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Genetic dissimilarity and relative importance of characteristics in banana cultivars through multivariate analysis

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ABSTRACT

One of the problems of banana cultivation is the reduced number of productive commercial cultivars, with fruits adequate to market requirements. This paper aimed to study the genetic divergence among 15 banana cultivars by multivariate analysis and determine the relative importance of 35 agronomic and nutritional characteristics. The data were subjected to multivariate analysis of variance. For the application of Tocher Optimization grouping method, the Mahalanobis distance was used. The hierarchical method UPGMA and canonical variables analysis were also used. For the identification of the most important characteristics for dissimilarity, was used the Singh method. There was a higher mean dissimilarity to cultivate Terrinha followed by Marmelo cultivars and Mysore. The smallest measure of dissimilarity was found between the Nanicão and Prata-Graúda cultivars. The characteristics vitamin A content, starch percentage, total soluble sugars, sugars, non-reducing, antioxidant potential, fresh weight of pulp, plant height and fresh weight of the fruit are the most important in the estimation of dissimilarity, are soon to be prioritized in studies future.

Key words: genetic diversity; Singh method; *Musa* spp.; variability

Dissimilaridade genética e importância relativa de características em cultivares de bananeira por meio de análise multivariada

RESUMO

Um dos problemas do cultivo da bananeira é o número reduzido de cultivares comerciais produtivas, com frutos adequados às exigências do mercado. Objetivou-se estudar a dissimilaridade genética entre 15 cultivares de bananeira por análise multivariada e determinar a importância relativa das 35características agronômicas e nutricionais. Os dados foram submetidos à análise de variância multivariada. Para a aplicação do método de agrupamento de Otimização de Tocher, foi utilizada a distância generalizada de Mahalanobis. O método hierárquico UPGMA e a análise de variáveis canônicas também foram utilizados. Para a identificação dos caracteres mais importantes para a dissimilaridade, utilizou-se o método Singh. Verificou-se maior dissimilaridade média para a cultivar Terrinha seguida das cultivares Marmelo e Mysore. A menor medida de dissimilaridade foi encontrada entre as cultivares Nanicão e Prata-Graúda. As características teor de vitamina A, percentual de amido, açúcares solúveis totais, açúcares não redutores, potencial antioxidante, matéria fresca de polpa, altura da planta e massa fresca do fruto são as mais importantes na estimação da dissimilaridade, logo devem ser priorizados em estudos futuros.

Palavras-chave: divergência genética; método de Singh; *Musa* spp.; variabilidade

Introduction

The banana tree (*Musa* spp.) is one of the most cultivated fruit plant in tropical and subtropical countries. In Brazil, banana production was 6.95 million tons in 478 thousand hectares harvested in 2014 (FAO, 2017). Due to its good organoleptic characteristics and low cost, bananas are accessible and consumed by people of all social classes in different regions of the world (Amorim et al., 2011). However, few cultivars are available for commercial exploitation with agronomic potential, pest and disease tolerance, and fruit suitable for the market (Silva et al., 2013). Therefore, it is of great importance the incentive of research in breeding programs in order to introduce new cultivars with agronomic characteristics favorable to the producer and consumer market.

Banana consumption is important because of the levels of carotenoids, minerals, carbohydrates and vitamins. In addition, phenolic compounds with a recognized antioxidant activity are present in the banana, providing to this fruit the characteristic of a functional food, which provides innumerable benefits to human health (Aquino et al., 2014; Aquino et al., 2016). In this way, considering the importance of banana farming, the increase in its consumption and commercialization and the growing search for nutritious fruits, it is necessary to obtain superior cultivars through genetic improvement (Alves et al., 2012).

The success of a breeding program depends, among other factors, on the availability of populations that present high genetic variability for the characteristics under selection (Cruz et al., 2012). For the quantification of genetic variability, morphological and agronomic characters are very accessible descriptors when compared with more advanced molecular techniques, and have been used in the evaluation of genetic divergence (Bertini et al., 2009; Alves et al., 2012; Azevedo et al., 2013).

When the evaluated characters are quantitative, the genetic divergence can be estimated from measures of dissimilarity, highlighting the generalized distance of Mahalanobis considering the possible dependencies between the characters (Cruz et al., 2012). Methods such as the hierarchical UPGMA, Tocher optimization and techniques of graphic dispersion by canonical variables are also useful, since they allow the grouping of cultivars (Azevedo et al., 2015).

