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Genetic divergence of sweet potato genotypes based on morpho-agronomic traits

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ABSTRACT

Sweet potato is a vegetable of great importance in human consumption, due to its high nutritional value. It presents high phenotypic variation, with different forms of roots, leaves and vines. Given the above, we aimed to study the genetic divergence of sweet potato genotypes based on morpho-agronomic traits. The experiment was conducted in an experimental area of Unesp, Ilha Solteira Campus, located in Selvíria-MS. The experimental design used was randomized blocks with two replicates, including 200 genotypes and 'Beauregard' as a biofortified control. Shoot morphological traits of the genotypes were evaluated at 110 days and root morphological traits were evaluated at 127 days after planting. We used the obtained data to perform descriptive analysis, in percentage of phenotypic classes. In order to study genetic divergence, multivariate analysis was performed, grouping was done using Tocher's and Ward's methods. We also analyzed relative contribution of each trait for genetic divergence. Statistical analyzes were performed using Genes software and SAS. Wide genetic variability could be verified in the population studied in this experiment, being possible to obtain genetic gains in recombination between genotypes. Grouping using Tocher's method was more effective for discriminating dissimilarity between genotypes. The low relative importance of leaf size, internode diameter and secondary peel color makes further evaluation of these traits unnecessary, reducing labor costs, cost and execution time.

Keywords: *Ipomoea batatas*, germplasm bank, breeding, biofortification.

RESUMO

Divergência genética de genótipos de batata-doce por meio de caracteres morfoagronômicos

A batata-doce é uma hortaliça de grande importância na alimentação humana, devido ao seu alto valor nutritivo. Apresenta alta variação fenotípica, com diversas formas de raízes, folhas e ramas. Diante do exposto, objetivou-se estudar a divergência genética de genótipos de batata-doce por meio de caracteres morfoagronômicos. O experimento foi conduzido em área experimental da Unesp, Câmpus de Ilha Solteira, situada no município de Selvíria-MS. O delineamento experimental utilizado foi de blocos casualizados com duas repetições, incluindo 200 genótipos e 'Beauregard' como testemunha biofortificada. Foram avaliados caracteres morfoagronômicos de parte aérea dos genótipos aos 110 dias e de raiz aos 127 dias, após o plantio. Com os dados obtidos realizou-se análise descritiva em percentual das classes fenotípicas. Para estudo da divergência genética realizou-se análise multivariada, agrupando-se pelos métodos de Tocher & Ward. Também realizou-se análise da contribuição relativa de cada carácter para a divergência genética. As análises estatísticas foram feitas utilizando-se o programa computacional Genes e SAS. Há ampla diversidade genética na população estudada, o que pode possibilitar ganhos genéticos na recombinação dos genótipos. O método de agrupamento de Tocher foi mais efetivo para discriminação da dissimilaridade entre os genótipos. A baixa importância relativa dos caracteres tamanho da folha, diâmetro do internódio e cor secundária da casca, permite dispensar a sua análise em futuros trabalhos, reduzindo gastos de mão-de-obra, custo e tempo de execução.

Palavras-chaves: *Ipomoea batatas*, banco de germoplasma, melhoramento genético., biofortificação.

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Sweet potato (*Ipomoea batatas*) is a tuberous vegetable of great economic and social importance. China is the largest worldwide producer, with annual production of approximately 72 million tons of roots and average yield of 21.4 t ha^{-1}. Brazil is the 15th worldwide producer, obtaining average productivities of 14.5 t ha^{-1} (FAO, 2019).

In human nutrition, sweet potato tuberous roots are used *in natura*, cooked, fried, baked and to make cakes, cookies and sweets. The tuberous roots have high nutritive value, due to its carbohydrate contents and sensory versatility, relating to pulp colors, flavor, texture, sugars, minerals (calcium, iron and potassium), precursors of vitamin A, C and B complex (Vizzotto *et al*., 2018).

Biofortified 'Beauregard' sweet potato cultivation has increased in the last years due to its nutritional potential and bioactive compounds which positively impact human health

(Vizzotto *et al*., 2018). Biofortified foods play a very important role in preserving health, as they have high levels of different micronutrients (Loureiro *et al.*, 2018). Biofortified sweet potatoes are rich in β-carotene, a precursor of vitamin A. The prevalence of vitamin A deficiency is a serious problem in developing countries (Park *et al*., 2014). This deficiency causes permanent and temporary eye damage and also increase mortality, especially among children and pregnant or breastfeeding women (Bovell Benjamin, 2007).

