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# Genetic dissimilarity among sweet potato genotypes using morphological and molecular descriptors

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**ABSTRACT.** This study aimed to evaluate the genetic dissimilarity among sweet potato genotypes using morphological and molecular descriptors. The experiment was conducted in the Olericulture Sector at Federal University of Jequitinhonha and Mucuri Valleys (UFVJM) and evaluated 60 sweet potato genotypes. For morphological characterization, 24 descriptors were used. For molecular characterization, 11 microsatellite primers specific for sweet potatoes were used, obtaining 210 polymorphic bands. Morphological and molecular diversity was obtained by dissimilarity matrices based on the coefficient of simple matching and the Jaccard index for morphological and molecular data, respectively. From these matrices, dendrograms were built. There is a large amount of genetic variability among sweet potato genotypes of the germplasm bank at UFVJM based on morphological and molecular characterizations. There was no duplicate suspicion or strong association between morphological and molecular analyses. Divergent accessions have been identified by molecular and morphological analyses, which can be used as parents in breeding programmes to produce progenies with high genetic variability.

**Keywords:** *Ipomoea batatas* (L.) Lam., morphological markers, microsatellites, genetic diversity, germplasm bank.

## Dissimilaridade genética entre genótipos de batata-doce por meio de descritores morfológicos e moleculares

**RESUMO.** Objetivou-se avaliar a dissimilaridade genética entre genótipos de batata-doce por meio de descritores morfológicas e moleculares. O experimento foi conduzido no Setor de Olericultura da Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) avaliando-se 60 genótipos de batata-doce. Na caracterização morfológica, utilizaram-se 24 descritores. Na caracterização molecular, utilizaram-se onze *primers* microssatélites específicos para batata-doce, obtendo-se 210 bandas polimórficas. A diversidade morfológica e molecular foi obtida por matrizes de dissimilaridade baseando-se no coeficiente de coincidência simples e índice de Jaccard, para dados morfológicos e moleculares, respectivamente. A partir destas matrizes foram construídos dendrogramas. Houve grande variabilidade genética entre os genótipos de batata doce do banco de germoplasma da UFVJM, tanto pela caracterização molecular como morfológica. Não houve suspeita de duplicata nem associação entre a análise molécular e morfológica. Foram identificados acessos divergentes pelas análises moleculares e morfológicas, os quais são indicados como genitores para compor programas de melhoramento a fim de obter progênies com alta variabilidade genética.

**Palavras-chave:** *Ipomoea batatas* (L.) Lam., marcadores morfológicos, microssatélites, diversidade genética, banco de germoplasma.

## Introduction

Sweet potato (*Ipomoea batatas* L. Lam.) is a tuberous vegetable of economic and social importance, grown mainly by small farmers and used as a staple food for the world's poorest regions (Roesler, Gomes, Moro, Kummer, & Cereda, 2008). Since it has high levels of carbohydrates, sugars, minerals, and vitamins A, C, and B complex, it can be used for multiple purposes (Azevedo, Andrade Júnior, Fernandes, Pedros, & Oliveira, 2015). Vale do Jequitinhonha is among the poorest regions in Brazil. It is diversified and has a poorly developed agriculture due to an arid climate. Consequently, sweet potato is among the most common crops grown in family farms in this region, due to its production ruggedness, resistance to pests and diseases, low demand in soil fertility and drought tolerance (Monteiro, 2007; Roesler et al., 2008).

Yield of sweet potato marketable roots can easily reach 45.4 t $ha^{-1}$ (Andrade Júnior et al., 2009; Azevedo et al., 2014); however, in Vale do Jequitinhonha, this productivity is much lower. Thus, obtaining agronomically superior cultivars adapted to Vale do Jequitinhonha through genetic improvement can be an important strategy in the region and throughout Brazil. Thus, a germplasm bank was created in the Federal University of Jequitinhonha and Mucuri Valleys (*Universidade Federal dos Vales do Jequitinhonha and Mucuri* - UFVJM). The bank contained more than 80 sweet potato accessions, mostly collected in rural properties in the Vale do Jequitinhonha region.

In any germplasm bank, an important step towards genetic improvement is to study dissimilarity among accessions; this enables the identification of possible duplicates and hybridizations that can generate high genetic variability in the offspring. Dissimilarity studies may be made by multivariate analysis based on morphological and/or molecular markers (Cruz, Regazzi, & Carneiro, 2012).

Morphological markers allow for easy discrimination between sweet potato accessions (Chávez et al., 2006; Veasey et al., 2007; Moulin et al., 2012). Similarly, genetic divergence can be evaluated by means of molecular markers, enabling germplasm characterization (Souza, Carvalho, Martins, Guedes, & Oliveira, 2008), phylogenetic analysis, DNA fingerprinting, gene link detection with mono and polygenic characters, identification of varieties, gene introgression, and indirect selection of agronomic traits. The effectiveness of different types of labels is confirmed by the wide use of these techniques in in genetic storage studies and for improving plants (Souza, 2015).

Simple sequence repeat (SSR) microsatellites are molecular markers that facilitate rapid differentiation between related individuals because they are abundant and evenly distributed throughout the genome. In addition, due to their high polymorphism rate, codominant inheritance and multiallelic nature, SSR markers are highly reproducibility and require small amounts of DNA (Oliveira, Pádua, Zucchi, Vencovsky, & Vieira, 2006; Oliveira & Silva, 2008). Several studies with sweet potato culture using SSR markers have observed high genetic diversity among accessions, including Veasey et al. (2008) and Koussao et al. (2014).

When considering the socioeconomic importance of sweet potatoes, it is essential to characterize the genotypes in germplasm banks for genetic dissimilarity in order to eliminate duplicates and guide further crosses in breeding programmes. Therefore, the objective of this work was to evaluate genetic dissimilarity among sweet potato genotypes of the germplasm bank of UFVJM through morphological and molecular descriptors.

