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Genetic divergence between half-sibling progenies of kale using different multivariate approaches

Orlando G Brito [1]; Valter C Andrade Júnior [2]; Alcinei M Azevedo [3]; Luan Mateus S Donato [1]; Antônio Júlio M Silva [1]; Altino Júnior M Oliveira [1]

[1]Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Diamantina-MG, Brasil; orlandocefet@yahoo.com.br; luan_mateus_sd@hotmail.com; antoniojulio.medina@hotmail.com; altinojrmendes@gmail.com; [2]Universidade Federal de Lavras (UFLA), Lavras-MG, Brasil; valter.andrade@ufla.br; [3]Universidade Federal de Minas Gerais, Instituto de Ciências Agrárias (UFMG/ICA), Montes Claros-MG, Brasil; alcineimistico@ufmg.br

ABSTRACT

The aim of this study was to evaluate the genetic dissimilarity between half-sibling progenies of kale in order to determine the most divergent progenies and, also, to select potential parents. Thirty-six kale genotypes were evaluated, being thirty-three half-sibling progenies and three commercial cultivars, in a randomized block design with four replicates and six plants per plot. Twenty-eight traits were evaluated in each plant per plot, thirteen quantitative and fifteen qualitative traits. Genetic divergence was studied using MANOVA and canonical variables for quantitative observations. In addition, dendrograms were made for quantitative, qualitative and joint analyses by UPGMA method, using Mahalanobis distance. Genetic divergence was observed between genotypes. Commercial cultivars were more divergent than half-sibling progenies. Among half-sibling progenies, the most divergent ones were P1, P21, P23, P25 and P30. We concluded that half-sibling progenies P1, P23 and P30 can be used as potential parents to compose the recombinant population.

Keywords: *Brassica oleracea* var. *acephala*, diversity, variability, canonical variables, grouping.

RESUMO

Divergência genética entre progênies de meios-irmãos de couve de folhas por diferentes abordagens multivariadas

Objetivou-se estudar a dissimilaridade genética entre progênies de meios-irmãos de couve de folhas para determinar as mais divergentes e para selecionar potenciais genitores. Foram avaliados 36 genótipos de couve de folhas, sendo 33 progênies de meios-irmãos e três cultivares comerciais, no delineamento experimental de blocos casualizados com quatro repetições e seis plantas por parcela. Foram avaliadas 28 características em cada planta por parcela, sendo treze quantitativas e quinze qualitativas. Procedeu-se o estudo da divergência genética por meio da MANOVA e variáveis canônicas para as observações quantitativas. Além disso, dendrogramas foram feitos para as observações quantitativas, qualitativas e em conjunto pelo método UPGMA, utilizando-se a distância de Mahalanobis. Foi observada divergência genética entre os genótipos. As cultivares comerciais foram mais divergentes que as progênies de meios-irmãos. Dentre as progênies de meios-irmãos, as mais divergentes entre si foram P1, P21, P23, P25 e P30. As progênies de meios-irmãos P1, P23 e P30 podem ser utilizadas como potenciais genitores na composição da população recombinante.

Palavras-chave: *Brassica oleracea* var. *acephala*, diversidade, variabilidade, variáveis canônicas, agrupamento.

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Kale(*Brassica oleracea* var. *acephala*) is widely grown in Brazil, mainly by family farmers. In these growing fields, kale cultivars and hybrids, such as Manteiga, Manteiga Portuguesa, Manteiga da Geórgia, Manteiga Legítima de Pé Alto, among others, besides creole genotypes, selected by the producers, stand out. However, the consumption of kale has been increasing, so that more productive cultivars have been researched and crop-breeding programs have been developed. Kale breeding program has prioritized higher leaf production, smaller plants, lower sprout production and resistance to main pests (Boiça Júnior *et al*., 2010; Azevedo *et al*., 2016).

