

Genetic divergence among garlic accessions grown in different environments in the state of São Paulo, Brazil

Divergência genética entre acessos de alho avaliados em ambientes distintos no estado de São Paulo, Brasil

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ABSTRACT - The objective of this study was to assess the genetic variability of 63 garlic accessions grown in two environments in the state of São Paulo, Brazil. A randomized block design with replications was used. Agromorphological evaluations were conducted based on descriptors defined by the International Plant Genetic Resources Institute (IPGRI), currently Bioversity International, encompassing 18 characteristics. The obtained data consisted of the mode of each variable and genetic distances from the complement arithmetic of the Jaccard coefficient. Cluster analysis was carried out using Tocher optimization and UPGMA methods; Mantel test was used to assess the correlation of genetic distances between locations. Garlic accessions exhibited genetic diversity for the studied qualitative variables. Correlations between distances of the evaluated accessions were found in both locations, although of low magnitude. Garlic characteristics vary according to growth environment, indicating divergence among accessions. This may be attributed to the plasticity of the species and genotype-environment interaction factors. Phenotypic variability of garlic germplasm observed in a particular environment cannot be extrapolated to other locations.

Keywords: *Allium sativum* L. Cluster analysis. Genetic variation. Multivariate analysis.

RESUMO - O objetivo deste trabalho foi avaliar a diversidade genética de 63 acessos de alho semi-nobre avaliados em dois ambientes no estado de São Paulo. Utilizou-se o delineamento de blocos casualizados, com repetições. As avaliações agromorfológicas foram realizadas com base nos descritores do *Plant Genetic Resources Institute* (IPGRI), atualmente *Bioversity International*, e totalizaram 18 características. Os dados foram obtidos pela moda de cada variável, sendo que a medida de divergência utilizada foi o complemento aritmético de Jaccard. As análises de agrupamento aplicadas foram o método de otimização de Tocher e UPGMA; bem como o teste de Mantel para avaliar a correlação das distâncias genéticas entre os locais. Os acessos apresentaram diversidade genética para as variáveis qualitativas estudadas. A correlação entre as distâncias dos acessos avaliados nos dois locais existiu, porém, sua magnitude foi baixa. As características do alho modificam-se de acordo com o ambiente, portanto, a divergência entre os acessos. Isso pode ser atribuído a plasticidade da espécie e aos fatores de interação genótipo-ambiente. A variabilidade fenotípica de germoplasma de alho observada em um determinado ambiente não pode ser extrapolado para outros locais.

Palavras-chave: *Allium sativum* L. Análise de agrupamento. Variabilidade genética. Análise multivariada.

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INTRODUCTION

Garlic (*Allium sativum* L.) is among the most important crops in olericulture in Brazil; however, the country is the second largest garlic importer in the world. The species has been described as a sterile vegetable or a typical obligate apomictic species. Its propagation is generally asexual, using cloves taken from the base of the plant or aerial inflorescences (BIESDORF et al., 2015; MAYER; GOLDSTEIN, 2019). Despite a long history of obligate apomixis, garlic exhibits high phenotypic diversity (VIANA et al., 2016).

Considering that the predominant vegetative propagation in the species, classical breeding through hybridization is not used in the search for new genotypes, resulting in limited mutations (BACELAR et al., 2021). Thus, expanding research is essential for identifying new genotypes (CHEN et al., 2014).

Several different genotypes of *Allium sp.* are conserved in germplasm banks in several countries, including Brazil. However, the potential use of germplasm accessions can only be achieved through a proper characterization (VIANA et al., 2016). Therefore, identifying the most promising genotypes, whether for breeding or direct use, is essential to benefit farmers by enabling a rational use of these genotypes in family farming. However, the observed plasticity in garlic clones is an important factor, which is affected by soil type and moisture, latitude, altitude, and cultural practices (VOLK; STERN, 2009; RESENDE et al., 2013). Plant plasticity, or the ability of plants to present functional adaptive responses to environmental conditions, tends to vary

according to the location. Thus, estimating genetic divergence in garlic through morphological characteristics in different environments is important.