The biofortification breeding program, in general, is based on a process of repeated crossing of plants of the same species until obtaining more nutritious cultivars, increasing contents of nutrients and vitamins (Loureiro *et al.*, 2018). The characterization of genetic resources of crops includes conventional approaches, such as the use of descriptive lists of morpho-agronomic traits or evaluation of agronomic performance (Gepts, 2006; Khoury *et al*., 2010).

Morpho-agronomic characterization facilitates the efficient use of genotypes in breeding programs, providing specific and important information about genetic relationships and specific agronomic important traits (Laurie *et al*., 2013). This is usually the most accessible way of quantifying genetic diversity (Ritschel & Huamán, 2002) and consists of providing an identity for each entry through the knowledge of a series of data, which allows to study the genetic variability of each sample (Daros *et al*., 2002).

Relatively high estimates of heritability of some of the traits used as sweet potato descriptors increase confidence in the use of these variables in characterization process (Ritschel & Huamán, 2002). For sweet potato, broad-sense heritability is important because the effects of dominance and epistasis are maintained by vegetative propagation (Gonçalves Neto *et al*., 2012).

Availability of genotypes adapted to regional climatic conditions depends on characterization, identification and selection of genotypes which show potential for cultivation and breeding, aiming to increase yield and quality of this crop (Moreira *et al*., 2009).

Considering socioeconomic importance of sweet potato, characterizing genotypes for genetic dissimilarity is essential, in order to guide new crossings in breeding programs (Andrade *et al*., 2017).

Given the above, this study aimed to characterize morphoagronomically biofortified sweet potato genotypes obtained by poly-crosses.

MATERIAL AND METHODS

The experiment was carried out in an experimental area, at Fazenda de Ensino, Pesquisa e Extensão da Faculdade de Engenharia (Teaching, Research and Extension Farm at Engineering College), UNESP, Ilha Solteira Campus, located in the municipality of Selvíria-MS (51º22′W, 20º22′S, altitude 335 m), from January to May, 2019.

According to Köppen, the local climate is Cwa, humid subtropical, annual rainfall is 1261 mm and temperature between 21.4 and 26.9ºC and 62.4% relative humidity (Portugal *et al*., 2015). The soil was classified as Dystrophic Red Latosol, clayey texture (Santos, 2013).

The experimental design was a randomized block design with two replicates, consisting of 200 treatments (genotypes), and Beauregard as biofortified control. Each experimental plot consisted of three plants, the spacing used was 1.20 between lines and 0.33 m between plants, according to methodology of Andrade *et al*. (2017). The genotypes of 15 half-sibling families (polycross) were obtained from the breeding program of *Centro Internacional de la Papa* (CIP).

Before planting, soil samples were collected from 0 to 20 cm layer depth in the experiment local. Afterwards, the samples were sent to Laboratório de Solos da Unesp (Soil Laboratory of UNESP), Ilha Solteira Campus, for chemical characterization, showing the following results: P (resin)= 23 mg dm^{-3}, MO= 20 g dm^{-3}, pH ($CaCl_2$)= 5.0, K= 2.3 $mmol_c$ dm^{-3}, Ca= 20 $mmol_c$ dm^{-3}, Mg= 17 $mmol_c$ dm^{-3}, H+Al= 34 $mmol_c$ dm^{-3}, SB= 39.3 $mmol_c$ dm^{-3}, CTC= 73.3 $mmol_c$ $dm^{-3}$and V= 54%.

Soil was plowed followed by two harrowings. Planting ridges were built mechanically, spaced 1.2 m among them, 0.40 m height. Meanwhile, planting fertilization was performed using 500 kg ha^{-1} fertilizer formula 4-14-8, 133 kg ha^{-1} potassium chloride and 166 kg ha^{-1} simple superphosphate, corresponding to 20 kg/ha N, 70 kg/ha P_2O_5 and 140 kg/ha K_2O (Monteiro & Peressin, 1997).

Vines were collected from a field grown mother plant. Each branch consisted of 8-10 buds. In planting, 2/3 of the branch was buried in pits previously made with an appropriate hoe. At 30 days after planting, top dressing fertilization was carried out with 30 kg ha^{-1} nitrogen (Monteiro & Peressin, 1997), using urea as source. Weed was mechanically controlled with the aid of a hoe and chemical control with applications of herbicide, with active ingredient Linurom, at a dose of 0.6 L ha^{-1} and Clethodim + Alkylbenzene at a dose of 0.20 L ha^{-1}. Irrigation was performed using a central pivot, 12 mm water depth, with a three-day watering shift. Pests and diseases were not controlled since no economic damage was verified.