In kale breeding program, the selection between and within half-sibling progenies has been frequently used since this is an allogamous plant which is difficult to be manually pollinated, which allows high morphological diversity (Azevedo *et al*., 2012; Liu *et al*., 2014). In any breeding program, understanding the genetic variability associated with the species is essential, since it determines strategies which enhance the selection gains (Cruz *et al*., 2012; Vaz-de-Melo *et al*., 2017). This variability can be evaluated studying genetic divergence, phenotypic and/or molecular traits. Nevertheless, studies on genetic divergence in plants are generally carried out using botanical, morphological and agronomic descriptors, due to its lower cost (Silva

et al., 2014).

Worldwide, few studies have been developed aiming to evaluate the genetic diversity among kale genotypes. These studies prioritized information for breeding programs, using their genetic diversity evaluation in relation to morphoagronomic (Singh *et al*., 2017; Lotti *et al*., 2018) and nutritional (Fadigas *et al*., 2010; Lotti *et al*., 2018) traits, through multivariate techniques similar to the ones used in this study. Despite the importance of this line of research, in Brazil, bibliographies aimed at evaluating genetic divergence in kale populations are also scarce, though (Sawazaki *et al*., 1997; Azevedo *et al*., 2014), which reinforces the importance of this study in order to offer information and improve this crop breeding.

Multivariate techniques are efficient tools to evaluate genetic divergence (Azevedo *et al*., 2013). Several multivariate procedures are used to determine genetics divergence, mainly principal component analysis (PCA), canonical analysis (VC) and hierarchical grouping methods (Fadigas *et al*., 2010; Benitez *et al*., 2011). The most appropriate method is related to the desired accuracy and used statistical design (Bezerra Neto *et al*., 2010). Besides, methods as PCA and VC cannot provide all the necessary information for genetic diversity study, so that hierarchical methods can give better interpretation of the results (Fadigas *et al*., 2010; Singh *et al*., 2017). This approach is not found in literature on kale genetic divergence studies.

Given the above, this study aimed to evaluate genetic divergence between half-sibling progenies of kale in order to select divergent parents using different multivariate approaches.

MATERIAL AND METHODS

The experiment was carried out in Diamantina-MG, at Setor de Olericultura, at Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM) (18º9'S, 43º21'W, 1400 m altitude). During the experiment, the average temperature was 21.3ºC and 67.0% relative humidity.

The experimental design was randomized blocks consisting of 33 half-sibling progenies (P1 to P33) and three commercial cultivars [Manteiga da Geórgia (P34), Manteiga (P35) and Manteiga Portuguesa (P36)], as controls. We used four replicates and six plants per plot. The seeds were used as half-sibling progenies obtained by Azevedo (2015) in Viçosa-MG, using parent plants of kale germplasm bank of UFVJM.

Seeds were sown in trays of 72 cells, filled with commercial substrate Plantmax® and taken to a greenhouse, closed laterally with 50% shading screen, automatic irrigation system and covered with diffuser plastic film (100 µm). Seedlings were kept in the greenhouse for 50 days, and then planted in the field. Soil was plowed, followed by two harrowings. The seedbeds were prepared measuring 1.2-meter wide, with six plants each, representing the plot of the experiment, being spaced 0.50 m among each other. Planting and topdressing fertilizations, in both stages, were performed according to the recommendations for the crop (Trani *et al*., 2015). Thirteen quantitative and fifteen qualitative traits were evaluated in each plant. The average values of each plot were, then, considered for the statistical analysis.

Thirty days after planting, eight harvests were performed at 18-day intervals, evaluating in each plant the number of sprouts, number of marketable leaves and total production of marketable leaves (g). For statistical analyses of these traits, we considered eight evaluations. Fresh mass per marketable leaves was estimated using the ratio between total production of marketable leaves and total number of marketable leaves. Leaves without any signs of senescence, damaged by pests and diseases which presented length greater than 15 cm were considered marketable (Azevedo *et al.*, 2012).

At 160 days after planting, leaf phenotyping was performed, evaluating five representative marketable leaves of each plant. We evaluated average values of leaf length (cm), leaf blade length (cm), petiole length (cm), petiole base diameter (mm), middle of the petiole diameter (mm) and leaf width (cm). At 170 days after planting, plant height (cm), stem height (cm) and stalk diameter (mm) were also evaluated.