Variations have been reported in the literature for several garlic characteristics, including flowering capacity, leaf characteristics, bulb characteristics (curvature, external leaf color, size, and clove arrangement), plant maturation, bulging response to daylight hours, resistance to cold, clove characteristics (number, size, color, and ease of removal), and flower characteristics (flowering season, number, size, color, distance between petals, and stigma height) (RESENDE et al., 2013; VIANA et al., 2016). Studies on garlic diversity in different environments have been conducted by Hoogerheide et al. (2017), Guimarães et al. (2019), and Polyzos et al. (2019).

These studies are important for improving productivity and identifying species traits, aiming to select desirable genotypes for each growing region. Additionally, studies considering more than one environment have shown the existence of genetic variability in genetic materials, environmental influence, and the potential of each genotype (GUIMARÃES et al., 2019; SANTOS, 2020).

Garlic is grown in 10 states of Brazil, including São Paulo, which is currently the ninth largest garlic producing state (IBGE, 2022). In 2021, garlic imports dropped by 35% compared to 2020 (CONAB, 2022), with an expected increase in area and production for 2022.

In view of the above and considering the need to characterize garlic accessions in different environments, along with the importance and prospects of garlic crops in Brazil, the objective of this study was to assess the genetic divergence among garlic accessions grown in two different environments in the state of São Paulo by using qualitative agromorphological variables.

MATERIALS AND METHODS

Sixty-three garlic accessions (Table 1) from the germplasm bank of the Instituto Agronômico de Campinas (IAC) and the Departamento de Genética e Melhoramento de Plantas of the Escola Superior de Agricultura Luiz de Queiroz (ESALQ/USP) were evaluated.

Table 1. Evaluated garlic accessions (Instituto Agronômico de Campinas - IAC, and Escola Superior de Agricultura Luiz de Queiroz - ESALQ), number of replications and location of the experimental areas.

Code	Accessions	Institution	MAS	PCBA
1	Canela de Ema	IAC	5	5
2	Cara	IAC	5	5
3	Mineiro	IAC	5	5
4	Mossoró	IAC	5	4
5	BGH-0525	IAC	5	5
6	BGH-4823	IAC	3	3
7	Andradas Manoel Lopez	IAC	2	2
8	Assai-3702	IAC	5	5
9	Piedade	IAC	5	5
10	Santa Catarina Roxo	IAC	5	5
11	Chinês-4653	IAC	5	5
12	Roxinho 5063	IAC	5	5
13	Peruano Bisão	IAC	5	5
14	Mexicano Br	IAC	3	3
15	Roxo Capim Branco	IAC	5	5
16	Cateto Precoco I-99	IAC	5	5
17	Alho Bepe	IAC	3	4
18	Formosa-4713	IAC	3	4
19	Lavínia-1632	IAC	5	5
20	BGH-5935	IAC	5	5
21	São José-4999	IAC	2	2
22	Catetinho do Paraná 1254	IAC	5	5
23	Mendonça-5062	IAC	5	5
24	Tatuí-3705	IAC	5	5
25	Gigante de Curitiba	IAC	5	5
26	Areal 23978	IAC	5	5
27	Vera Cruz-5004	IAC	5	5
28	BGH-4814	IAC	5	5
29	BGH-5947	IAC	5	5
30	Chinês ESALQ	IAC	5	4

Table 1. Continuation.