Although the evaluation of a greater number of characteristics makes possible a more complete study of the dissimilarity, the evaluation of many characteristics can be infeasible when the number of cultivars is large, being necessary to know the most important ones (Azevedo et al., 2015). Thus, the objective of this study was to study genetic dissimilarity among 15 banana cultivars by multivariate analysis and to determine the relative importance of the characteristics evaluated in this study.

Material and Methods

Genetic material

The plants and fruit analyzed were obtained in a 7-year-old experimental orchard in spaced 3.5 m x 2.5 m plants at the Universidade Federal de Viçosa (UFV), in Viçosa, Minas Gerais, located at 20°45' S and 42°52' W, with an altitude of 648 m. The cultivars Ouro (AA), Nanica (AAA), Nanicão (AAA), Caru-Verde (AAA), Caru-Roxa (AAA), Caipira (AAA), Prata (AAB), Prata-Anã (AAB), Maçã (AAB), Mysore (AAB), Pacovan (AAB), Terrinha (AAB), Marmelo (ABB), Prata-Graúda (AAAB) and Caju (unknown genomic group) were evaluated.

Characteristics evaluated

Five plants per cultivar were selected, which had already emitted inflorescence, in which they were evaluated for height, measured from the soil level to the inflorescence insertion and pseudostem diameter at 30 cm from the soil level. From each cultivar, the third leaf from the inflorescence was taken to determine the length of the petiole and the leaf blade and the length/width ratio of the blade.

The bunches were harvested when the first signs of appearance of the yellow color in the fruit peel were observed. Then they were plucked, counting the number of cluster per bunch, number of fruit per cluster, number of fruit per bunch and bunch weight without rachis. Subsequently, the second, third and fourth stem were removed from each bunch and immediately transported to the Fruit Analysis Laboratory, at the UFV campus. The fruit were cut close to the floral cushion, eliminating the damaged, diseased and malformed fruit. They were then washed in tap water and allowed to stand on absorbent paper for a few minutes to coagulate the latex. Six fruit were selected per bunch, making 24 fruit per cultivar, being immersed in ethephon solution (1.2 g L^{-1}) for eight minutes, to standardize ripening. On average, fruit reached the color 6 stage (fruit with completely yellow bark) (Dadzie & Orchard, 1997) four days after harvest.

After fruit ripening, the following determinations were made: fresh fruit and pulp weight, fruit diameter, pulp color, pulp firmness, soluble solids content, fresh pulp mass and pulp/peel ratio. Soluble solids content was determined with the aid of an Atago portable digital refractometer model N1, with reading in the range of 0 to 32 ºBrix. The fruit were then peeled and processed in a domestic blender, with pulp samples packed in aluminum wrap, freezed in liquid nitrogen and kept in an ultra-freezer at -80 °C until analysis. In the pulp the content of vitamin A, total soluble, reducing and non-reducing sugars, starch, titratable acidity, phenolic compounds, antioxidant potential and mineral content (P, K, Ca, Mg, Fe, Zn, Cu and Mn) were evaluated. Carotenoids α-carotene, β-carotene and lutein were analyzed by high-performance liquid chromatography with diode array detection (HPLC-DAD) according to Pinheiro-Sant'Ana et al. (1998). The levels of α-carotene and β-carotene were used only for determining vitamin A content, and the value was expressed as retinol activity equivalents (RAE) in 100g of fresh pulp.

The total soluble, reducing and non-reducing sugars and starch contents were extracted with 80% hot ethanol (Hodge & Hofreiter 1962). The quantification of total soluble sugars and starch was determined by the reaction with anthrone (9,10-dihydro-9-oxoanthracene), changing the concentration of the reagent to 0.1% in sulfuric acid (28 N). The quantification of reducing sugars was performed by the method of Nelson

(Nelson 1944, Somogy 1952). For the titratable acidity the titrations were carried out until pH 8.2 under constant stirring with 0.05 N NaOH solution, previously standardized with potassium biftalate.