## Material and methods

The experiment was conducted in the Olericulture Sector of the Federal University of Jequitinhonha and Mucuri Valleys, in JK Campus, in Diamantina city, Minas Gerais State, Brazil (18º12'01"S, 43º34'20"W and 1,400-m altitude). Sixty sweet potato genotypes were assessed, which belong to the germplasm bank of UFVJM. They are named UFVJM (01, 02, 03, 04, 05, 06, 07, 08, 09, 10, 13, 14, 16, 17, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 34, 35, 37, 38, 39, 41, 42, 43, 44, 45, 46, 48, 49, 50, 51, 52, 54, 55, 56, 57, and 58), Arruba, Cariru Vermelha, Brazlândia-Branca, Cambraia, Espanhola, Licuri, Palmas, Princesa, Tomba Carro 1, Tomba Carro 2, and Coquinha. Sweet potato branches containing three to five buds were planted in expanded polystyrene trays containing commercial substrate and were kept for 35 days in a greenhouse. Subsequently, seedlings containing eight nodes were transplanted to the field, with the burial of four nodes in prepared areas. The planting of sweet potato seedlings was performed on December 18, 2012.

In the field, the area was plowed and harrowed, followed by the incorporation of fertilizers (20, 90, and 90 kg $ha^{-1}$ of N, $P_2O_5$ and $K_2O$, respectively). Subsequently, another plowing was performed to create windrows with heights between 30 cm and 40 cm. Plots consisted of a row that was 2.4-m long, with spaces of 1.0 metres between planting rows and 0.3 metres between plants, totalling eight plants per section. Culture management was performed according to Filgueira (2008).

At 90 days after planting (DAP), the morphological characteristics of plant shoots were evaluated; and at 150 DAP, roots were assessed. Evaluations were made according to the descriptors and scoring scales that were recommended by the International Board for Plant Genetic Resources (IBPGR), which were designed by Huamán (1991) and are listed in Table 1.

**Table 1.** Scoring scale related to the evaluated morphological descriptors in 60 accessions of sweet potato (*Ipomoea batatas*). Source: Huamán (1991). UFVJM, Diamantina, Minas Gerais State, 2016.

| Characteristics | Score |
|---|---|
| 1. Predominant foliage colour | 1-Green; 3-green with a few purple stains; 4-green with many purple stains; 5- green with many dark purple stains; 6-predominantly purple; 7-predominantly dark purple; 8-completely purple; 9-completely dark purple. |
| 2. Secondary foliage colour | 0-Absent; 1-green base; 2-green apex; 3-green nodes; 4-reddish base; 5-reddish apex; 6-reddish nodes; 7-other. |
| 3. Foliage apex pubescence | 0-Absence of hairiness; 3- thin hairiness; 5- moderate; 7-dense; 9- very dense. |
| 4. Principal foliage length | 3-Erect (<75 cm); 5-semi-erect (75-150 cm); 7-scattered (150-250 cm); 9-extremely scattered (>250 cm). |
| 5. Leaf lobule types | 0-Absence of lobules; 1-very superficial lobules; 3-superficial lobules; 5-moderate; 7-deep; 9-very deep. |
| 6. Number of leaf lobules | 0- Absence of lobules- round and smooth leaf; 1-absence of lateral lobules; 3-three lobules; 5-five lobules; 7-seven lobules; 9-nine lobules. |
| 7. Central lobule format | 0-Absent; 1-jagged; 2-triangular; 3-semi-circular; 4-semi-elliptical; 5-elliptical; 6-lanceolate; 7-oblong and lanceolate; 8-linear and wide; 9-linear and narrow. |
| 8. Pigmentation of veins | 1-Yellow; 2-green; 3-purple stains in principal base of veins; 4-purple stains in many veins; 5-principal vein predominantly purple; 6-principal vein completely purple; 7-partially purple veins; 8-veins totally purple; 9-lower surface and veins completely purple. |
| 9. Petiole length | 1-Very short (<10 cm); 3-short (10-20 cm); 5-intermediate (21-30 cm); 7-long (31-40 cm); 9-very long (>40 cm). |
| 10. Petiole pigmentation | 1-Green; 2-green with purple base; 3-green with purple leaf insertion; 4-green with purple in both extremities; 5-green with purple stains in all petiole; 6-green with purple stripes; 7-purple with green leaf insertion; 8-some green and some purple petioles; 9-completely of almost purple. |
| 11. Mature leaf colour | 1-Greenish yellow; 2-green; 3-green with purple ends; 4-greyish green; 5-green with purple veins; 6-smooth purple; 7-predominantly purple; 8-green upper surface and purple lower surface; 9-purple in both surfaces. |
| 12. Immature leaf | 1-Greenish yellow; 2-green; 3-green with purple ends; 4-greyish green; 5-green with purple veins; 6-smooth purple; 7-predominantly purple; 8-green upper surface and purple lower surface; 9-purple in both surfaces. |
| 13. Mature leaf size | 3-Small (<8 cm); 5-average (8-15 cm); 7-large (16-25 cm); 9-very large (>25 cm). |
| 14. Mature leaf general format | 1-Round; 2-reniform; 3-cordate; 4-triangular; 5-lanceolate; 6-lobed; 7-almost separated. |
| 15. Internode diameter | 1-Very thin (<4 mm); 3-thin (4-6 mm); 5-intermediate (7-9 mm); 7-thick (10-12 mm); 9-very thick (>12 mm). |
| 16. Internode length | 1-Very short (<3 cm); 3-short (3-5 cm); 5-intermediate (6-9 cm); 7-long (10-12 cm); 9-very long (>12 cm). |
| 17. Flaws in root surface | 0-Absent; 1-alligator skin; 2-prominent veins; 3-shallow horizontal constrictions; 4-deep horizontal constrictions; 5-shallow longitudinal slots; 6-deep longitudinal slots; 7-constrictions and deep cracks; 8-other. |
| 18. Root format | 1-Round; 2-round and elliptical; 3-elliptical; 4-oval; 5-oboval; 6-oblong; 7-oblong and long; 8-long and elliptical; 9-irregular and long. |
| 19. Predominant colour of root bark | 1-White; 2-cream; 3-yellow; 4-orangish; 5-orangish brown 6-pinkish; 7-red; 8-reddish purple; 9-dark purple. |
| 20. Root bark colour intensity | 1-Pale; 2-intermediate; 3-dark. |
| 21. Secondary colour of root bark | 0-Absent; 1-white; 2-cream; 3-yellow; 4-orangish; 5-orangish brown; 6-pinkish; 7-red; 8-reddish purple; 9-dark purple. |
| 22. Pulp predominant colour | 1-White; 2-light cream; 3-dark cream; 4-light yellow; 5-dark yellow; 6-light orange; 7-orangish brown; 8-dark orange; 9-strongly purple. |
| 23. Secondary pulp colour | 0-Absent; 1-white; 2-cream; 3-yellow; 4-orangish brown; 5-pinkish; 6-red; 7-reddish purple; 8-purple; 9-dark purple. |
| 24. Distribution of secondary pulp colour | 0-Absent; 1-ring close to the bark; 2-large ring close to the bark; 3-intense sparse stains; 4-thin ring in the pulp; 5-large ring in the pulp; 6-ring and other stains in the pulp; 7-stains in longitudinal sections; 8-stains covering the surface almost completely; 9-stains covering the hole pulp. |

