessions. This method involves observing and measuring plant traits, such as leaf shape, stem color, and tuber shape, to distinguish one accession from another (Karuri, *et al*., 2010). It is cost-effective, simple, and does not require specialized equipment or technical expertise (Govindaraj, *et al*., 2015).

Although molecular markers provide information that is more detailed by detecting genetic variation at the DNA level, their analysis requires specialized equipment, technical expertise, and can be costly. Hence, Morphological characterization continues to be a widely used method for assessing sweet potato germplasm, particularly in resource-limited countries.

The identification of duplicates and assessment of genetic diversity have significant implications for sweet potato breeding programs. A diverse germplasm collection provides a broad range of genetic traits, enabling breeders to develop new cultivars with desirable traits such as high yield, disease resistance, and nutritional quality. A germplasm collection with low genetic diversity can hinder breeding progress and increase the risk of inbreeding depression (Begna & Teressa, 2024).

Moreover, conserving sweet potato genetic resources contributes to food security and sustainable development. Sweet potato is a climate resilient crop that can withstand harsh environmental conditions such as drought and heat stress (Alam, 2021). Therefore, conserving sweet potato germplasm provides a valuable resource

for farmers in regions prone to climate variability, contributing to the sustainability of agricultural production (Bhattarai, *et al*., 2017).

Characterizing 100 accessions helps to investigate the diversity of the collection for gap analysis and core collection development. This improves the efficiency of the collection by eliminating duplicate accessions and identifying gaps in unique traits that are not represented. Despite the considerable diversity in sweet potato with many beneficial traits, these have not been well-analyzed, resulting in low credibility of available information. Therefore, detailed morphological characterization of sweet potato accessions is a timely requirement.

This study is aimed at assessing the genetic diversity and identifying duplicates of sweet potato accessions conserved at the PGRC using morphological characterization. This information will be valuable for the efficient management and sustainable use of sweet potato genetic resources.

Materials and methods

Experimental site and field operations

The study was conducted in the PGRC experimental field at Gannoruwa, in the WM2b Agro-Ecological Zone at 479 meters above sea level. During the study, average temperatures ranged from 22-28 °C, with rainfall amounts of 829.4 mm in the 2019 *Yala* season and 1018.7 mm in the 2019/20 *Maha* season. The relative humidity was 82%, and the average soil temperature at a depth of 5 cm was 28.6 °C (DOA, 2019).

Plant materials

For this study, 100 sweet potato accessions from the PGRC collection were selected. These accessions were previously uncharacterized, ensuring that no two accessions shared the same code (Table 1).

Morphological characterization

This study was conducted during the 2019 *Yala* and 2019/20 *Maha* seasons. A total number of 100 sweet potato accessions, including eight DOA (Department of Agriculture) recommended check varieties were used (Table 2).

Eight-inch cuttings were rooted in water for four days before planting in an augmented plot design. Each plot consisted of five holes with three cuttings per hole, making a total of 15 cuttings per plot. Accessions and check varieties were randomly assigned within the blocks.

Data collection

Data for each morphological trait were recorded using "The Standardized Descriptor for Sweet Potato" by CIP, AVRDC, and IBPGR (1991). A total of 42 traits, including 9 quantitative and 33 qualitative characters, were recorded to differentiate accessions (Table 3). Color related qualitative characters in tubers, vines, and leaves were measured using the Royal Horticultural Society (RHS) color chart (2015).