Morpho-agronomic traits of genotype shoots were evaluated at 110 days after planting and roots at 127 days after planting. We evaluated leaf type, general leaf outline, leaf lobe type, the number of lobes per leaf, lobe shape, mature leaf color, immature leaf color, petiole color, petiole length, abaxial central vein pigmentation, predominant leaf color, secondary leaf color, leaf pubescence type, haulm length, haulm diameter, root shape, root surface defects, cortex thickness, predominant peel color, intensity of the peel color, secondary peel color, predominant pulp color, secondary pulp color and secondary pulp color distribution. Shoot attributes were characterized using the central part of the vines, using three leaves per plant and one plant per plot. To characterize the roots, we selected two roots showing commercial standard, weight over 80 g, which represented the entire plot, in relation to size, shape and mass.

Morpho-agronomic characterization of shoots and roots was done according to the methodology described by Huamán (1992). Using the data of morpho-agronomic traits measured in this study, descriptive analysis, in percentage of phenotypic classes was done using Microsoft Excel graphs, version 2010.

Using morpho-agronomic and production data, the authors performed individual analysis of response variable. Clonal progeny test was carried out, it means, half-sibling families and clones were considered as progenies. Using the analysis results, we verified the proportional magnitudes of genetic variances between and within families. These analyses were carried out with the aid of SAS statistical software (Khattree & Naik, 2018). Due to the fact that we verified that the main genotypic variation was between the clones within the families, the authors carried out the individual analysis for 24 morpho-agronomic traits, following the model of clonal tests, obtaining the averages and matrices of variances and covariance. Then, the distances from the average Euclidean distance was calculated (Cruz, 2013).

For groupings using Tocher's and Ward's methods, 76 genotypes were selected. These genotypes showed marketable root productivity higher than cultivar Beauregard (847 g/plant). The authors decided to exclude genotypes which presented productivity lower than the control cultivar, since the aim of this study was to recognize genetic variability among high productive genotypes for future actions in a breeding program. Thus, genetic distance matrix was estimated using multi-categorical variables. Afterwards, data were grouped by Tocher's optimization method and the modified Ward's hierarchical method. In Ward's hierarchical method, data were grouped with a cut made at 69% similarity, based on the permutation test.

We also analyzed relative contribution of each trait to genetic divergence, according to the method proposed by Singh (1981). Statistical analyses were carried out using Genes software (Cruz, 2013).

RESULTS AND DISCUSSION

We evaluated 181 morpho-agronomic traits, distributed in 24 morpho-agronomic descriptors of roots and shoots of sweet potato, being verified an expression of 90.61% of these traits. For shape, defect, cortex thickness, predominant peel color, peel color intensity, predominant pulp color, type of plant, predominant leaf color, secondary leaf color, type of pubescence, general leaf outline, number of lobes, lobe shape, immature leaf color and petiole pigmentation, 100% of the possible classes were expressed within each descriptor (Figure 1).

For secondary peel color, secondary pulp color, color distribution, haulm diameter, haulm length, type of leaf, mature leaf color and petiole length, we verified an expression ranging from 70 to 83%, of possible classes within each descriptor (Figure 1). Vargas *et al.* (2018), evaluating 172 morpho-agronomic classes, distributed in 23 morpho-agronomic descriptors, verified 70.35% of expression of these classes, values which were below those found in this study. Andrade *et al.* (2017), studying 60 accessions and 24 descriptors of shoots and roots, verified wide variability among the accessions. Thus, we can infer that the population used in this study shows wider phenotypic variability than the studies mentioned above, and that, *a priori*, genetic gains with this selection can be gained.

Despite the difficulties in classifying morpho-agronomic traits according to subjectivity, which was verified by Vargas *et al.* (2018), this methodology for investigating germplasm diversity is often carried out (Fongod *et al.*, 2012), since this is a low-cost technique as well as easy to be applied (Vieira *et al*., 2008).

Grouping analysis using Tocher´s method provided the division of 76 genotypes, into 14 groups, confirming the existence of high genetic variability between genotypes (Table 1). As based on the formation of these 14 groups, it is possible to select divergent genotypes for hybridization. According to Casassola *et al.* (2013), parental selection is an important step for any breeding program. The genetic diversity found in this study makes it possible to cross divergent genotypes for an expression of a wide genetic variability in segregating descendants, which will allow the selection of promising genotypes for superior agronomic traits.