Code	Accessions	Institution	MAS	PCBA
31	Cateto Roxo 99	IAC	5	5
32	BGH-5936	IAC	5	5
33	BGH-6394	IAC	4	5
34	BGH-5952	IAC	5	5
35	Chinês Esalq 2	IAC	5	5
36	Andradas Manoel Lopez 2	IAC	5	5
37	Roxo de Ouro Fino	IAC	5	5
38	Sr. Wilson (bairro Godói)	IAC	5	5
39	B. aéreo Gig. Curitibanos	IAC	5	5
40	Chinês Mogi	IAC	5	5
41	Gigante	ESALQ	5	5
42	Centenário	ESALQ	5	5
43	Gigante Dez	ESALQ	5	5
44	São José	ESALQ	5	5
45	Lavínia	ESALQ	5	5
46	Caiano Branco	ESALQ	5	5
47	Cateto Branco	ESALQ	5	5
48	Crespo	ESALQ	5	5
49	Gigante Vinte	ESALQ	5	5
50	Cajuru	ESALQ	5	5
51	Peruano	ESALQ	5	5
52	Ouro Fino	ESALQ	5	5
53	Sergipe	ESALQ	5	5
54	Mineiro Branco	ESALQ	5	5
55	Babás	ESALQ	5	5
56	Chinês Quatapara	ESALQ	5	5
57	A	IAC	5	5
58	B	IAC	5	5
59	C	IAC	5	5
60	D	IAC	5	5
61	E	IAC	5	5
62	F	IAC	5	5
63	Embrapa Cateto roxo (free of virus)	IAC	5	5

Experiments were conducted at two locations in the state of São Paulo: Monte Alegre do Sul (MAS) (22°24'S, 46°24'W, and altitude of 760 m) at the APTA - Polo Regional do Leste Paulista; and in Piracicaba (PCBA) (22°25'S, 47°22'W, and altitude of 554 m) at the Department of Genetics of ESALQ/USP. The climate of these locations was classified as Cwa, characterized as a high-altitude tropical climate, with rainy summers and dry winters, according to the Köppen Classification, with the mean air temperature in the warmest month exceeding 22 °C.

MAS has a mean annual air temperature of 20.8 °C (minimum of 17 °C and maximum of 24 °C) and a mean annual rainfall depth of 1,548 mm. The soil of the experimental area in MAS was classified as Ultisol. PCBA has a mean annual air temperature of 21.6 °C (minimum of

17 °C and maximum of 25 °C) and a mean annual rainfall depth of 1,328 mm (CEPAGRI, 2009). The soil of the experimental area in PCBA was classified as Oxisol.

Planting took place on April 24th and 25th, 2007 in MAS; and on May 7th, 2007 in PCBA. The mean rainfall depths and air temperatures in 2007 were 51.98 mm and 18.33 °C in MAS, and 53.73 mm and 18.42 °C in PCBA, respectively (Figure 1).

The experiments were conducted in a randomized block design. The experimental plot consisted of four one-meter rows, with spacing of 0.10 m between plants and 0.25 m between rows; the plot evaluation area consisted of the two central rows (Figure 2). The number of replications varied from two to five, depending on the number of garlic bulbs/seeds available (Table 1).