Table 1. Sweet potato accessions used for the morphological characterization

No.	Accession No.	Source	No.	Accession No.	Source	No.	Accession No.	Source
1	BTSP 01	HORDI	35	BTSP 57	HORDI	68	BTSP 95	CIP
2	BTSP 04	HORDI	36	BTSP 58	RARDC Bandarawela	69	BTSP 96	CIP
3	BTSP 05	HORDI	37	BTSP 59	PGRC	70	BTSP 97	CIP
4	BTSP 08	HORDI	38	BTSP 60	HORDI	71	BTSP 98	CIP
5	BTSP 09	HORDI	39	BTSP 61	HORDI	72	BTSP 99	CIP
6	BTSP 13	HORDI	40	BTSP 62	CIP	73	BTSP 100	CIP
7	BTSP 14	CIP	41	BTSP 63	PGRC	74	BTSP 101	PGRC
8	BTSP 16	HORDI	42	BTSP 66	PGRC	75	BTSP 102	CIP
9	BTSP 17	HORDI	43	BTSP 67	PGRC	76	BTSP 103	CIP
10	BTSP 19	CIP	44	BTSP 68	PGRC	77	BTSP 104	CIP
11	BTSP 20	CIP	45	BTSP 69	PGRC	78	BTSP 105	CIP
12	BTSP 21	HORDI	46	BTSP 70	PGRC	79	BTSP 106	CIP
13	BTSP 23	HORDI	47	BTSP 72	PGRC	80	BTSP 108	CIP
14	BTSP 24	HORDI	48	BTSP 73	HORDI	81	BTSP 109	CIP
15	BTSP 25	HORDI	49	BTSP 74	PGRC	82	BTSP 111	CIP
16	BTSP 29	HORDI	50	BTSP 76	HORDI	83	BTSP 112	CIP
17	BTSP 30	HORDI	51	BTSP 77	HORDI	84	BTSP 115	CIP
18	BTSP 31	HORDI	52	BTSP 78	HORDI	85	BTSP 116	CIP
19	BTSP 32	HORDI	53	BTSP 79	HORDI	86	BTSP 117	CIP
20	BTSP 35	HORDI	54	BTSP 80	HORDI	87	BTSP 118	CIP
21	BTSP 37	HORDI	55	BTSP 81	HORDI	88	BTSP 119	HORDI
22	BTSP 38	HORDI	56	BTSP 82	PGRC	89	BTSP 120	HORDI
23	BTSP 39	HORDI	57	BTSP 83	PGRC	90	BTSP 121	HORDI
24	BTSP 40	HORDI	58	BTSP 84	PGRC	91	BTSP 123	HORDI
25	BTSP 41	HORDI	59	BTSP 85	HORDI	92	BTSP 124	HORDI
26	BTSP 44	HORDI	60	BTSP 87	CIP	93	BTSP 125	RARDC Bandarawela
27	BTSP 46	RARDC Bandarawela	61	BTSP 88	CIP	94	BTSP 126	HORDI
28	BTSP 49	PGRC	62	BTSP 89	CIP	95	BTSP 130	HORDI
29	BTSP 50	PGRC	63	BTSP 90	CIP	96	BTSP 133	PGRC
30	BTSP 52	PGRC	64	BTSP 91	CIP	97	BTSP 134	HORDI
31	BTSP 53	PGRC	65	BTSP 92	CIP	98	BTSP 135	HORDI
32	BTSP 54	PGRC	66	BTSP 93	CIP	99	BTSP 136	Badulla
33	BTSP 55	PGRC	67	BTSP 94	CIP	100	BTSP 137	Kurunegala
34	BTSP 56	PGRC						

Notes: BTSP-Biotechnology Sweet Potato, HORDI-Horticultural Crop Research and Development Institute, CIP-International Potato Centre, RARDC- -Regional Agriculture Research and Development Centre

Table 2. Used DOA recommended sweet potato varieties

Vernacular name	Accession No.	Vernacular name	Accession No.
Shanthi	BTSP-58	Wariyapola Red	BTSP-119
Gannoruwa Sudu	BTSP-120	Ranabima	BTSP-121
CARI - 273	BTSP-123	Chithra	BTSP-125
CARI-426	BTSP-126	HORDI Malee	BTSP-134