For qualitative observations, at 165 days after planting, the note scale proposed by IBPGR (1990) was used by three panelists, for leaf blade angle; leaf blade shape; leaf margin; leaf incision; apex shape; leaf blade diameter; bubbles in the leaf blade; leaf tip; leaf color; widening of the petiole; petiole section; color of the main vein; petiole color; stem lengthening and stem color.

Statistical analyses were done with the aid of R software (R Core Team, 2016). For quantitative observations, statistical analysis was performed at average plot level. Multivariate analysis of variance (MANOVA) was done with the aid of manova function in the stats package, using Pillai test at 5% significance. Subsequently, the information regarding the observations was submitted to multivariate analysis using canonical variables (VC) with the aid of the candisc package. After calculating the dissimilarity matrix by the Mahalanobis distance, a dendrogram was made by the UPGMA (Unweighted Pair Group Method with Arithmetic Mean), function hclust.

Qualitative observations, referring to note scales, were transformed into a percentage matrix of occurrence of each note, considering each evaluated trait (Cruz *et al.*, 2012). To calculate genetic dissimilarity ($d_{ii'}$) between the genoptypes i and i' (i=1,2,3...36 genotypes) we used the estimator:

$$d_{ii'} = \sqrt{\sum_{j=1}^{J}\left[\frac{\sum_{k=1}^{K}\left(F_{ijk} - F_{i'jk}\right)^2}{JK}\right]}$$

where F_{ijk} is the frequency in which the genotype i (i=1,2,3...36) received for the descriptor j (j=1,2....15) the note associated to its class k, with F_{ijk} values ranging from 0 to 1; K refers to the number of classes of the descriptor; J is the number of descriptors (15).

After obtaining this matrix, we made a dendogram using the UPGMA method, function hclust. In order to study the join dissimilarity (quantitative and qualitative traits) the two dissimilarity matrices were standardized in order to have their values varying between 0 and

1. Thereunto, we used the following equation: $V_n = [1+(V_{obs}-V_{max})]/(V_{max})$ in which: V_n is the standardized value; V_{obs} the observed value and V_{max} is the maximum value of the matrix. Afterwards, the weighted average was calculated considering the number of descriptors evaluated in each matrix. Then, another dendogram was created using the UPGMA method. The cut-off point of the dendrograms was established according to Mojena (1977). The correlation among the three obtained matrices of dissimilarity was estimated, testing the significance using the Mantel test with 1,000 simulations, "mantel. rtest" function in ade4 package.

RESULTS AND DISCUSSION

Multivariate analysis of variance (MANOVA) showed significant effect ($p<0.05$) of genotypes, considering multivariate quantitative traits, which highlights genetic variability within the studied population (Rigão *et al*., 2009). So, the breeder can establish parent combinations which promote greater heterotic effect in the descendant populations and, also, greater gains from selection.

The dispersion of genotypes presented in composition VC1 x VC2 (Figure 1A), showed the formation of five different groups. The most divergent group was established by the commercial cultivars Manteiga Georgia (P34) and Manteiga Portuguesa (P36), followed by the group formed exclusively by Manteiga cultivar (P35). The height of plants and width of