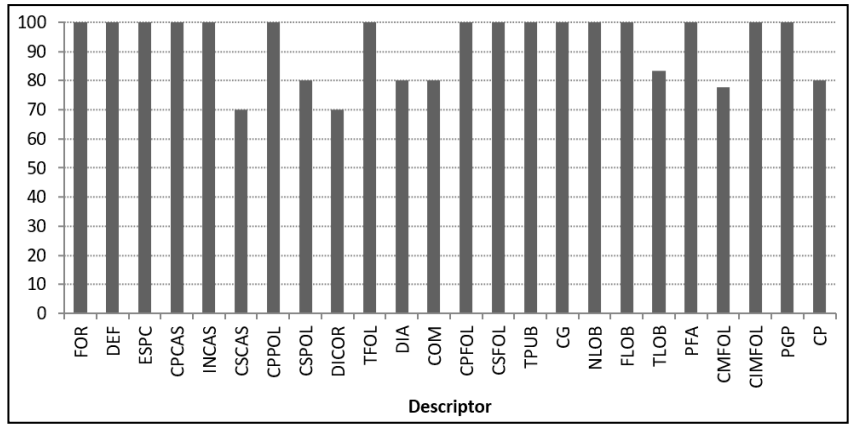

Figure 1. Percentages of expression of morpho-agronomic classes. FOR= shape; DEF= defect; ESPC= cortex thickness; CPCAS= predominant peel color; INCAS= intensity of peel color; CSCAS= secondary peel color; CPPOL= predominant pulp color; CSPOL= secondary pulp color; DICOR= secondary pulp color distribution; TFOL= type of plant; DIA= haulm diameter; COM= haulm length; CPFOL= predominant leaf color; CSFOL= secondary leaf color; TPUB= type of pubescence; CG= general leaf outline; NLOB= number of lobes; FLOB= lobe shape; TLOB= leaf type; PFA= abaxial central vein pigmentation; CMFOL= mature leaf color; CIMFOL= immature leaf color; PGP= petiole pigmentation; CP= petiole length. Ilha Solteira, Unesp, 2019.

The authors observed groupings of 30 genotypes in group 1 and 24 in group 2. Vargas *et al.* (2018), studying 96 accessions of sweet potato, verified 11 groups formed, being the first group with 70 accessions, showing existence of great genetic variability. In group 3, we can verify 7 grouped genotypes. Group 4 showed 3 genotypes, groups 5 and 6 showed 2 genotypes, and groups 7, 8, 9, 10, 11, 12, 13 and 14 (Table 2) showed only 1 genotype. These genotypes can be considered divergent in the evaluated groups, and they can be recommended for directed hybridization programs, as long as good performance per se and genetic complementation between chosen genotypes are observed. According to Dias & Kageyama (1997), parental divergence should not be the only criterion when deciding for crossings.

The number of groupings formed by Tocher's method, in this study, was higher than the one found by Oliveira *et al*. (2000). These authors used 51 accessions of different regions of the country and observed the formation of seven groups using the same method. Maquia *et al*. (2013) reported the formation of six groups with similarity, based on morpho-agronomic descriptors and a grouping of 44 accessions of different countries, using UPGMA method.

Grouping analysis using Ward's methodology, made possible the ordering of 76 genotypes, in 11 groups, corroborating the existence of high genetic variability among genotypes (Figure 2), since based on the formation of these 11 groups, we could select divergent genotypes for hybridization. Sousa *et al.* (2019), studying 19 shoot morpho-agronomic descriptors in 102 sweet potato clones, verified 17 groupings using unweighted pair group method with arithmetic average.

The authors observed 13 genotypes in group 1 and 9. In group 2 and 7, 3 genotypes were grouped. Group 3 and 6 showed 9 genotypes. Groups 4 and 11 showed 6 genotypes. Groups 5 and 8 showed 5 genotypes and group 10 showed only 4 genotypes, showing genetics diversity (Figure 2).

The authors verified high number of groups formed using both methods, Tocher´s and Ward´s methods, among the genotypes used in this study (Figure 2 and Table 1). Differences in estimating genetic divergence between Tocher's method and the dendrogram were also observed by Azevedo *et al*. (2013), showing difference between the methods concerning accuracy and criterion. According to Azevedo *et al*. (2013), differences among the results obtained from different methods of multivariate analysis is common, since the methods are based on different grouping techniques. Thus, compare the results obtained by different multivariate analysis methodologies, in order to obtain a more accurate interpretation of the results, is important (Azevedo *et al.*, 2015).