Notes: CARI - Central Agriculture Research Institute

Table 3. Morphological characters recorded in the study

Character type	Quantitative traits	Qualitative traits
Plant characters	Twining	Plant type, Ground cover
Vine characters	Internode length, Internode diameter	Primary vine color, Secondary vine color, Vine tip pubescence
Leaf characters	Leaf lobes Number, Mature leaf size, Petiole length	General outline of the leaf, Leaf lobes type, Shape of central leaf lobe, Abaxial leaf vine pigmentation, Mature leaf color, Immature leaf color, Petiole pigmentation
Floral characters	Flower length, Flower width,	Flowering habit, Flower color, Shape of limb, Equality of sepal length, Number of sepal veins, Sepal shape, Sepal apex, Sepal pubescence, Sepal color, Color of stigma, Color of style, Stigma exertion, Seed capsule set
Tuber characters	Storage root cortex thickness	Storage root shape, Storage root surface defects, Predominant skin color, Intensity of predominant skin color, Secondary skin color, Predominant flesh color, Secondary flesh color, Distribution of secondary flesh color

Statistical analysis

Out of 42 characters recorded only 27 were used for data analysis across all 100 accessions. Fifteen characters, related to flowering habit, flower color, flower width, limb shape, equality of sepal length, number of sepal veins, sepal shape, sepal apex, sepal pubescence, sepal color, stigma color, style color, stigma exertion, and seed capsule set were excluded due to the absence of flowering in most accessions during the tested seasons.

The genetic diversity of these 27 characters included both quantitative and qualitative traits and it consisted of 3 plant characters, 10 leaf characters, 9 tuber characters, and 5 vine characters (Table 3). Cluster analysis was performed on standardized

morphological data using the Euclidean distance matrix with the complete linkage method in MINITAB 17.1, which was also used to generate a dendrogram. Additionally, Principal Component Analysis (PCA) was conducted to identify the highly influential characters contributing to variation among the accessions. Before PCA analysis, a dummy data set was created, incorporating the 27 characters and the data were entered as the binary numbers.

Results & Discussion

In the characterized germplasm collection, 44% of the plants showed a bushy growth habit, while the remaining 56% were vine types. The DOA recommends bush-type sweet potatoes for growing in poly-sack bags in home gardens. These bushy type accessions can be evaluated, selected and recommended for growing in poly-sack bags.

Regarding flowering, out of 100 accessions, 70% did not flower in either of the two seasons tested. The other 30% did flower, but only 10% accessions formed seeds. These seed-producing accessions; BTSP - 13, BTSP - 16, BTSP - 25, BTSP - 31, BTSP - 39, BTSP - 41, BTSP - 68, BTSP - 90, BTSP - 95, and BTSP - 96 are valuable for future breeding programs. It's suggested to evaluate all flowering accessions in different agro-ecological zones to test seed formation in various climatic conditions.

For leaf shape, 42% of the accessions had a lobed shape. The next most common leaf shape was triangular, found in 35% of the accessions. Heart-shaped or cordate leaves appeared in 17%, while hastate (trilobed) and spear shapes represented 5%. Only 1% had a kidney-shaped (or reniform) outline, and no round or deeply divided leaf shapes were observed (Figure 1).

As for skin color, 34% of the collection had cream-colored skin, 18% had pink skin, and 10% had brownish-yellow skin. White and orange skin colors each represented 4% of the collection, while yellow skin appeared in only 1%. No red-skinned tubers were found in the collection (Figure 2).

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) was conducted on 27 characteristics, excluding flower traits, across 100 accessions. To explain 70.3% of the total variation, 10 principal components (PCs) were required. All 27 PCs were needed to account for the total variation in the 27 characteristics.