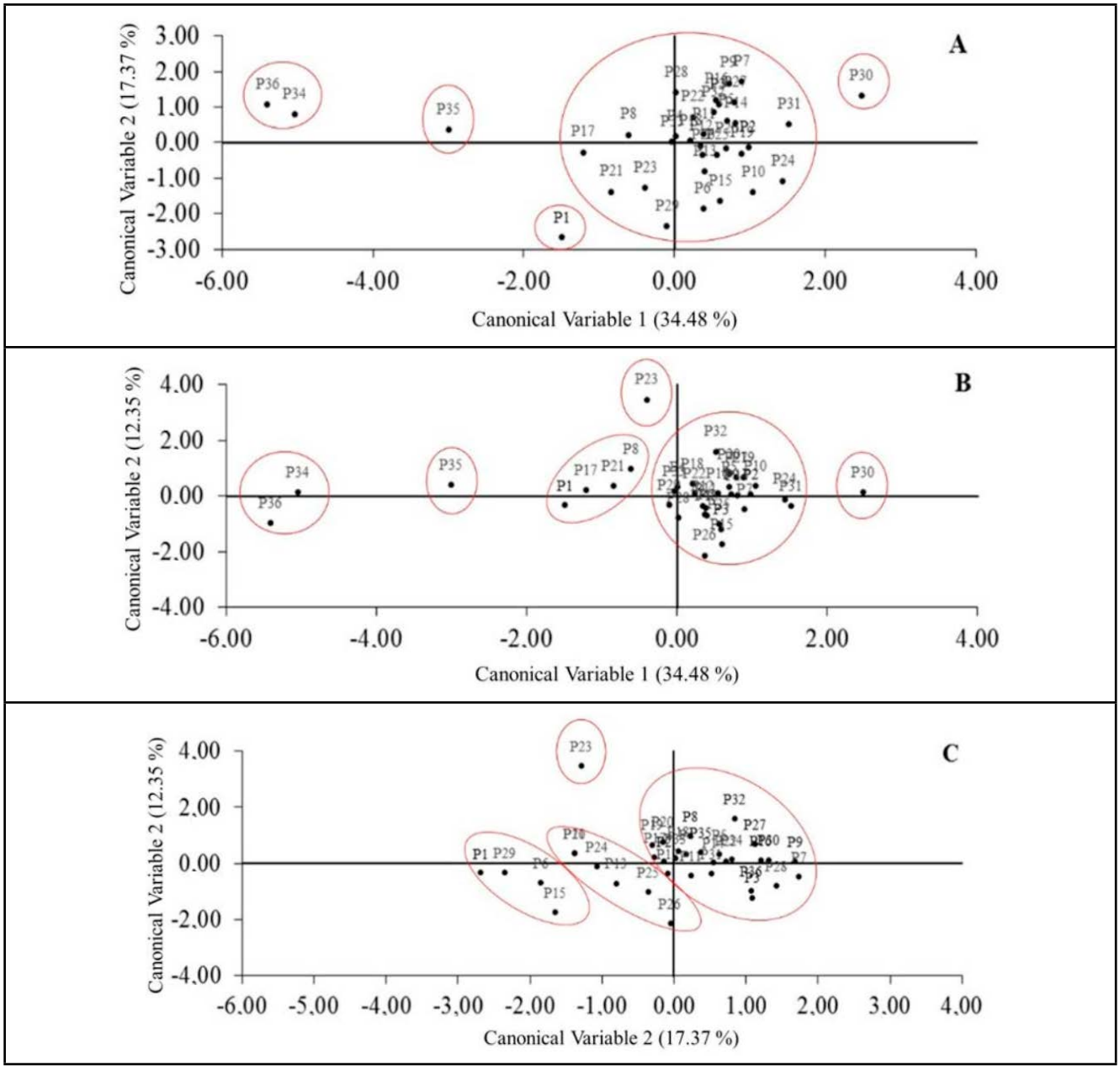

Figure 1. Dispersion of canonical scores for the first three canonical variables studying thirty-six kale genotypes. Diamantina, UFVJM, 2020.

Table 1. Correlations between the first three canonical variables and the thirteen quantitative traits evaluated in thirty-six kale genotypes. Diamantina, UFVJM, 2020.

Quantitative descriptors	Canonical variable		
	1	2	3
Plant height	0.82	0.07	-0.63
Stem height	-0.09	-0.53	0.08
Stem diameter	-0.29	0.57	-0.31
Leaf length	0.29	0.25	-0.10
Leaf blade	0.26	0.39	-0.24
petiole length	0.21	-0.10	0.18
petiole base diameter	0.01	0.26	0.22
middle of the petiole diameter	0.18	0.32	-0.04
Leaf width	0.59	0.49	-0.01
number of sprouts	0.10	0.24	-0.10
Number of leaves	-0.06	-0.22	-0.07
Leaf production	-0.04	-0.15	-0.22
Average weight per leaf	-0.02	-0.01	-0.18

leaves were the traits which showed greater correlation with VC_1 (Table 1). The commercial cultivars Manteiga da Geórgia, Manteiga and Manteiga Portuguesa showed average heights of 37.8, 36.8 and 42.7 cm, whereas the widths of the leaves were 15.9, 16.3 and 14.7 cm, respectively. In relation to other formed groups, their heights were of progenies 74.7 (P1), 75.4 (P30) and 67.9 cm (other progenies) and leaf width was 17.0 (P1), 24.0 (P30) and 20.3 (other progenies). So, the dispersions presented in Figures 1A and 1B showed that the commercial cultivars showed shorter plants with narrower leaves.