Genotypes belonging to more distant groups are different and can be considered as promising artificial crosses to obtain superior segregating populations (Sousa *et al.*, 2019). However, in addition to the genetic divergence, the selected clones must present gene complementation associated with high variability for the evaluated traits (Martins *et al*., 2012). Among the advantages of using multivariate analysis techniques, the possibility of assessing the importance of each trait studied on the total variation available among the evaluated genotypes stands out (Azevedo *et al.*, 2015).

We observed low amplitude

Table 1. Grouping established by Tocher's method among 76 sweet potato genotypes evaluated by 24 morpho-agronomic traits. Ilha Solteira, Unesp, 2019.

Groups	Genotypes			
I	CERAT31-02	CERAT35-18	CERAT35-22	CERAT16-04
	CERAT55-20	CERAT21-08	CERAT34-20	CERAT25-11
	CERAT35-13	CERAT25-01	CERAT31-06	CERAT21-05
	CERAT21-04	CERAT34-22	CERAT29-13	CERAT52-02
	CERAT21-17	CERAT52-25	CERAT51-12	CERAT21-02
	CERAT51-28	CERAT51-05	CERAT34-04	CERAT55-17
	CERAT25-24	CERAT16-25	CERAT34-14	CERAT51-30
	CERAT56-31	CERAT21-01		
II	CERAT24-26	CERAT26-01	CERAT31-04	CERAT52-22
	CERAT31-22	CERAT34-07	CERAT29-26	CERAT21-06
	CERAT60-05	CERAT24-08	CERAT52-24	CERAT56-34
	CERAT52-26	CERAT35-20	CERAT24-03	CERAT24-31
	CERAT24-09	CERAT29-23	CERAT31-15	CERAT34-06
	CERAT34-18	CERAT24-04	CERAT60-03	CERAT24-25
III	CERAT29-04	CERAT31-16	CERAT16-06	CERAT21-18
	CERAT56-23	CERAT60-02	CERAT34-15	
IV	CERAT16-15	CERAT52-15	CERAT60-07	
V	CERAT16-20	CERAT31-01		
VI	CERAT31-09	CERAT35-09		
VII	CERAT16-03			
VIII	CERAT24-32			
IX	CERAT16-02			
X	CERAT56-32			
XI	CERAT55-01			
XII	CERAT37-07			
XIII	CERAT55-11			
XIV	CERAT29-16			