Extraction of genetic material (DNA) was performed according to the protocol proposed by Veasey et al., (2008) with some modifications. DNA samples were analysed for the quality and quantity that were required for molecular procedures. Each sample was subjected to electrophoresis on 0.8% agarose gel in 1x TAE buffer under a constant voltage of 100 $\mu$V for 90 minutes. A size and weight molecular standard (1-kb DNA Ladder, BioLabs Inc.) was used with the samples and served as a standard to estimate the size (in base pairs) and weight (in nanograms that was present in each sample. After the run, the gel was examined under ultraviolet light, and the image was scanned with LpixImage (Loccus Biotecnologia). PCR reactions were performed according to the protocol presented by Veasey et al. (2008). Thirty-two oligonucleotide primers were used; Jarret and Bowen (1994), Buteler, Jarret, and La Bonte (1999), describe them. Initially, ten sweet potato accessions with different morphological characteristics were examined in order to choose those with good amplification, high resolution, reproducibility and polymorphisms. The optimal annealing temperature was 50ºC for amplification using all selected oligonucleotides.

PCR amplifications were performed in an Eppendorf thermocycler, model Mastercycler Gradiente, under the following conditions: 94ºC for 3 minutes, followed by 40 cycles at 94ºC for 45 sec., 50ºC for 30 sec., and 72ºC for 1 minute. At the end of 40 cycles, an additional step at 72ºC for 1 minute was used followed by a 4ºC hold

Products resulting from amplification were separated by electrophoresis on 6% polyacrylamide gels in a TBS running buffer under a constant voltage of 80 V for 4 hours in a vertical vessel (20x20 cm). Then, 7 $\mu$L of PCR product and 2 $\mu$L of loading buffer were loaded in each well. A size marker (50-pb Ladder, Biolabs Inc.) was used as standard. Amplified fragments were detected by staining with silver nitrate.

For multicategoric data (Table 1), a dissimilarity matrix was obtained based on a coefficient of simple matching. Sweet potato is a hexaploid species (Azevedo et al., 2015); there are many different band patterns. From the presence and absence of each DNA fragment, the dissimilarity matrix was designed with a Jaccard similarity coefficient (Jaccard, 1901). For multicategoric, molecular and combined data, a cutoff equal to the average dissimilarity was performed. The R program (R Core Team, 2014) and ade4 package (Dray & Dufour, 2007) were used for the estimates of dissimilarity matrices. To combine molecular and multicategoric data, an algebraic sum of these two matrices was calculated (Alves et al., 2013), generating a new matrix for joint analysis. The correlation between matrices and their significance were estimated by Mantel test with 1,000 permutations using package ade4 with 'mantel.rtest' function in R software (R Core Team, 2014). To obtain the dendrogram, the unweighted pair group method with arithmetic mean (UPGMA) grouping method was used, with 'hclust' function.

## Results and discussion

The grades of the 24 evaluated descriptors are presented in Table 2. A large variability among accessions was observed, which was not expected given the large number of phenotypic classes with a high percentage of hits in each category. In studies of genetic dissimilarity, the evaluation of the largest possible number of morphological descriptors is indicated, in order to obtain a more complete dissimilarity analysis. Variables that showed little morphological variability were the secondary colour of the branches (76.7% absent of colour); the pubescence of the branch tip (98.33% absent); the mature leaf (90% green-coloured); the size of the mature leaf (93.3% with medium size between 8 to 15 cm); the secondary colour of the root bark (98.33% absent of colour); the secondary colour of the root pulp (78.3% absent of colour) and the distribution of the pulp colour (76.7% without ring formation) (Table 2).