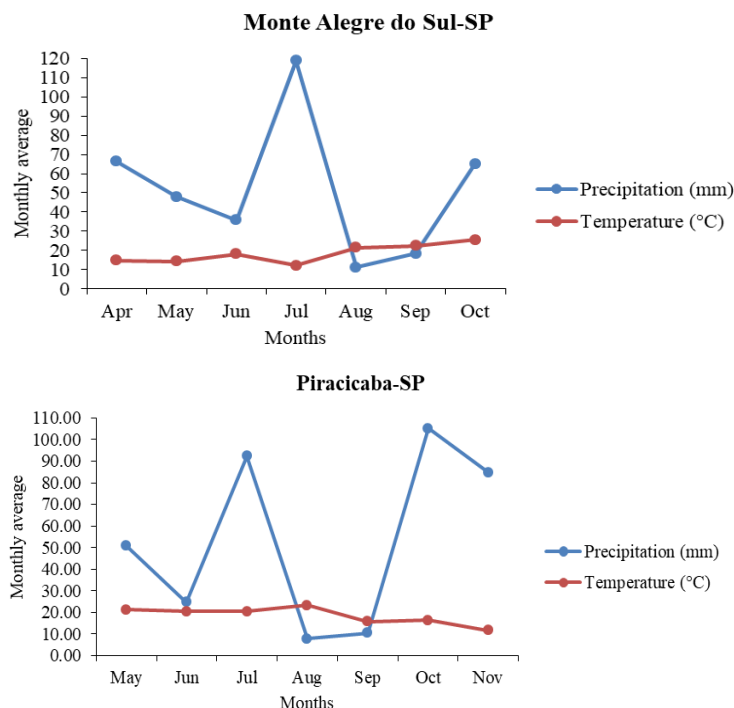


Figure 1. Rainfall depths and air temperatures in Monte Alegre do Sul (MAS) and Piracicaba (PCB), SP, Brazil, in 2007.



Figure 2. Experimental area in Monte Alegre do Sul (A) and Piracicaba (B) in the state of São Paulo, Brazil.

The soil was chemically analyzed, and technical recommendations for garlic growing were followed. The beds were raised using a 1.10 m wide rotary hoe. Basal dressing was applied the week before planting. Soon after planting, the beds were covered with a 2.0 cm layer of chopped *Pennisetum purpureum* Schum plants. Irrigation was carried out twice a week, using a water depth of 40 mm; irrigation was interrupted when it rained. Topdressing was applied at 50 and 70 days after planting, using 60 kg ha⁻¹ of N and 40 kg ha⁻¹ of K₂O. No fungicides or insecticides were applied to any of the experimental areas.

Agromorphological evaluations were conducted based on descriptors defined by the International Plant Genetic Resources Institute (IPGRI), currently Bioversity International (IPGRI, 2001), including others considered relevant, totaling 18 qualitative characteristics (Table 2).

The mode of each variable and accession was used for

the analyses (FARIAS, 2016; SILVA, 2011); values were converted into binary data, 0 and 1, attributed to the absence and presence of the category in the accession, respectively, as proposed by Sneath and Sokal (1973). Subsequently, the complement arithmetic of the Jaccard coefficient was calculated. The distance matrix for the 63 accessions was then estimated for each location. The matrix for both locations was obtained by summing the dissimilarity matrices of MAS and PCBA; this matrix will be referred to hereafter as sum of matrix (SEARLE, 1966). The magnitude and significance of the correlations of dissimilarity measures between locations were estimated using the Z statistic or Mantel test (DINIZ-FILHO et al., 2013). Accessions were grouped using the Tocher optimization method and the hierarchical method of the arithmetical means of dissimilarities (UPGMA), as described by Cruz (2008). All analyses were conducted using the software GENES (CRUZ, 2013).

Table 2. Agromorphological descriptors used to evaluate 63 garlic accessions in 2007.

Descriptors	Classes
1 Plant foliage density	(1) very sparse, (3) sparse, (5) medium, (7) dense
2 Leaf habit	(3) erect, (5) semi-erect, (7) horizontal
3 Leaf green intensity	(3) light, (5) medium, (7) dark
4 Leaf waxiness	(3) weak, (5) medium, (7) strong
5 Leaf cross-sectional shape	(1) strongly concave, (2) slightly concave, (3) flat
6 Anthocyanin pigmentation intensity at the base of the pseudostem	(1) absent or very weak, (3) weak, (5) medium, (7) strong, (9) very strong
7 Aerial bulbs	(1) presence; (0) absence
8 Bulb uniformity	(1) uniform, (2) normal, (3) very uniform
9 Position of the bulb root disk	(1) depressed, (2) flat, (3) protuberant
10 Bulb base shape	(1) depressed, (2) flat, (3) rounded;
11 Bulb longitudinal shape	(1) short elliptical transverse, (2) wide elliptical transverse, (3) circular;
12 Bulb cross-sectional shape	(1) elliptical, (2) circular;
13 Adherence of bulb tunics	(3) weak, (5) medium, (7) strong
14 Bulb tunic back color	(1) white, (2) yellowish-white, (3) reddish-white
15 Clove pulp color	(1) white, (2) yellowish
16 Ease of clove removal	(1) difficult, (2) normal, (3) easy
17 Clove tunic color intensity	(3) weak, (5) medium, (7) strong
18 Bulb firmness	(1) soft, (2) normal, (3) very firm

Descriptors 1 to 6 were evaluated at 90 days after planting; descriptor 7 was evaluated at harvest; descriptors 8 to 17 were evaluated after harvest and after 30 days of storage; and descriptor 18 was evaluated at 120 days after harvest.