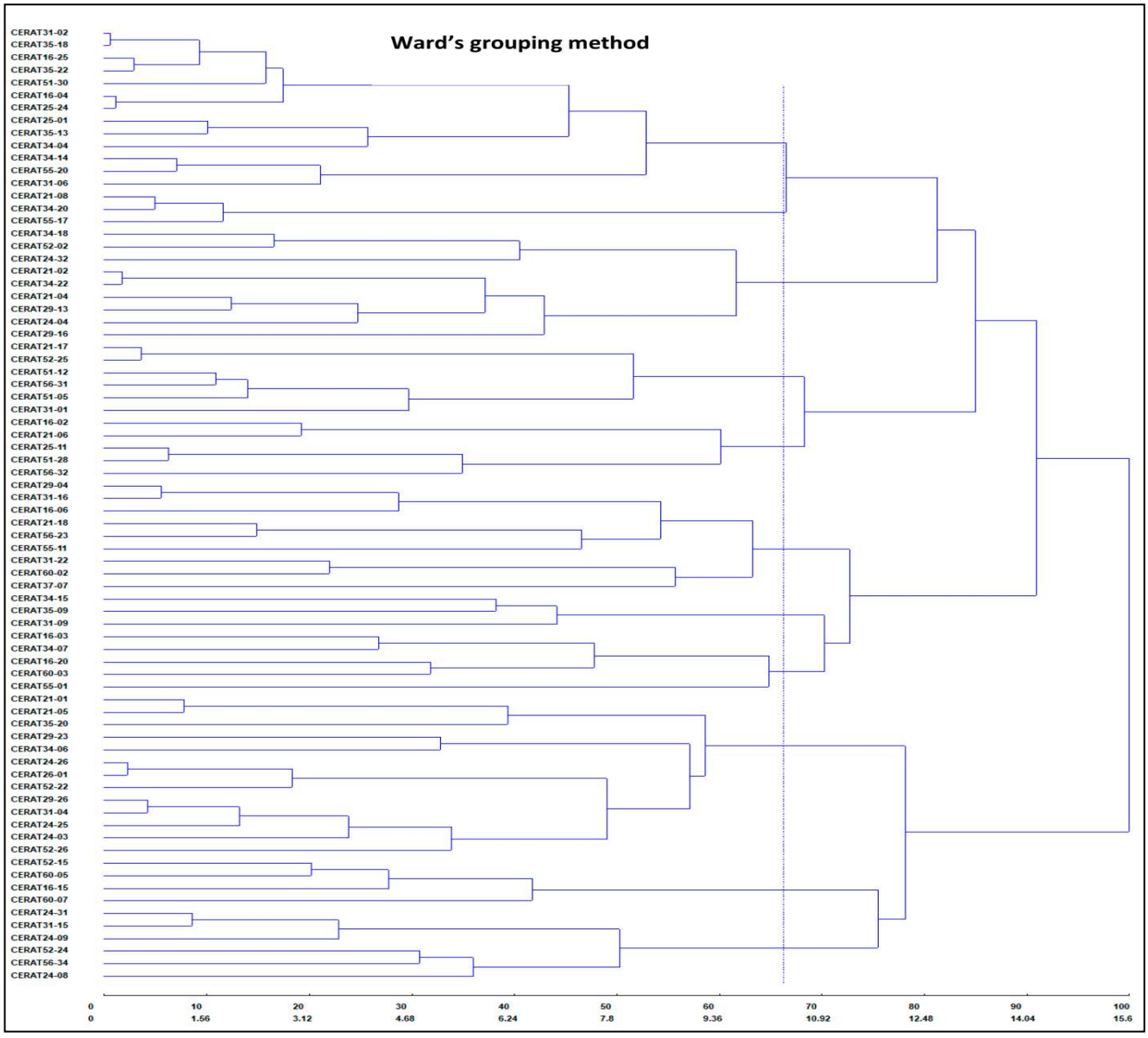

Figure 2. Dissimilarity dendrogram of 76 sweet potato genotypes, established by Ward's method using Euclidean distance. Ilha Solteira, Unesp, 2019.

among traits which most contribute for divergence (pigmentation of the lower ribs) and which contributed least (leaf size) (Table 2). The traits which more contributed for genetic diversity among genotypes were pigmentation of the inferior veins (8.19%), petiole pigmentation (7.85%), primary leaf color (7.54%), secondary leaf color (7.32%), secondary pulp color (6.49%), petiole length (5.06) and number of lobes per leaf (5.02%). Traits which contributed least were leaf size (1.33%), internode diameter (1.56%), secondary peel color (1.94%), internode length (2.42%), predominant

Table 2. Relative contribution of 24 sweet potato morpho-agronomic descriptors to genetic divergence by the methodology method proposed by Singh. Ilha Solteira, Unesp, 2019.

Morpho-agronomic descriptors	Relative contribution (%)
Leaf size	1.33
Internode diameter	1.56
Secondary peel color	1.94
Internode length	2.42
Predominant peel color	2.93
General outline	3.00
Immature leaf color	3.07
Plant type	3.10
Mature leaf color	3.17
Predominant pulp color	3.20

Table 2. continuation

Morpho-agronomic descriptors	Relative contribution (%)
Cortex thickness	3.32
Central lobe shape	3.37
Root defect	3.50
Root shape	3.65
Color distribution	3.76
Pubescence type	4.56
Color intensity	4.67
Number of leaf lobes	5.02
Petiole length	5.06
Secondary pulp color	6.49
Secondary leaf color	7.32
Primary leaf color	7.54
Petiole pigmentation	7.85
Pigmentation of the lower veins	8.19

peel color (2.93%) and general outline (3.00%). In this study, the seven main traits represented 47.47% of relative contribution for genetic divergence. Azevedo *et al.* (2015) verified that the three main traits represented 63.58% of contribution and for Oliveira *et al.* (2000) the contribution was 79.53%.

Wide genetic diversity among sweet potato genotypes in the studied population could be noticed; this genetic diversity allows genetic gains by recombining genotypes.

Tocher's grouping method was the most effective to identify dissimilarity among genotypes.

The low relative importance of leaf size, internode diameter and secondary peel color allows to verify that these evaluations can be unnecessary for further studies, reducing labor costs, cost and execution time of programs.

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