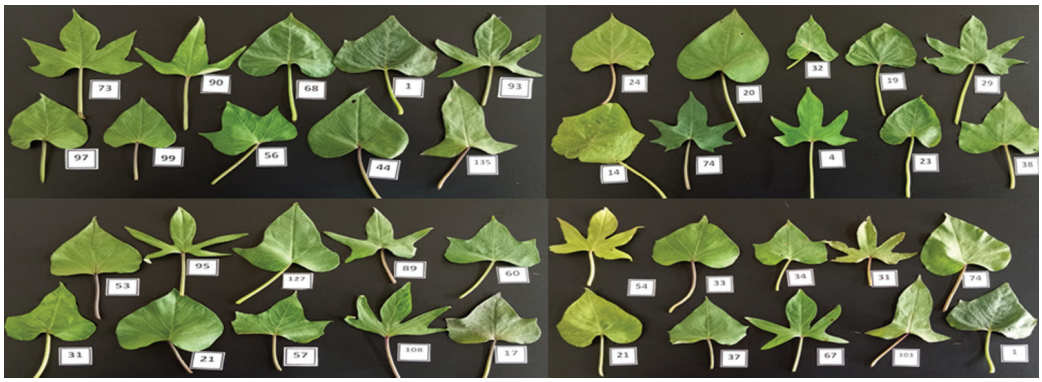

Figure 1. Leaf diversity of sweet potato accessions

Figure 2. Tuber diversity of sweet potato accessions

PC1 accounted for only 36.7% of the variation among the accessions. In the PCA of 27 plant characters, PC1was primarily influenced by positive loadings from plant type, ground cover, and immature leaf color. Conversely, its major negative loadings included vine tip pubescence, general leaf outline, leaf lobe type, shape of the central leaf lobe, abaxial leaf vein pigmentation, and predominant skin color. PC2 accounted

for only 28.7% of the variation among the accessions. The PC2had significant negative contributions from storage root shape, while internode diameter, primary vine color, abaxial leaf vein pigmentation, petiole pigmentation, secondary flesh color, storage root surface defects, predominant flesh color, and distribution of secondary flesh color had higher positive loadings. The PCA results highlighted considerable variability among the 100 accessions, indicating that nearly all characters must be considered for accurate characterization. This suggests that most characters are relatively and equally important for characterization studies. Therefore, when characterizing these accessions again under similar experimental conditions, characters with low values could potentially be omitted without compromising the overall analysis.

In the scree plot (Figure 3), the elbow point is at the 4th component, represented by secondary skin color. The first four components; petiole pigmentation, primary vine color, predominant flesh color, and secondary skin color are significant and explain most of the variation. These traits have the highest eigenvalues, reflecting their importance in characterizing the accessions.

After the 4th component, the eigenvalues drop sharply, and the slope of the curve flattens. Components 5 through 27, covering all other analyzed traits, contribute much less to the total variation. Their lower eigenvalues indicate that these traits add minimal information to the analysis and are less critical for understanding the variation.

This pattern shows that focusing on the first four components is sufficient for capturing the most meaningful differences among the accessions, while the remaining components add little value.

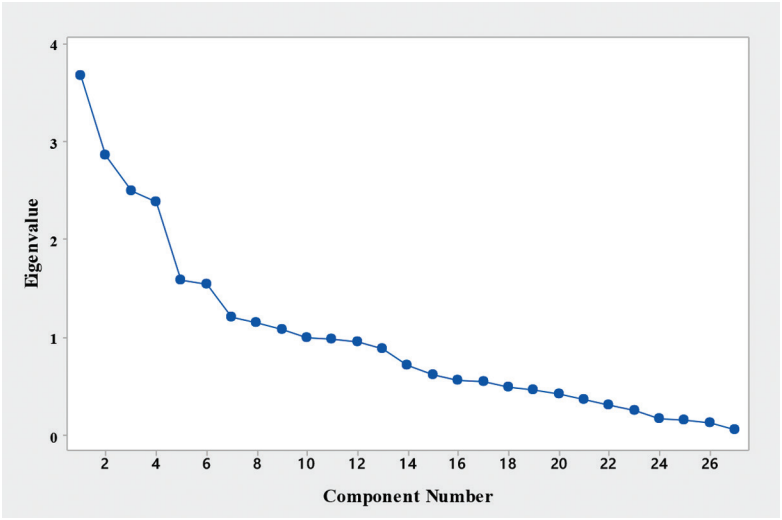

Figure 3. Scree plot diagram of 100 sweet potato accessions based on 27 morphological