Considering only the dispersion of half-sibling progenies in Figure 1A, the authors verified that P1 and P30 progenies were the most divergent from each other, forming isolated groups. Correlations with VC_1 and VC_2 (Table 1) showed that progeny P1 represented the lowest values for plant height (74.7 cm) and stem diameter (29.7 mm), whereas P30 showed the highest average value for plant height (75.4 cm). Due to this greater divergence, these two progenies have the potential to compose the recombination population, since they are the most distant genetically speaking, considering a set of traits and can contribute to increase the genetic variability in the descendant population. Thus, we strongly believe that more productive genotypes can be obtained: genotypes with lower heights and higher stem diameter, for instance. We highlight that the genetic variability with superior-average genotypes are essential for selection gains and breeding program development obtained through hybridization (Souza *et al*., 2008).

In VC_1 x VC_3 composition VC_1 x VC_3 (Figure 1B) six groupings were formed, where commercial cultivars (P34, P35 and P36) strongly diverged from half-sibling progenies. The progenies P1, P8, P17 and P21, which formed just one group, with progenies P23 and P30, also formed individual groups divergent from the other genotypes. The correlations among the studied traits and the VC_1 and VC_3 (Table 1) showed that the main trait associated with progenies P1, P8, P17, P21 and P23, as well as with commercial cultivars, was the lowest height of the plant. The group formed by P1, P8, P17 and P21 showed average height of 61.3 cm, whereas the group formed by P23 was 46.2 cm. The largest group, formed by the other progenies, except for P30, showed average height of 69.3 cm. On the other hand, the positive correlation established with VC_2 showed that progeny P30 differed from the other progenies mainly for its highest height (75.4 cm).

In composition VC_2 x VC_3 (Figure 1C), the authors observed the formation of four groupings. The group with the greatest divergence was composed of the progenies P1, P6, P15 and P29, followed by the grouping formed by progeny P23. According to the correlations established with VC_2 and VC_3 (Table 1), the first grouping was characterized by genotypes with smaller stem diameter (29 mm) and plants showing bigger stems (78.3 cm) in relation to the other groups, whereas progeny P23, despite having an average diameter of the lower stem (24.34 mm), presented lower average stem height (48.3 cm). The group formed by greater amount of progenies showed average values for stalk diameter and stem height 31.6 mm and 63.4 cm, respectively.

Generally, the estimates of correlations between traits and canonical variables were low (Table 1). This fact explained the low phenotypic variation accumulated by the three first canonical variables (64.20%). However, this was an efficient analysis, and it even allowed the discrimination between the commercial cultivars and the half-sibling progenies. This methodology has been used successfully in studies on genetic divergence in several vegetables, such as lettuce (Azevedo *et al.*, 2013), pepper and green pepper (Martins *et al*., 2013), pumpkin (Ferreira *et al*., 2016) and carrot (Carvalho & Silva, 2017).

Considering the above, in general, the progenies P1 and P30 should be prioritized in the composition of the recombinant population with any of other progenies, since they may provide greater heterotic effect in the segregating population. Besides, the analysis through canonical variables allowed to identify the importance of quantitative variables for genetic divergence in the studied population, highlighting leaf production, plant height, leaf width and stem diameter. This corroborates what was verified by Singh *et al*. (2017) evaluating the genetic variability among 87 kale genotypes collected in the valley of Caxemira. These mentioned authors also observed that traits like leaf production, leaf width and plant height are traits which significantly contribute for the variability among kale genotypes. Okumus & Balkaya (2007) state that qualitative and quantitative