**Table 2.** Scores of morphological descriptors used in the characterization of 60 accessions of sweet potato. UFVJM, Diamantina-MG, 2016.

| Genotypes | Morphological descriptors [1] | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| Arruba | 1 | 0 | 0 | 6 | 5 | 5 | 6 | 7 | 2 | 5 | 3 | 5 | 5 | 3 | 3 | 3 | 1 | 9 | 5 | 1 | 0 | 1 | 0 | 0 |
| Braz.-Branca | 2 | 0 | 0 | 3 | 0 | 1 | 1 | 8 | 5 | 6 | 2 | 5 | 5 | 3 | 5 | 7 | 1 | 4 | 2 | 1 | 0 | 1 | 0 | 0 |
| Cambraia | 3 | 0 | 0 | 5 | 7 | 5 | 6 | 7 | 2 | 6 | 4 | 3 | 5 | 5 | 3 | 3 | 0 | 3 | 2 | 1 | 0 | 1 | 0 | 0 |
| C. Vermelha | 9 | 0 | 0 | 5 | 7 | 9 | 6 | 5 | 2 | 6 | 9 | 5 | 5 | 5 | 3 | 3 | 1 | 3 | 2 | 1 | 0 | 1 | 0 | 0 |
| Coquinha | 1 | 0 | 0 | 6 | 5 | 5 | 6 | 7 | 2 | 1 | 1 | 5 | 5 | 5 | 3 | 3 | 0 | 4 | 5 | 1 | 0 | 2 | 0 | 0 |
| Espanhola | 1 | 0 | 0 | 6 | 5 | 7 | 6 | 6 | 2 | 6 | 3 | 3 | 5 | 3 | 1 | 3 | 0 | 5 | 2 | 1 | 0 | 1 | 0 | 0 |
| Licuri | 1 | 0 | 0 | 6 | 7 | 5 | 6 | 4 | 2 | 1 | 4 | 5 | 7 | 5 | 1 | 3 | 0 | 7 | 5 | 1 | 0 | 1 | 0 | 0 |
| Palmas | 1 | 0 | 3 | 4 | 1 | 1 | 2 | 3 | 2 | 2 | 3 | 5 | 5 | 3 | 3 | 5 | 0 | 7 | 6 | 2 | 0 | 2 | 0 | 0 |
| Princesa | 9 | 4 | 0 | 7 | 9 | 5 | 9 | 6 | 2 | 3 | 9 | 3 | 5 | 3 | 3 | 7 | 0 | 8 | 2 | 1 | 0 | 1 | 0 | 0 |
| T Carro 1 | 7 | 5 | 0 | 5 | 7 | 5 | 5 | 7 | 2 | 1 | 8 | 3 | 5 | 3 | 3 | 7 | 0 | 8 | 2 | 1 | 0 | 1 | 0 | 0 |
| T Carro 2 | 1 | 0 | 0 | 4 | 1 | 1 | 2 | 3 | 2 | 1 | 3 | 3 | 5 | 3 | 1 | 5 | 1 | 6 | 2 | 1 | 0 | 9 | 6 | 1 |
| UFVJM-01 | 1 | 0 | 0 | 6 | 7 | 5 | 4 | 5 | 2 | 1 | 1 | 5 | 7 | 5 | 3 | 5 | 1 | 8 | 5 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-02 | 1 | 0 | 0 | 6 | 7 | 5 | 4 | 7 | 2 | 1 | 4 | 3 | 5 | 3 | 1 | 3 | 0 | 9 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-03 | 3 | 4 | 0 | 6 | 5 | 5 | 4 | 5 | 2 | 5 | 3 | 5 | 5 | 3 | 3 | 3 | 0 | 8 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-04 | 3 | 0 | 0 | 6 | 7 | 1 | 6 | 7 | 2 | 2 | 4 | 5 | 5 | 3 | 1 | 3 | 1 | 8 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-05 | 1 | 0 | 0 | 4 | 3 | 5 | 1 | 3 | 2 | 1 | 1 | 5 | 5 | 3 | 1 | 5 | 1 | 8 | 2 | 2 | 0 | 9 | 8 | 1 |
| UFVJM-06 | 9 | 0 | 0 | 6 | 5 | 7 | 4 | 6 | 2 | 1 | 9 | 3 | 5 | 3 | 3 | 3 | 1 | 4 | 4 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-07 | 1 | 0 | 0 | 3 | 1 | 5 | 4 | 4 | 2 | 1 | 3 | 5 | 5 | 5 | 3 | 3 | 0 | 8 | 5 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-08 | 5 | 5 | 0 | 6 | 5 | 9 | 4 | 7 | 5 | 3 | 4 | 5 | 5 | 3 | 3 | 5 | 1 | 5 | 5 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-09 | 1 | 0 | 0 | 4 | 1 | 5 | 3 | 4 | 2 | 2 | 3 | 5 | 5 | 3 | 3 | 3 | 0 | 8 | 2 | 2 | 0 | 9 | 8 | 1 |
| UFVJM-10 | 1 | 0 | 0 | 4 | 1 | 5 | 1 | 5 | 2 | 1 | 3 | 5 | 5 | 3 | 3 | 3 | 1 | 5 | 6 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-13 | 1 | 0 | 0 | 4 | 1 | 1 | 2 | 5 | 2 | 1 | 3 | 5 | 5 | 5 | 3 | 3 | 2 | 3 | 6 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-14 | 1 | 0 | 0 | 4 | 1 | 5 | 1 | 8 | 2 | 1 | 3 | 5 | 7 | 3 | 3 | 5 | 1 | 3 | 2 | 1 | 0 | 4 | 0 | 0 |
| UFVJM-16 | 3 | 0 | 0 | 6 | 5 | 5 | 4 | 8 | 2 | 5 | 3 | 5 | 5 | 3 | 3 | 3 | 1 | 5 | 5 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-17 | 1 | 0 | 0 | 4 | 1 | 5 | 3 | 4 | 2 | 1 | 3 | 5 | 5 | 3 | 3 | 3 | 0 | 4 | 6 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-19 | 3 | 0 | 0 | 4 | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 3 | 5 | 3 | 1 | 5 | 0 | 4 | 2 | 2 | 0 | 9 | 8 | 1 |
| UFVJM-20 | 3 | 0 | 0 | 6 | 7 | 7 | 6 | 8 | 2 | 5 | 3 | 3 | 5 | 3 | 3 | 3 | 4 | 9 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-21 | 9 | 5 | 0 | 6 | 7 | 5 | 6 | 5 | 7 | 8 | 3 | 7 | 5 | 3 | 5 | 7 | 0 | 9 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-22 | 1 | 0 | 0 | 5 | 3 | 3 | 4 | 5 | 2 | 1 | 4 | 3 | 5 | 3 | 3 | 3 | 1 | 4 | 2 | 2 | 0 | 4 | 0 | 0 |
| UFVJM-23 | 1 | 0 | 0 | 5 | 7 | 5 | 6 | 7 | 2 | 1 | 4 | 5 | 5 | 3 | 1 | 3 | 1 | 9 | 5 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-24 | 1 | 4 | 0 | 6 | 5 | 5 | 4 | 8 | 2 | 7 | 2 | 5 | 5 | 3 | 3 | 5 | 2 | 5 | 5 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-25 | 1 | 0 | 0 | 3 | 1 | 5 | 1 | 4 | 2 | 2 | 1 | 3 | 5 | 5 | 3 | 3 | 0 | 9 | 6 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-26 | 1 | 0 | 0 | 4 | 1 | 3 | 1 | 5 | 2 | 2 | 1 | 7 | 5 | 5 | 3 | 3 | 2 | 5 | 6 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-27 | 1 | 0 | 0 | 4 | 1 | 5 | 1 | 3 | 2 | 2 | 1 | 3 | 5 | 5 | 3 | 3 | 0 | 8 | 6 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-28 | 1 | 0 | 0 | 3 | 1 | 0 | 1 | 8 | 2 | 6 | 4 | 3 | 5 | 3 | 5 | 7 | 0 | 9 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-29 | 1 | 0 | 0 | 3 | 1 | 3 | 2 | 5 | 2 | 1 | 2 | 5 | 5 | 5 | 3 | 3 | 0 | 8 | 6 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-30 | 1 | 0 | 0 | 4 | 0 | 1 | 1 | 3 | 2 | 1 | 1 | 3 | 5 | 3 | 3 | 5 | 3 | 3 | 2 | 2 | 0 | 9 | 0 | 1 |
| UFVJM-31 | 1 | 0 | 0 | 4 | 0 | 1 | 1 | 2 | 2 | 2 | 1 | 3 | 5 | 3 | 3 | 5 | 0 | 4 | 2 | 2 | 0 | 9 | 8 | 1 |