The extraction of the phenolic compounds was done according to Bloor (2001), the content being determined using the Folin-Ciocalteu reagent (Sigma-Aldrich, USA), according to Singleton et al. (1999). The capacity of free radical sequestration was evaluated using the DPPH (2,2-diphenyl-2-picrylhydrazyl radical) test (Sigma-Aldrich, USA), according to Blois (1958). The P was determined by colorimetry, according to Braga & Defelipo (1974), and the other nutrients were quantified by spectrophotometry of atomic absorption in spectrophotometer model SpectrAA220 FS (Varian Medical Systems, Belrose, Australia).

Statistical analysis

For the analysis of variance we considered the model: $y_{ij} = \mu + t_i + e_{ij}$; where y_{ij} = observation of the i-th treatment; μ = general mean; t_i = effect of the i-th treatment (1, ..., 15 cultivars); and e_{ij} = effect of the experimental error on the j-th repetition (1,..., 4 bunches) that received treatment i. All these parameters were considered as random effects, with the exception of the general mean. For the application of the Tocher Optimization grouping method, the generalized distance of Mahalanobis was used as a measure of dissimilarity. The hierarchical UPGMA method and the analysis of canonical variables were also used in the study of genetic dissimilarity. To establish the cut-off point in the dendrogram, the Mojena test (1977) was used. Subsequently, for the identification of the most important characteristics for the divergence, the relative contribution to the divergence was estimated, estimated by the method proposed by Singh (1981). Statistical analyzes were performed using the Genes program (Cruz, 2013).

Results and Discussion

There was a higher mean dissimilarity (13383.36) for the cultivar Terrinha (Table 1), followed by the cultivars Marmelo (6629.01) and Mysore (3690.46). The lowest dissimilarity measure was found between the cultivars Nanicão and Prata-Graúda (606.76), indicating that these cultivars are very close as to the morphological characters of the plant and the characters related to fruit production.

The highest estimate of dissimilarity was found between the cultivar Terrinha in relation to all other cultivars (Tables 1 and 2, Figures 1 and 2). The main peculiarities of the cultivar Terrinha are its greater firmness of pulp, higher content of soluble solids and higher content of Vitamin A (Retinol Activity Equivalent - RAE). 'Terrinha' presented a mean of 132.36 RAE in 100g of fresh pulp, whereas the other cultivars presented a mean of 13.53 RAE in 100g of fresh pulp (Table 2).

High levels of Vitamin A are desired because its deficiency in human nutrition is considered a serious nutritional disease, and it is the most frequent cause of avoidable blindness in the world (Santos et al., 2010). Therefore, plants of the cultivar Terrinha are important for cultivation and for integrating breeding programs in order to obtain the segregating population with high content of vitamin A.

Table 1. Establishment of the most similar and dissimilar banana cultivars, based on estimates of the generalized distances of Mahalanobis (D^2).

Cultivar	D^2 (mean)	More similar		More distant	
		D^2 (smaller)	Cultivar	D^2 (greater)	Cultivar
Caipira	3253.54	1279.26	Maçã	14376.98	Terrinha
Caju	2761.23	805.99	Prata-Anã	15099.16	Terrinha
Caru-Verde	2570.78	944.77	Caru-Roxa	9571.81	Terrinha
Caru-Roxa	2475.04	944.77	Caru-Verde	9157.27	Terrinha
Maçã	2534.39	703.76	Pacovan	13540.84	Terrinha
Marmelo	6629.01	3555.27	Maçã	16643.71	Terrinha
Mysore	3690.46	1049.62	Prata-Anã	17026.03	Terrinha
Nanica	3142.99	661.54	Nanicão	11904.47	Terrinha
Nanicão	2405.70	606.76	Prata-Graúda	12084.36	Terrinha
Ouro	2966.46	1522.42	Prata	11922.09	Terrinha
Pacovan	2481.02	703.76	Maçã	14112.85	Terrinha
Prata	2648.32	790.31	Pacovan	14115.96	Terrinha
Prata-Anã	2424.80	608.75	Nanicão	14031.31	Terrinha
Prata-Graúda	2519.79	606.76	Nanicão	13780.15	Terrinha
Terrinha	13383.36	9157.27	Caru-Roxa	17026.03	Mysore

Table 2. Mean of each of the evaluated characteristics of the mature fruit and the plant for the groups formed by the Tocher method based on the generalized distance of Mahalanobis.