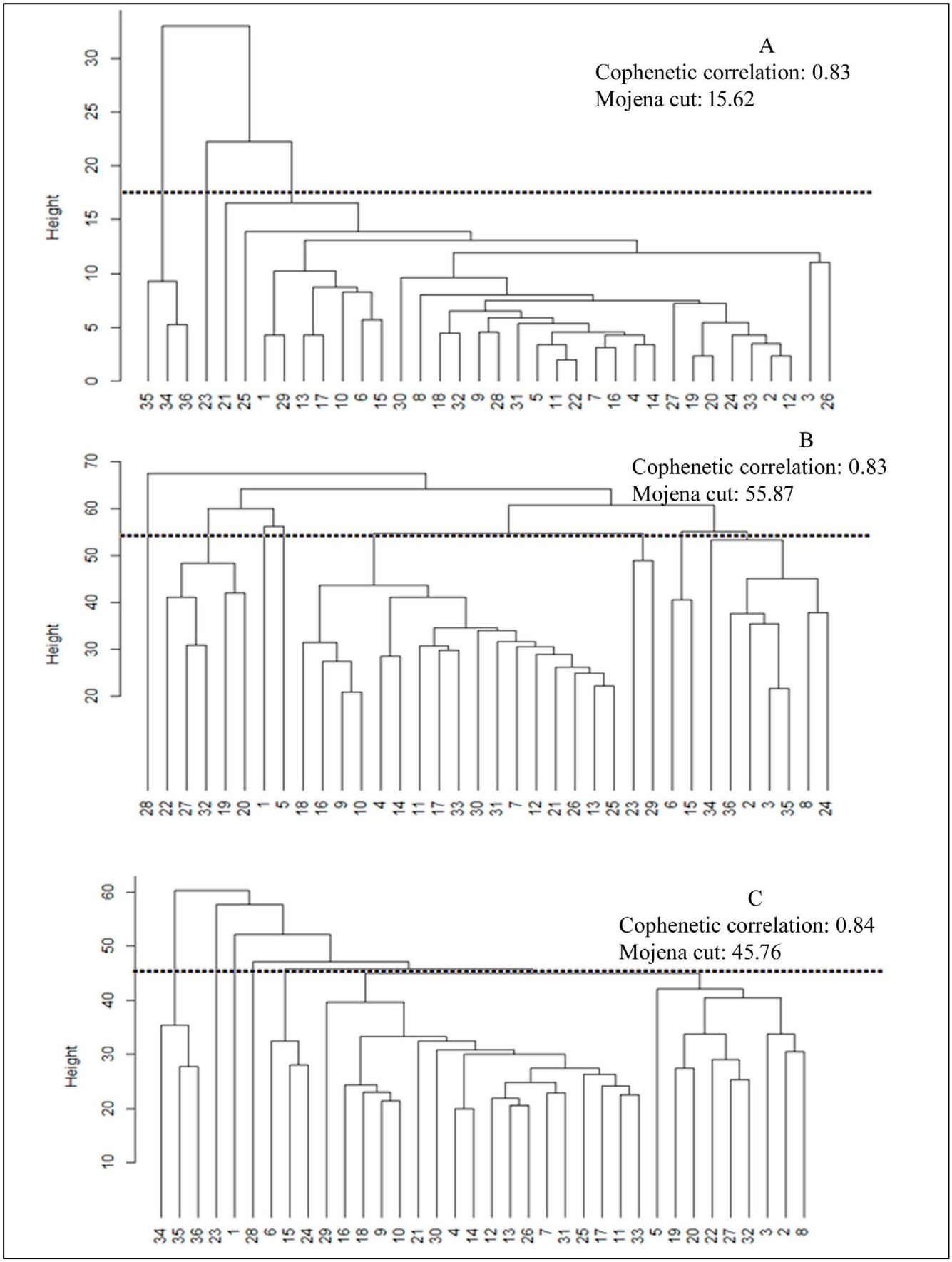

Figure 2. Dendrograms obtained by the UPGMA method using dissimilarity matrices based on thirteen quantitative traits (A), fifteen qualitative traits (B) and set (quantitative + qualitative) (C) in thirty-six kale genotypes. Diamantina, UFVJM, 2020.

traits of the plant are still more important to determine genetic variability than the traits associated with geographical location of the accessions.