Continue...

Continuation...

| Genotypes | Morphological descriptors [1] | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| UFVJM-33 | 1 | 0 | 0 | 4 | 1 | 1 | 2 | 4 | 2 | 2 | 1 | 5 | 5 | 5 | 3 | 3 | 0 | 8 | 6 | 2 | 0 | 1 | 0 | 0 |
| UFVJM-34 | 1 | 2 | 0 | 4 | 1 | 5 | 2 | 5 | 1 | 1 | 3 | 7 | 5 | 5 | 3 | 3 | 0 | 3 | 5 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-35 | 1 | 0 | 0 | 4 | 1 | 5 | 2 | 7 | 2 | 2 | 3 | 5 | 5 | 5 | 3 | 3 | 0 | 8 | 6 | 2 | 0 | 2 | 0 | 0 |
| UFVJM-37 | 1 | 0 | 0 | 4 | 1 | 5 | 2 | 3 | 2 | 2 | 1 | 5 | 5 | 3 | 3 | 5 | 0 | 5 | 2 | 2 | 0 | 9 | 8 | 1 |
| UFVJM-38 | 1 | 0 | 0 | 4 | 1 | 1 | 2 | 3 | 2 | 1 | 2 | 5 | 5 | 3 | 1 | 5 | 0 | 4 | 2 | 2 | 0 | 9 | 8 | 1 |
| UFVJM-39 | 1 | 0 | 0 | 4 | 1 | 1 | 1 | 3 | 2 | 1 | 1 | 3 | 5 | 3 | 3 | 3 | 1 | 5 | 2 | 2 | 0 | 9 | 8 | 1 |
| UFVJM-41 | 4 | 0 | 0 | 4 | 0 | 1 | 2 | 8 | 2 | 2 | 3 | 3 | 5 | 5 | 5 | 7 | 2 | 6 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-42 | 1 | 0 | 0 | 4 | 1 | 1 | 2 | 3 | 2 | 2 | 1 | 5 | 5 | 3 | 3 | 3 | 0 | 5 | 2 | 2 | 0 | 9 | 8 | 1 |
| UFVJM-43 | 1 | 0 | 0 | 4 | 1 | 3 | 1 | 7 | 2 | 3 | 1 | 5 | 5 | 5 | 3 | 3 | 4 | 3 | 6 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-44 | 1 | 0 | 0 | 4 | 1 | 1 | 2 | 8 | 2 | 5 | 3 | 5 | 5 | 5 | 5 | 5 | 0 | 3 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-45 | 1 | 0 | 0 | 3 | 1 | 5 | 3 | 4 | 2 | 1 | 3 | 3 | 5 | 5 | 1 | 3 | 1 | 2 | 6 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-46 | 1 | 0 | 0 | 4 | 1 | 3 | 1 | 3 | 2 | 1 | 3 | 3 | 5 | 3 | 1 | 3 | 1 | 8 | 2 | 2 | 0 | 9 | 8 | 1 |
| UFVJM-48 | 3 | 5 | 0 | 4 | 1 | 1 | 1 | 8 | 2 | 5 | 8 | 5 | 5 | 5 | 1 | 3 | 0 | 8 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-49 | 1 | 0 | 0 | 6 | 3 | 5 | 2 | 3 | 2 | 2 | 1 | 3 | 5 | 5 | 3 | 3 | 1 | 9 | 6 | 2 | 0 | 1 | 0 | 0 |
| UFVJM-50 | 6 | 1 | 0 | 6 | 3 | 3 | 4 | 8 | 2 | 5 | 9 | 3 | 5 | 3 | 3 | 3 | 1 | 8 | 8 | 3 | 0 | 9 | 8 | 1 |
| UFVJM-51 | 1 | 0 | 0 | 6 | 3 | 3 | 2 | 2 | 2 | 2 | 1 | 3 | 5 | 3 | 1 | 3 | 3 | 8 | 6 | 2 | 0 | 1 | 0 | 0 |
| UFVJM-52 | 4 | 4 | 0 | 6 | 5 | 5 | 2 | 6 | 2 | 5 | 4 | 3 | 5 | 3 | 1 | 3 | 1 | 4 | 2 | 1 | 0 | 2 | 0 | 0 |
| UFVJM-54 | 1 | 2 | 0 | 6 | 7 | 5 | 5 | 3 | 2 | 5 | 1 | 3 | 5 | 3 | 3 | 3 | 1 | 3 | 2 | 1 | 0 | 1 | 0 | 0 |
| UFVJM-55 | 8 | 0 | 0 | 3 | 1 | 1 | 4 | 8 | 5 | 5 | 9 | 5 | 7 | 5 | 1 | 3 | 3 | 5 | 8 | 3 | 0 | 9 | 8 | 1 |
| UFVJM-56 | 1 | 2 | 0 | 4 | 1 | 5 | 4 | 3 | 2 | 2 | 1 | 5 | 5 | 3 | 3 | 3 | 3 | 7 | 5 | 1 | 3 | 0 | 0 | 0 |
| UFVJM-57 | 9 | 6 | 0 | 6 | 3 | 5 | 4 | 8 | 5 | 5 | 9 | 3 | 5 | 5 | 3 | 5 | 0 | 9 | 8 | 3 | 0 | 9 | 8 | 1 |
| UFVJM-58 | 5 | 2 | 0 | 4 | 0 | 1 | 1 | 7 | 2 | 2 | 5 | 3 | 5 | 3 | 1 | 3 | 1 | 7 | 2 | 2 | 0 | 7 | 0 | 0 |