Variable	Groups[1] formed by the Tocher method		
	1		2
	A	B	
Vitamin A (RAE in 100g of dry matter)	14.38	2.54	132.36
Starch content (%)	2.91	13.33	12.61
Total soluble sugars (%)	20.78	11.51	10.91
Non-reducing sugars (%)	12.01	0.29	0.43
Antioxidant potential (%)	69.41	44.41	79.86
Pulp fresh weight (g)	87.64	130.47	97.18
Plant height (m)	4.56	4.42	3.80
Frui fresh weight (g)	126.94	221.99	149.85
P content of the pulp (g kg^{-1} of dry matter)	2.86	3.70	2.49
Ca content of the pulp (g kg^{-1} of dry matter)	0.18	0.13	0.09
Firmness of the pulp (N)	5.08	8.22	12.51
Pulp color	84.44	94.62	72.10
Pupl dry mass (g)	27.57	33.63	37.38
Fruit per bunch (kg)	130.63	62.52	91.06
Length/width of the blade	3.23	3.02	2.71
Mn content of the pulp (mg kg^{-1} of dry matter)	11.90	4.87	20.67
Pulp/peel	2.44	1.43	1.84
Bunch weight (kg)	16.62	17.33	19.09
Phenolic compounds (mg of EGA)[2]	53.82	50.29	73.28
Fruit diameter (mm)	36.21	44.13	36.92
Reducing sugars (%)	8.76	11.22	10.48
Fruits per cluster (unit)	14.80	9.97	11.38
Clusters per bunch (unit)	8.92	6.50	8.00
Total soluble solids (º Brix)	25.43	22.41	29.53
Mg content of the pulp (g kg^{-1} of dry matter)	0.86	0.86	0.72
Lutein content (μg 100 g^{-1} of dry matter)	198.40	111.29	192.50
Width of the blade (cm)	82.06	75.25	82.50
Titratable acidity (%)	0.50	0.62	0.73
K content of the pulp (g kg^{-1} of dry matter)	8.35	8.22	6.31
Fe content of the pulp (mg kg^{-1} of dry matter)	29.50	25.36	25.42
Length of leaf petiole (cm)	53.65	50.00	46.00
Cu content of the pulp (mg kg^{-1} of dry matter)	4.22	3.86	2.83
Zn content of the pulp (mg kg^{-1} of dry matter)	21.73	20.52	16.89
Length of leaf blade (cm)	263.62	227.75	222.75
Diameter of the pseudostem (cm)	30.72	24.75	23.16

[1]Groups of cultivars formed by the Tocher method: 1a (Caipira, Caju, Caru-Roxa, Maçã, Mysore, Nanica, Nanicão, Pacovan, Prata, Prata-Anã, Prata-Graúda, Caru-Verde and Ouro), 1b (Marmelo) and 2 (Terrinha). [2]EGA (equivalents of gallic acid per 100g of mature pulp, based on fresh matter).

Group 1b, in spite of presenting smaller number of fruits per cluster, per bunch the cultivar Marmelo was distinguished by high content of starch, which is important in the process of cooking and frying, since it is the form that generally some types of banana are eaten (Aquino et al., 2016). This high content of starch in the mature banana pulp (13.33%) is 4.6 times higher than the average of the cultivars that composed the group 1a, and close to the percentage found for the cultivar Terrinha (12.60%) (Table 2). According to Emaga et al. (2007), bananas present high levels of starch in the green pulp and, even when mature, they maintain a high content. The high content of starch, especially resistant starch, provides beneficial effects on human health due to its action as dietary fiber (Rodríguez-Ambriz et al., 2008). Thus, cultivars with a high percentage of starch can be used to obtain flour to be used as a functional ingredient in several foods, providing health benefits to humans because they play an important role in the prevention and treatment of obesity, atherosclerosis, coronary diseases, colorectal cancer and diabetes (Juarez-García et al., 2006).

The same grouping tendency observed for Tocher (Table 2) was found by the hierarchical dendrogram using the UPGMA algorithm (Figure 1) and based on the study of the first two canonical variables (Figure 2).