The dendrogram for quantitative traits formed three different groups considering cutoff point of 15.62 (Figure 2A). The first group was formed by commercial cultivars (P34, P35 and P36), the second group by progeny P23 and the third group by the other genotypes. Half-sibling progenies P21, P23 and P25 were the ones which showed to be more genetically distant from the others (Figure 2A). Such genotypes showed potential to compose the recombinant population, so that the authors recommend priority crosses with other genotypes which are more genetically distant (P2, P11, P12, P19, P20 and P22). In this method, approximately 89% of the genotypes are in one group. Through the canonical variable method, the largest group formed in each composition (VC_1 x VC_2, VC_1 x VC_3 and VC_2 x VC_3) showed 86%, 75% and 69% of the genotypes, respectively. This can be a limiting factor when choosing genitors, considering greater proximity between genotypes, especially half-sibling progenies.

Table 2. Genotype distances (dendrogram with joint observations) for thirty-six kale genotypes. Diamantina, UFVJM, 2020.

Genotype	Quantitative			Qualitative			Joint		
	DM[1/]	MP	MD	DM	MP	MD	DM	MP	MD
P1	18.95	29	30	64.31	22	16	52.97	22	30
P2	13.00	12	36	55.78	3	28	43.49	8	23
P3	16.40	7	34	55.40	35	23	45.69	35	23
P4	9.71	22	36	50.58	14	19	37.95	14	36
P5	11.28	20	36	71.89	8	23	52.15	8	36
P6	14.45	15	36	60.04	15	5	47.14	15	36
P7	13.02	16	36	47.93	26	5	38.69	31	36
P8	11.15	33	36	56.53	35	23	42.63	2	23
P9	13.05	14	36	50.97	10	5	40.58	10	36
P10	13.70	11	36	50.09	9	19	40.51	9	36
P11	9.30	22	36	50.47	17	5	37.59	33	36
P12	9.55	2	36	51.36	21	5	38.32	13	36
P13	11.30	17	34	46.73	25	5	36.73	26	36
P14	9.95	22	36	53.32	4	19	39.81	4	36
P15	14.68	13	36	60.39	24	19	47.52	24	23
P16	10.48	22	36	54.22	10	1	40.74	9	36
P17	11.55	13	34	48.63	25	5	38.07	13	5
P18	10.02	22	36	55.54	10	5	41.22	9	36
P19	11.27	20	36	69.24	20	23	50.51	20	36
P20	10.67	19	36	66.44	19	23	48.37	19	36
P21	17.80	8	36	48.25	25	5	42.30	12	36
P22	8.40	11	36	55.73	32	23	40.18	32	36
P23	23.71	32	36	68.85	29	19	59.15	17	36
P24	12.28	2	36	61.55	8	23	46.52	15	34
P25	16.55	11	36	47.89	13	5	41.18	13	36
P26	14.80	12	36	46.59	13	5	39.14	13	36
P27	14.29	33	36	55.50	32	23	44.24	32	36
P28	11.62	22	36	67.50	7	23	49.70	7	23
P29	15.38	1	36	59.00	11	19	47.16	4	36
P30	17.25	31	36	50.56	26	5	43.32	31	36
P31	12.47	11	36	49.65	26	5	39.35	7	36
P32	12.20	22	36	53.46	27	23	41.49	27	34
P33	9.20	12	36	52.98	25	5	39.06	11	36
P34*	35.69	36	30	60.86	14	19	62.81	36	30
P35**	18.88	17	30	53.31	3	23	46.17	36	23
P36***	40.17	34	30	62.79	35	28	67.18	35	30

[1/]DM= average distance; MP= closer; MD= more distant; *Manteiga da Geórgia;**Manteiga; ***Manteiga Portuguesa.

Grouping based on qualitative observations using UPGMA method formed eight different groups (Figure 2B), considering half-sibling progenies P2, P3, P8 and P24 which are more similar to commercial cultivars (P34, P35 and P36). The joint dendrogram allowed the formation of six distinct groups, the first being formed by commercial cultivars, the second group formed by progeny P23, the third group formed by progeny P1, the fourth group by progeny P28, the fifth group by progenies P6, P15 and P24, and the last group formed by the other half-sibling progenies (Figure 2C). The number of groups formed in the dendrogram for the qualitative traits was higher than that observed for the quantitative ones. This stronger discrimination could indicate its best use in the study of genetic divergence. However, we highlight that in kale breeding program, the quantitative traits are of more interest, so that, divergence based on this type of information should be prioritized.