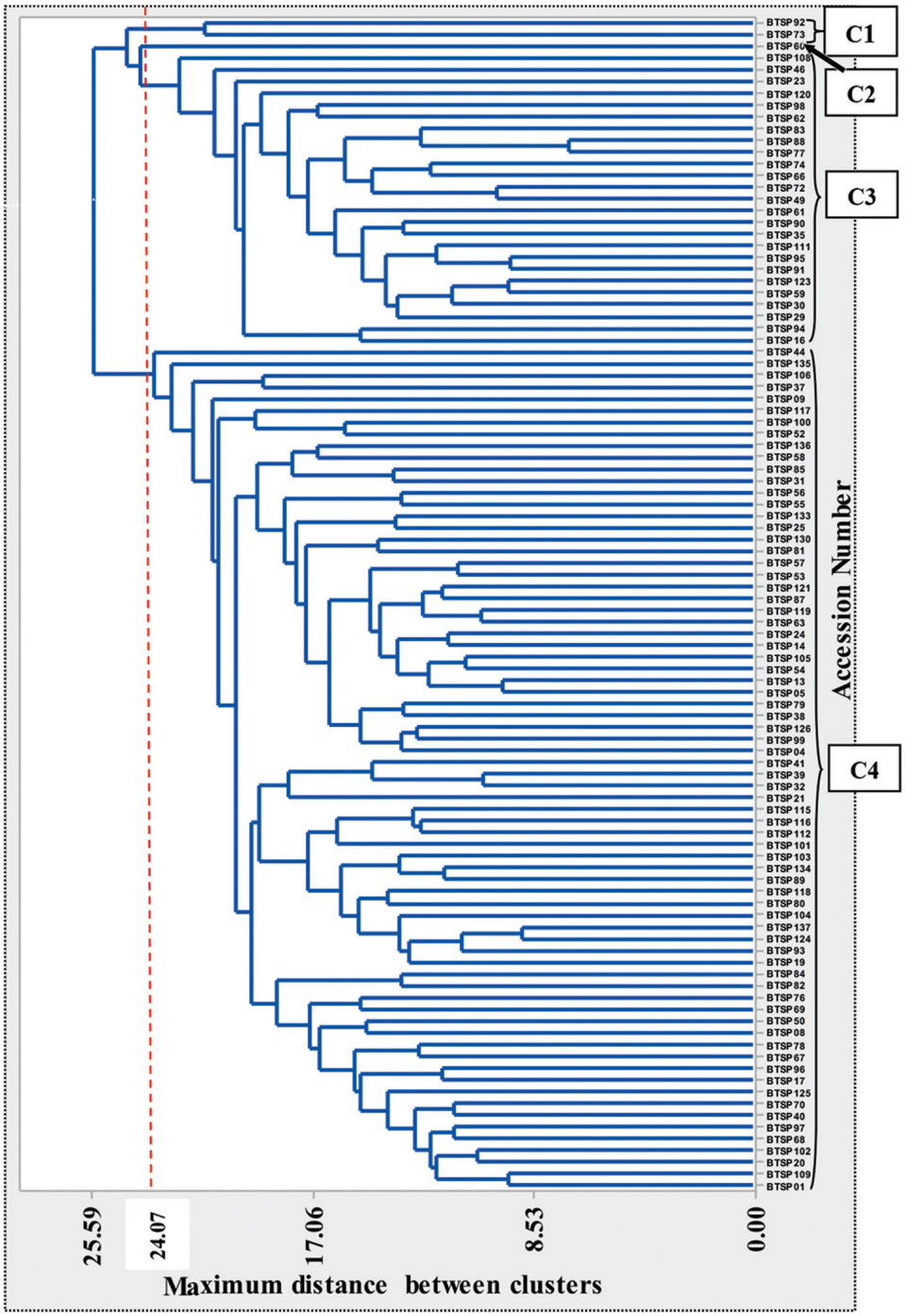

Figure 4. Dendrogram of sweet potato accessions based on morphological characters generated by average linkage method and Euclidian distance

Cluster Analysis

The 27 phenotypic characters separated the accessions into four major clusters (C1-C4) with Euclidean distances ranging from zero to 25.59 (Figure 4). When the dendrogram was truncated at a distance of 24.07, four main clusters had resulted. Cluster 1 (C1) consisted of only two orange-fleshed accessions; BTSP-92 (CIP 420027), received from CIP and described as a pro-vitamin A (beta-carotene) rich accession in 2002, and BTSP-73, received from HORDI. Hence, it was evident that BTSP-73 also possessed pro-vitamin A characteristics, a fact has substantiated by similar tuber traits (Figure 5) but it is need to be characterized using the molecular tools to understand the real genetic diversity. Further it can be confirmed by physicochemical analysis of the tubers. This cluster formed at a distance of 24.51comparted to other clusters and these two accessions showed significant similarity in predominant flesh color, secondary skin color, primary vine color, and petiole pigmentation characters. Specially both share the same predominant flesh color; dark orange and neither exhibits a secondary flesh color too (Figure 5)