From the Tocher method, it was possible to group the 15 cultivars into two groups (Table 2). Group I was composed of 14 cultivars, totaling 93% of the cultivars. Group II was formed only by the cultivar Terrinha. This information of dissimilarity of the cultivar Terrinha in relation to the others is in agreement with that verified in Table 1. When the Tocher grouping was performed only for the cultivars belonging to the group I, two subgroups were obtained, named 1a and 1b (Table 2).

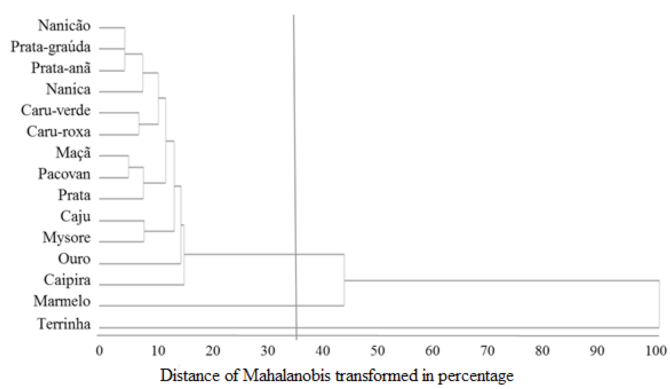

Figure 1. Dendrogram illustrating the dissimilarity pattern obtained by the UPGMA method, based on the generalized distance of Mahalanobis in banana cultivars.

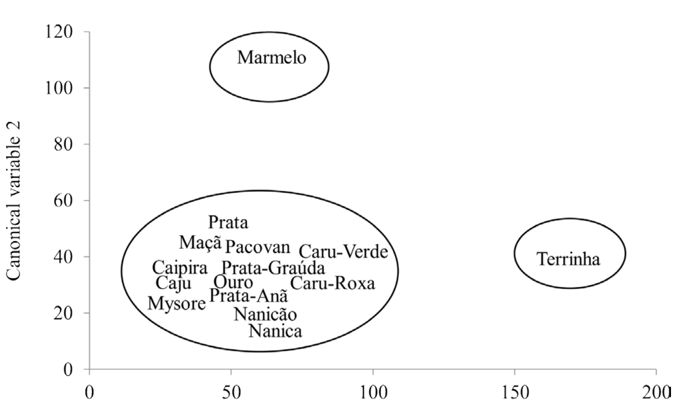

Figure 2. Graphical dispersion of the scores in relation to the first two canonical variables (VC1 and VC2) in banana cultivars.

The study of different methodologies in the evaluation of dissimilarity provides greater reliability in the study of dissimilarity, since each method is based on distinct grouping techniques. This statistical methodology used is important in the study of genetic variability for the genetic improvement of plants (Azevedo et al., 2013).

Among the three clusters formed (groups 1a, 1b and 2) there was a greater distance between group 2 and group 1b (16643.71) (Table 3), a distance close to that found between groups 2 and 1a (13132.56). The smallest distance was found between groups 1a and 1b (5858.65). These observations confirm the information presented in Table 1 and Figure 1, confirming the dissimilarity between the cultivars Terrinha and Marmelo and the others. These two cultivars (Terrinha and Marmelo) present high starch content (Table 2) even after fruit ripening, being important for pulp firmness at the time of frying or cooking of fruit, besides the benefits of resistant starch. In addition, the 'Terrinha' banana has high levels of phenolic compounds and pro-vitaminic carotenoids, such as α-carotene and β-carotene, both in the pulp and in the bark, providing to this cultivar a great antioxidant potential (Aquino et al., 2016). The presence of antioxidant components in the human diet is associated with protective effects against some chronic degenerative diseases, including cancer, stroke, cardiovascular diseases, Parkinson's disease and Alzheimer's disease (Isabelle et al., 2010). In addition, antioxidant compounds have proven action on synergistic effects and protective properties against various degenerative diseases (Abdel-Hameed, 2009). The use of 'Terrinha' and 'Marmelo' in breeding programs could contribute to obtain plants with good characteristics of nutritional quality and fruit market.