[1]Morphological descriptors: 1-predominant foliage color; 2-secondary color of foliage; 3-pubescence point in foliage apex; 4-mature leaf general format; 5-foliage lobule types; 6-number of foliage numbers; 7-central lobule format; 8-pigmentation of veins; 9-mature leaf color; 10-immature leaf color; 11-petiole pigmentation; 12-petiole length; 13-mature leaf size; 14-internode diameter; 15-internode length; 16-principal foliage length; 17-root surface flaws; 18-format of roots; 19-predominant color of root bark; 20-intensity of root bark color; 21-secondary color of bark; 22-predominant color of pulp; 23-secondary color of pulp; 24-pulp color distribution. The description of each score is shown in the Material and Methods section.

Molecular characterization involved a screening with 32 primers (Jarret & Bowen, 1994; Buteler, Jarret, & La Bonte, 1999), of which 11 were selected and evaluated (Table 3). These primers were chosen because they had a high average number of evident bands and because they are polymorphic and present a high reproducibility. A total of 210 polymorphic bands were obtained with the 11 selected primers. The average number of polymorphic bands per primer was 19.09. The primer that had the most polymorphic bands was IBSSR02, which amplified 28 bands, while the least polymorphic primer was Ib-255, which amplified 10 polymorphic bands. Moulin et al. (2012) studied sweet potato and performed a screening with 32 primers, from which 18 were selected and evaluated.

Genetic dissimilarity estimated from multicategoric data ranged from 0.08 to 0.92, averaging 0.52 (Table 4). The most divergent genotypes was the pair of UFVJM-55 accessions and Tomba Carro 1; UFVJM-37 and UFVJM-21 were considered the most similar with 0.08 distance (Table 4). Similarly, Veasey et al. (2007) observed variations of 0.12 to 1 in the morphological characterization of sweet potato plants demonstrating large diversity in the traditional variety group in Vale do Ribeira. Longer distances obtained from molecular data ranged from 0.14 to 1.00 with a dissimilarity average of 0.76, indicating high genetic diversity. Through this analysis, it was determined that UFVJM-42 and Espanhola accessions and UFVJM-07 and UFVJM-44 accessions with a distance equal to 1.00 were the farthest. Moreover, the genotype pairs of UFVJM-26 and UFVJM-27 were the closest to each other, with a distance equal to 0.14 (Figure 1).

**Table 3.** List of SSR primers selected for DNA amplification of sweet potato genotypes (*Ipomoea batatas*) with its base sequences. UFVJM, Diamantina, Minas Gerais State, 2016.

| *Primer* (SSR) | Sequence (5' → 3') | Number of Polymorphic Bands | Percentage of Polymorphic bands |
|---|---|---|---|
| Ib-242 | F: GCGGAACGGACGAGAAAA<br>R: ATGGCAGAGTGAAAATGGAACA | 21 | 10,0 |
| Ib-255 | F: TGGGCATTCTCATATTTTGCT<br>R: GCCACTCCAACAGCACATAA | 10 | 4,8 |
| Ib-316 | F: CAAACGCACAACGCTGTC<br>R: CGCGTCCCGCTTATTTAAC | 14 | 6,7 |
| Ib-318 | F: AGAACGCATGGGCATTGA<br>R: CCCACCGTGTAAGGAAATCA | 22 | 10,5 |
| IBSSR02 | F: GTAACTGAAGTAGTTGGCGAGG<br>R: CACTGTGGCTCAATGTAGGG | 28 | 13,3 |
| IBSSR14 | F:CAAAACTCAGACGACCCATGATG<br>R: CCCCGAGGTATTTATATCACACCC | 26 | 12,4 |
| IBSSR15 | F: TGGAGCAGATGTTCCTGGAC<br>R: GATTTGAAGCAGTTCATCC | 16 | 7,6 |
| IBSSR18 | F: GATCTTGAATTAGCCCAC<br>R: AGATGGATGACCGTATGC | 19 | 9,0 |
| IBSSR19 | F: GCGAATCAAGTCTTTTGTCCAC<br>R: GGGACTGTCCTTTGGGTATG | 19 | 9,0 |
| IBSSR21 | F: AAACAACCAACGGGTCTTTGC<br>R: CTCTAGGGTCGCCATAAAAATCAC | 17 | 8,1 |
| IBSSR27 | F: GTGTTTATCACATCGTTTTCTG<br>R: GGCTCGTACAATTTTCAAAG | 18 | 8,6 |
| Total | | 210 | |