Additionally, C1 exhibited more similarity in characters of plant type, internode diameter, general outline of the leaf, leaf lobe type, shape of the central leaf lobe, mature leaf size, mature leaf color, storage root surface defects, storage root cortex thickness, and distribution of secondary flesh color. Based on figure 5, where the tuber flesh is concerned, these two are genetically distinct accessions. These have the potential to be valuable breeding sources along distinct accessions such as with BTSP-01 and BTSP-109.

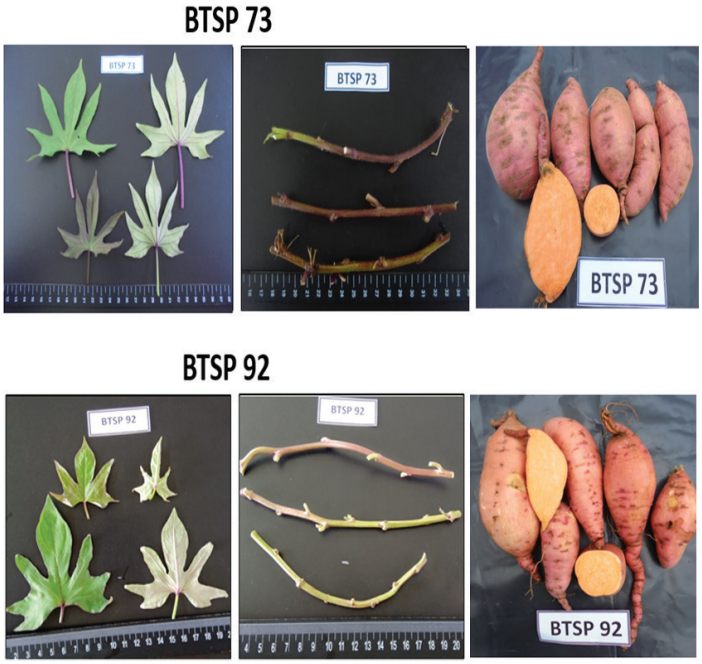

Figure 5. Leaf, vein and tuber variation in BTSP-92 and (CIP 420027) and BTSP-73

Cluster 2 (C2) was formed at a distance of 24.29 and contained a single accession, BTSP-60, which grouped alone in the cluster. Specifically, BTSP-60 possessed a twining vine, a cordateor heart-shaped general outline of the leaf, absence of leaf lobes, very thin (<1mm) storage root cortex, and pale orange predominant flesh color (Figure 6). BTSP-60 showed variation in twining, primary vine color, secondary vine color, general outline of the leaf, leaf lobe type, shape of the central leaf lobe, abaxial leaf vein pigmentation, petiole pigmentation, storage root cortex thickness, predominant skin color, secondary skin color, predominant flesh color, secondary flesh color, and distribution of secondary flesh color compared to C1 (Figure 5). It exhibited differences in the general outline of the leaf, leaf lobe number, shape of the central leaf lobe, storage root cortex thickness, intensity of predominant skin color, and predominant flesh color compared to Cluster 3 (C3).

The C3 was formed at a distance of about 23.52 and contained 25 accessions including two recommended varieties; Gannoruwa Sudu (BTSP-120) and CARI 273 (BTSP-123) divided into several sub-clusters. These accessions exhibited more similar characteristics; slightly twining vines with short internodes (3-5 cm), thin internode diameters (4-6 mm), lobed leaf outlines, moderate leaf lobes, mature leaves that are green in color and measure 8-15 cm in size. These traits were notably more consistent among these accessions compared to accessions in other three clusters.