Among the advantages of using multivariate analysis techniques, we highlight the possibility of evaluating the importance of each characteristic studied in the estimation of dissimilarity (Azevedo et al., 2015). Thus, based on the criterion proposed by Singh (1981), in terms of relative contribution, it was evidenced greater importance for the characteristics of the vitamin A content, starch content, total soluble sugars, non-reducing sugars, antioxidant potential, pulp fresh mass, plant height and fresh fruit mass. These eight characteristics add up to the relative contribution of 79% (Table 4). Alves et al. (2012) verified that the number of clusters, number of fruit, number of days from planting to harvest, weight of the clusters and average fruit weight were the variables that contributed the most to genetic divergence. Likewise, Mattos et al. (2010) observed that the variable number of fruit contributed with 83.80% of variation, whereas the fresh fruit and pulp masses contributed with 59.42% and 30.32% of the variation, respectively, in 26 accesses of banana tree, being responsible for the greatest variation found.

The characteristics of Vitamin A content, percentage of starch, total soluble sugars, non-reducing sugars, antioxidant

Table 3. Distances within (diagonal) and between (off-diagonal) clusters formed by the Tocher method based on the generalized distance of Mahalanobis.

Clusters	Group 1A	Group 1B	Group 2
Group 1A	1636.91	5858.65	13132.56
Group 1B	5858.65	-	16643.71
Group 2	13132.56	16643.71	-

potential, fresh pulp matter, plant height, fresh fruit mass, phosphorus and calcium content in pulp and pulp firmness are the most important in the estimation of dissimilarity and should, therefore, be prioritized in future studies. The relative contribution of these 11 characteristics amounts to 85.85%. Brandão et al. (2013) found that the elimination of some descriptors, such as fruit diameter, leaf blade length, petiole length and pseudostem diameter, used to characterize the banana germplasm, did not cause loss of information for the formation of the groups formed in 77 banana tree accesses evaluated. According to Brandão et al. (2013) and Azevedo et al. (2015), the analysis of less important characteristics for the study of divergence is not necessary in future studies, which reduces labor costs, cost of analysis and time.

Table 4. Relative contribution (%) of characteristics for genetic divergence in banana cultivars, estimated by the method proposed by Singh (1981).

Variable	S.j[1]	S.j.	Cumulative-S.j.
		(%)	
Vitamin A	174959.75	30.41	30.41
Starch content	82406.62	14.32	44.73
Total soluble sugar	66119.32	11.49	56.22
Non-reducing sugars	43653.85	7.59	63.81
Antioxidant potential	27625.29	4.8	68.61
Pulp fresh weight	26533.90	4.61	73.22
Plant height	17018.47	2.96	76.18
Fruit fresh weight	16250.05	2.82	79.00
P content of the pulp	13732.49	2.39	81.39
Ca content of the pulp	13204.57	2.29	83.68
Firmness of the pulp	12482.97	2.17	85.85
Pulp color	11366.98	1.98	87.83
Pulp dry mass	9182.36	1.6	89.43
Fruit by bunch	8944.73	1.55	90.98
Length/width of the blade	6677.64	1.16	92.14
Mn content of pulp	5691.19	0.99	93.13
Pulp/peel	5546.37	0.96	94.09
Bunch weight	4558.19	0.79	94.88
Phenolic compounds	3783.21	0.66	95.54
Fruit diameter	3571.37	0.62	96.16
Reducing sugars	3310.27	0.58	96.74
Fruits per cluster	3297.73	0.57	97.31
Clusters per bunch	3174.09	0.55	97.86
Total soluble solids	2509.53	0.44	98.30
Mg content of the pulp	2209.32	0.38	98.68
Lutein content	1742.30	0.3	98.98
Width of the blade	1041.10	0.18	99.16
Titratable acidity	917.42	0.16	99.32
K content of the pulp	880.32	0.15	99.47
Fe content of the pulp	787.02	0.14	99.61
Petiole length	659.09	0.11	99.72
Cu content of the pulp	610.03	0.11	99.83
Zn contente of the pulp	434.33	0.08	99.91
Length of blade	303.33	0.05	99.96
Diameter of the pseudostem	242.38	0.04	100.00

[1]Contribution of the variable x to the generalized distance of Mahalanobis between i and i'

Conclusions

The cultivars Terrinha and Marmelo are the most recommended to integrate breeding programs.

The characteristics Vitamin A content, percentage of starch, total soluble sugars, non-reducing sugars, antioxidant potential, fresh pulp matter, plant height, fresh fruit mass, phosphorus and calcium content in the pulp and firmness of the pulp are the most important in the estimation of dissimilarity and should, therefore, be prioritized in future studies.

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