*F = Forward; R = Revers

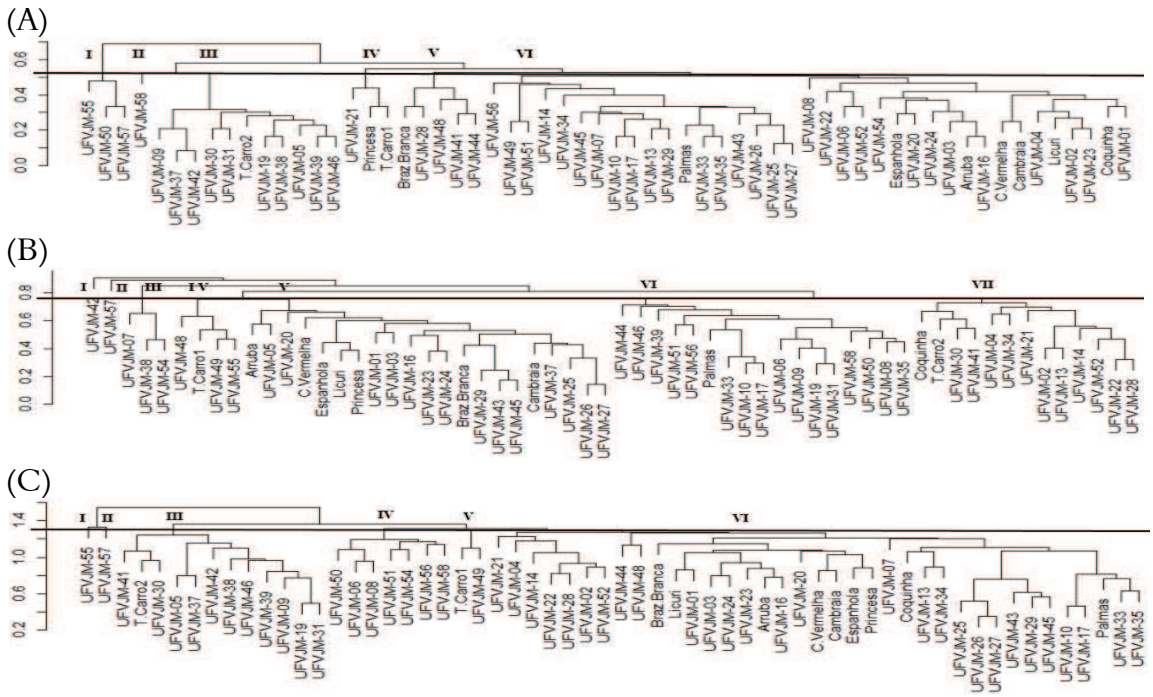


**Figure 1.** Dendrograms obtained by UPGMA method from dissimilarity matrices based on: multicategoric (A), molecular (B) and combined data (multicategoric + SSR molecular) (C), among 60 sweet potato genotypes (*Ipomoea batatas*). UFVJM, Diamantina, Minas Gerais State, 2016.

Cophenetic correlation estimates larger than 0.70 were found for all dissimilarity matrices considering the UPGMA dendrogram (Table 4). This indicated the viability of using dendrograms to summarize the information of dissimilarity matrices (Cruz et al., 2012). For multicategoric data, a cutoff equal to an average dissimilarity of 0.52 was applied, which allowed the formation of six groups (Figure 1). Group I (UFVJM-55, UFVJM-50, UFVJM-57) is characterized by having a semi-elliptical shape of the central lobe, petiole completely or almost purple, root bark predominantly purple-red with an intense dark colour. Group II consisted only of a UFVJM-58 accession. This differed from other groups by being the only one with pigmentation of green and purple spots throughout the petiole and predominantly orange brown pulp. Group III met eleven hits that generally had triangular-shaped mature leaves, thin diameter of the internode, predominantly cream-coloured root bark, and strong purple with absence of colour distribution in pulp. Group IV (UFVJM-21, Princesa, Tomba Carro 1) was formed by accessions characterized by having five lobes dispersed in the sheet length of the stem and no defects on the surface of roots. Group V was composed of five hits (Brazlândia Branca, UFVJM-28, UFVJM-48, UFVJM-41, UFVJM-44) that had completely purple veins, median mature leaf size, and cream-coloured bark and pulp. Group VI gathered the largest number of accessions, 37, which was 62% of the total. This group was characterized by the absence of colour distribution in pulp and secondary bark and pulp colour.