RESULTS AND DISCUSSION

The genetic distances between pairwise accessions in MAS ranged from 0.8750 to 0.1053, with a mean of 0.6017. The smaller distance was found between accessions 22 (Catetinho do Paraná 1254) and 19 (Lavinia-1632), whereas accessions 38 (Sr. Wilson – Bairro Godói) and 8 (Assai-3702) formed the most divergent pairs. In PCBA, distances ranged from 0.9412 to 0.1053, with a mean of 0.5803. The closest pairs consisted of accessions 9 (Piedade) and 24 (Tatuí-3705); and the most distant consisted of accessions 48 (Crespo) and 49 (Gigante Vinte). The pairs with maximum and minimum

divergence differed between the experimental locations.

The first 20 pairs with greater and lower dissimilarity were compared between locations and showed practically no similarity. Such dissimilarity may be attributed to the environmental effect on the genetic material, which is a phenomenon observed in garlic, as mentioned by Volk and Stern (2009), Resende et al. (2013), and Viana et al. (2016).

There was an asymmetry to the right in the distribution in the genetic dissimilarity estimates of (Figure 3), indicating that the largest classes were formed by genetically more distant accessions; 80.7% of the accessions were concentrated in classes with distances from 7.06 to 11.41.

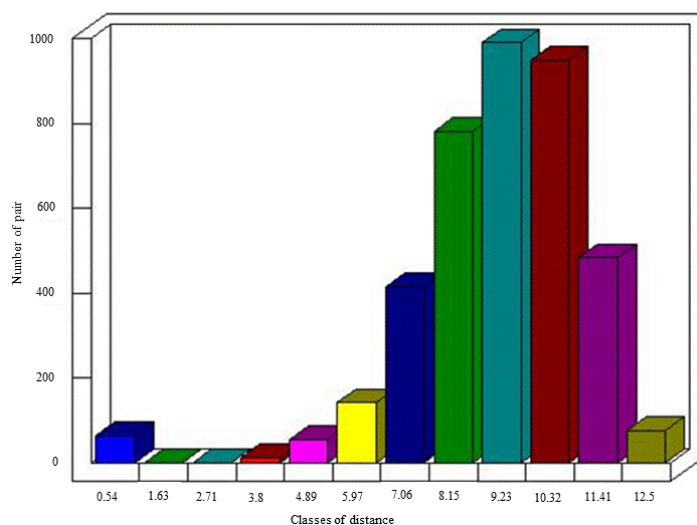


Figure 3. Distribution of the distances between pairs of garlic accessions for qualitative variables estimated through the complement arithmetic of the Jaccard coefficient.

Compilation of MAS and PCBA

The dissimilarity found represents the genetic variability within the group of studied garlic accessions, the different genotypic response to the environment, or seedling quality (TCHORZEWSKA et al., 2018). The greater the distance, the greater the divergence among accessions. Information on genetic variability through dissimilarity studies is important in breeding processes that can promote satisfactory genetic gains, as well as reduce the vulnerability of crops to biotic and abiotic factors (COSTA; SILVA; LÉDO, 2011).

Considering the pairs of accessions in the sum of qualitative matrix, i.e., the matrix considering both study locations (MAS and PCBA), the distances ranged from 13.0463 to 3.6609. The greatest discrepancy was found for pairs 26 (Areal 23978) and 48 (Crespo); and 14 (Mexicano Br) and 51 (Peruano). The smallest discrepancy was found for 10 (Santa Catarina Roxo), 22 (Catetinho do Paraná 1254), and 23 (Mendonça-5062); 10 (Santa Catarina Roxo) and 25 (Gigante de Curitiba); and 47 (Cateto Branco) and 53 (Sergipe). Regarding clusters, the Tocher optimization method distributed the 63 accessions into 12 and 11 groups for MAS and PCBA, respectively (Table 3).