Comparing the groups using the canonical variable analysis with dendrogram for quantitative variables (Figure 2A), some differences, in the established groups, were verified. However, other studies have shown discordant groups with regard to multivariate techniques when evaluating genetic dissimilarity (Azevedo *et al*., 2013; Sulzbacher *et al*., 2017). Fadigas *et al*. (2010) and Lotti *et al*. (2018), studying the genetic diversity between kale accessions through principal components and dendrograms, also

Table 3. Correlation between the dissimilarity matrices and the number of groups established for thirty-six kale genotypes. Diamantina, UFVJM, 2020.

Dendrogram	Correlation between dissimilarity matrices			Number of groups
	Quantitative	Qualitative	Joint	
Quantitative	-	0.182^{ns}	0.75**	3
Qualitative		-	0.79**	8
Joint			-	6

$^{ns, **}$Not significant and significant by the Mantel test with 1000 simulations.

verified differences in the composition of groups, which is associated with methodological characteristics of each technique.

Average dissimilarity estimation ranged from 8.40 to 40.17 for quantitative observations, from 46.59 to 71.89 for qualitative observations and from 36.73 to 67.18 for joint observations (Table 2). The authors verified that for quantitative traits, cultivar Manteiga Portuguesa (P36) was the most divergent (the most distant) in relation to most genotypes (80%), followed by half-sibling progeny P30, which was more distant, in relation to 11% of evaluated genotypes. For qualitative observations, the half-sibling progenies P5 and P23 were more divergent in relation to 38% and 31% of the genotypes, respectively. Considering the joint dendrogram, Manteiga Portuguesa cultivar was also distant in relation to most genotypes (67%), followed by progeny P23, which was the most distant in relation to 17% of genotypes. These divergences can be verified by the dispersion based on the canonical variables.

The dissimilarity matrix of the joint dendrogram showed a high correlation with the dissimilarity matrices of the quantitative and qualitative dendrograms, however between the quantitative and qualitative dendrograms the correlations were low (Table 3). This low correlation between dendrograms from different sources of variables was also reported by other authors. Andrade *et al*. (2017), evaluating the genetic distance in sweet potato genotypes using morphological and molecular information, observed that the genetic variability characterization showed a differentiated response depending on the type of data, identifying different groupings by the UPGMA method. The same was verified by Sawazaki *et al*. (1997), characterizing the genetic diversity in kale genotypes using information obtained through isoenzymes and RAPD using the UPGMA method. We highlight that the three dendrograms formed showed high cophenetic correlations (superior to 80%) (Figure 2); this value is higher than the minimum desired value, 70% (Streck *et al*., 2017). This indicates high agreement between the original value matrices and the representation in the dendrogram (Cruz & Carneiro, 2006; Cabral *et al*., 2011). Besides, the UPGMA method has also been recommended and described as highly accurate when using the Mahalanobis distance measurement compared to other genetic distances (Azevedo *et al.*, 2014; Bertan *et al.*, 2006).

The difference between groups formed by quantitative and qualitative variables, and the low correlation between dendrograms can be explained by the fact that quantitative traits are controlled by a large number of genes, whereas qualitative traits are determined by one or few genes (Cruz *et al.*, 2012). Therefore, a correlation between these traits from different origins is not expected, resulting in different groups. Such situation can be a limiting factor for the breeder when specific traits, concerning quantitative and qualitative traits, would be of simultaneous interest in breeding, since an association between each other is not expected.

Thus, genetic divergence in the studied population of kale and commercial cultivars is quite dissimilar in relation to half-sibling progenies. Progenies P1, P21, P23, P25 and P30 are the most divergent in relation to most of the studied genotypes. The progenies of half-sibling P1, P23 and P30 can be used as parents to compose the recombinant population. In addition, plant height, stem height, stem diameter and leaf width are the quantitative traits which strongly contribute to the genetic divergence in the population studied in this experiment.

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