For molecular data, the grouping of accessions, which are represented by dendrogram (Figure 1), resulted in seven groups based on a cutoff equal to the average dissimilarity between all genotype pairs (0.76). Groups I and II were formed by only one accession each. Accession UFVJM-42 (group I) showed 19 specific polymorphic bands (9% of total). Accession UFVJM-57 (group II) amplified only 10 specific polymorphic bands, which corresponded to 4.8% of total. Group III gathered three accessions: UFVJM-38, UFVJM-54, UFVJM-07, which shared 8 common polymorphic bands. Group IV was formed by four accessions: UFVJM-48, Tomba Carro 1, UFVJM-49, and UFVJM-55, which showed 20 polymorphic bands for all primers used. Group V was formed by the largest number of accessions, totalling 21, which was 35% of studied accessions. Thirty-four polymorphic bands were observed for most accessions, which was more than 16% of the total polymorphic bands. Group VI was formed by 17 accessions, and most accessions present in this group showed 29 similar polymorphic fragments. Group VII gathered 13 accessions (Coquinho, Tomba Carro 2, UFVJM-30, UFVJM-41,

UFVJM-04, UFVJM-34, UFVJM-21, UFVJM-02, UFVJM-13, UFVJM-14, UFVJM-52, UFVJM-22, and UFVJM-28). In this group, there were 17 similar polymorphic fragments.

Based on molecular data, most accessions had a dissimilarity distinct from that represented by morphological characteristics. This dissimilarity demonstrates the importance of complementation of multicategoric data with molecular data. Genetic dissimilarity estimated by combined analysis (multicategoric + SSR molecular) varied from 0.35 to 1.8 with an average of 1.28 (Table 3). Accessions UFVJM-55 and UFVJM-21 had the largest dissimilarity. Accessions UFVJM-55 and UFVJM-21 had distances of 1.80, while accessions UFVJM-26 and UFVJM-27 were the most similar among each other with a distance of 0.35 (Figure 1).

The correlation between the distance matrix that was obtained with multicategoric data and the matrix of SSR marker was 0.133, which indicates a low association between these two methodologies (Table 4). Similarly, Maric, Bolaríc, Martincic, Pejić, and Kozumplik (2004) reported a low correlation (r = 0.12) using RAPD markers and 12 morphologic characters in wheat. The correlation coefficient among combined and multicategoric matrices (r= 0.764) was similar to that of combined and molecular matrices (r = 0.74). Both correlations were significant, p = 0.01, by the Mantel test (Table 4).

**Table 4.** Descriptive statistics, correlations among dissimilarity matrices estimated and correlation cophenetic from multicategoric, molecular and combined data (multicategoric + SSR molecular), in genetic divergence study on 60 sweet potato genotypes (*Ipomoea batatas*). UFVJM, Diamantina, Minas Gerais State, 2016.

| Matrices | Descriptive analysis | | |
|---|---|---|---|
| | Minimum | Maximum | Average |
| $D_{multicategoric}$ | 0.08 | 0.92 | 0.52 |
| $D_{molecular}$ | 0.14 | 1.00 | 0.76 |
| $D_{(mult.+molec.)}$ | 0.35 | 1.80 | 1.28 |
| Matrices | Correlation coefficient | | |
| | $D_{multicategoric}$ | $D_{molecular}$ | $D_{(mult.+molec.)}$ |
| $D_{multicategoric}$ | - | 0.13 | 0.76 |
| $D_{molecular}$ | ** | - | 0.74 |
| $D_{(mult.+molec.)}$ | ** | ** | - |
| Matrices | Correlation cophenetic | | |
| $D_{multicategoric}$ | 0.77** | | |
| $D_{molecular}$ | 0.87** | | |
| $D_{(mult.+molec.)}$ | 0.72** | | |

**Significant correlation at 1% probability by Mantel test with 1.000 permutations.

For combined data (multicategoric + molecular), a cutoff equal to the average dissimilarity (1.28) was applied, which allowed the formation of seven groups (Figure 1). The use of multivariate techniques that simultaneously analyse morphologic and molecular data has been increasing in studies focused on genetic divergence among accessions of germplasm banks (Machado, Jesus, & Ledo, 2015). Groups I and II were formed by a single accession each. Access UFVJM-55 (group I) was the only one that had predominantly purple foliage. Fifty-three specific bands were observed, and they were polymorphic for all oligonucleotide primers. Accession UFVJM-57 (group II) was the only one with a secondary colour of foliage with reddish nodes, and the only one that only amplified 10 specific polymorphic bands, which corresponds to 4.8% of the total. Group III was formed by 12 accessions with an absence of secondary colour of foliage, absence of pubescence point in the apex, triangular mature leaf with average size, predominantly cream-coloured root bark and absence of secondary colour of bark. In this group, seven polymorphic bands were observed for most accessions.

Group IV was formed by seven accessions (Figure 1) and reunited genotypes with absence of pubescence points in foliage apex, mature leaves with average size and fine diameter of internode. This group was not polymorphic to primers Ib-255 and Ib316, and eight polymorphic fragments were observed. Group V was formed by only two accessions: Tomba Carro 1 and UFVJM-49. These genotypes were grouped by characteristics such as five leaf lobes, green-coloured mature leaf, petiole and internode with short length, predominantly white pulp, and absence of a secondary colour in bark and pulp. In this group, 31 polymorphic bands were observed. Group VI gathered the largest number of accessions, totalling 37, which was 61.7% of studied accessions. Cultivars from Embrapa, Brazilândia Branca, Princesa Coquinho, and Palmas were in this group.

The importance of using molecular markers is evident by the large differences that SSR markers have revealed, within groups that were initially formed based only on multicategoric descriptors. The possibility to join multicategoric and molecular data provides reproducibility in analysis, demonstrating that the entire information set can be used.

## Conclusion

There is large amount of genetic variability among sweet potato genotypes of the germplasm bank at UFVJM; this variability is detected by both morphological and molecular characterizations. There was no strong association between morphological and molecular analyses of sweet potato accessions. There was no duplicate suspicion

in dissimilarity studies. Access pairs UFVJM-55 and Tomba Carro 1, UFVJM-55, and UFVJM-21 of multicategoric data and accession pairs UFVJM-42 and Espanhola, UFVJM-07, and UFVJM-44 for molecular data are the most dissimilar. These pairs can be used as genitors to compose breeding programs and can provide progenies with high genetic variability.

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