Cluster 4 (C4) was the largest cluster, forming at a distance of about 23.96. It included 72 accessions, among them six check accessions: Shanthi (BTSP-58), Wariyapola Red (BTSP-119), Ranabima (BTSP-121), Chithra (BTSP-125), CARI 426 (BTSP-126), and HORDI14 Malee (BTSP-134). This cluster consisted of many sub-clusters, and all accessions exhibited similar characteristics in twining, internode length, internode diameter, storage root surface defects, secondary skin color, mature leaf size, and mature leaf color.

The assessment of genetic diversity in sweet potato germplasm conserved at the PGRC using morphological traits revealed that these traits are informative for identifying duplicates and assessing diversity.

Figure 6. Leaf, vein and tuber variation of accession no. BTSP-60

The results provided valuable information for understanding genetic diversity among the conserved sweet potato accessions. In the cluster analysis, accessions grouped closely within the same clusters exhibited similar morphological traits, whereas those clustered further apart displayed unique morphological characteristics. When the distance between two accessions within the same sub-cluster was zero, they were considered duplicates. However, no duplicates were observed among the 100 characterized accessions (Figure 4), indicating that each accession had sufficient variation to be considered unique.

This information is essential for guiding the conservation and management of sweet potato genetic resources and for developing effective breeding strategies. Morphological characterization for sweet potato germplasm conservation has been validated by several studies (Dufour *et al*., 2021a). Genetic diversity, which refers to the variability among individuals of a genotype, species, or population, provides adaptability to erratic environmental conditions and the potential to develop new genotypes (Brown& Hardner, 2000). This variability is expressed through molecular, biochemical, physiological, cytogenetic, and morphological traits (Ramanatha & Hodgkin, 2002). For breeding and hybridization programs, selecting distantly clustered accessions with unique traits as parental lines can enhance hybrid vigor in subsequent generations. Reliable categorization of germplasm is crucial, as genetic resources have traditionally been stored and categorized in conservation centers according to vernacular names used by farmers (Dansi *et al*., 2013).

In PCA analysis, using both qualitative and quantitative data helps in better understanding the genetic diversity of a population. Mixed data analysis is a valuable tool in agricultural research, as it combines numbers and categories to give a complete picture of complex15 systems (Mohebodini *et al*., 2017). This approach supports to decision-making in breeding, conservation, crop management, pest control, and climate adaptation, leading to more sustainable and efficient farming.

When measuring qualitative traits, it is important to record accurate values for color in leaves, vines, plants, and tubers. Using subjective color charts often leads to inconsistent results because color perception can differ from one person to another. To avoid this problem, it is better to use objective methods. Techniques such as spectrophotometry, colorimetry, digital image analysis, and high-performance liquid chromatography (HPLC) provide more precise and reliable measurements for the accurate characterization and comparison of different accessions.

Further analysis of these accessions, including molecular, biochemical and physio-chemical analysis and quality assessments with yield characteristics, are essential for their proper utilization and germplasm management. Accessions with highly favorable traits should be characterized again in different agro-ecological regions to obtain reliable and credible data on their performance.

Additionally, evaluating their response to biotic and abiotic stress is critical for crop improvement programs. Based on the tuber colour The accessions BTSP-73 and BTSP-92, guessed as having high carotene content, require further evaluation of their physiochemical and sensory properties. If confirmed, they can be evaluated for DOA recommendation and release as new varieties.

Moreover, conducting melecular analysis can validate the results obtained from morphological characterization, ensuring the reliability of the identified superior accessions. This comprehensive approach will facilitate the development of new cultivars with desirable traits and contribute to sustainable agriculture. All the 100 characterized accessions are essential to conserve due to the absence of duplicates. After food component analysis for beta-carotene accessions BTSP-92 and BTSP-73 can be recommended for breeding programs with morphologically distant accessions to develop new cultivars with desirable traits, contributing to sustainable agriculture.

Conclusions

Significant morphological diversity was observed particularly in traits such as flesh color, skin color, vine color, and petiole pigmentation, although 72% of the collection showed less16 diversity. Accessions BTSP-73 and BTSP-92 show greater diversity and more prominent traits compared to others.

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