Table 3. Clustering of 63 garlic accessions through the Tocher optimization method in Monte Alegre do Sul (MAS) and Piracicaba (PCBA) and sum of matrix (MAS and PCBA).

Groups	MAS	PCBA	Sum of matrix
1	19 22 10 23 12 25 7 21 8 30 35 29 40 56 62 18 24 4 34 13 9 32 58 59 57 26 3 61 20	9 24 27 10 46 16 36 55 17 12 56 98 32 19 44 25 52 43 39 40 13 21 35 22 60 18 26	10 22 23 19 12 25 24 9 16 8 27 36 32 18 56 17 21 13 35 30 46 44 62 40 55 60 52 39
2	44 47 53 48 45 51 41 15 54 46 49	31 42 50 51 48 54 33 47 53 41 45	47 53 48 51 54 41 45 31 50 42
3	52 60 5 39 42 55	1 2 4 3	26 34 7 14 6 20
4	17 36 27	6 14 20 7 34 62	57 59 61 58
5	50 63	15 63 29 5	29 63 15 5
6	14 16	23 38 57 59 61	1 2 4 3
7	31 43	28 30	11
8	1 6	11	37
9	2 37	58	43
10	11 33	49	33
11	38	37	28
12	28		49
13			38

There was a similarity in the pattern of distribution of the number of groups between locations. As for the accessions, some remained together, as the accession groups [10 (Santa Catarina Roxo), 22 (Catetinho do Paraná 1254), 19 (Lavínia-1663), 25 (Gigante de Curitiba), 24 (Tatuí-3705), 9 (Piedade), 18 (Formosa-4713), 21 (São José-4999), 13 (Peruano Bisão), 40 (Chine Mogi), 12 (Roxinho-5063), 56 (Chinês Quatapara), 32 (BGH-5936)] and [47 (Cateto Branco), 48 (Crespo), 51 (Peruano), 54 (Mineiro Branco), 41 (Gigante), 45 (Lavínia)]. Siqueira et al. (1984) found similar

response for accessions 22 (Catetinho do Paraná 1254) and 9 (Piedade), which were grouped through isoenzymatic and morphological characterizations of 72 accessions.

Thirteen groups were formed by the sum of matrix, seven of them formed by a single accession. Intragroup distances showed that group one presented the greatest genetic divergence; and the greatest intergroup dissimilarity was found between groups 1 and 2. These results indicate genetic variability within and between the groups of garlic accessions evaluated through qualitative variables, in both tested

environments. Studies have reported phenotypic variation in garlic for both quantitative and qualitative characteristics (SAIF et al., 2020; SANTOS, 2020); the evaluation of these variables is important for breeders to explore the gene banks and develop new genotypes (SANTOS, 2020).

Several groups with subgroups were formed, except for accessions 37 (Roxo de Ouro Fino) and 38 (Sr. Wilson (bairro Godói). The cutoff point in the dendrogram based on the overall mean ($dg_E = 9.02$) formed eight groups (Figure 4). These accessions were grouped similarly when using the Tocher method and were grouped with few or no accessions in the clustering. Accession 37 (Roxo de Ouro Fino) remained isolated in the population evaluated in PCBA when using the

Tocher method and also remained isolated in the clustering by the UPGMA method. This denotes the greater dissimilarity of accession 37 (Roxo de Ouro Fino) for the evaluated qualitative variables compared to the other accessions. Considering the absence of meiotic recombination in the species due to an exclusively propagative reproduction with the presence of apomixis, identifying accessions with wide genetic dissimilarity is essential for breeding programs to obtain new commercial genotypes. Thus, accessions such as 37 (Roxo de Ouro Fino) are important when identified within the collection and need to be evaluated in agronomic experiments to identify superior genotypes.

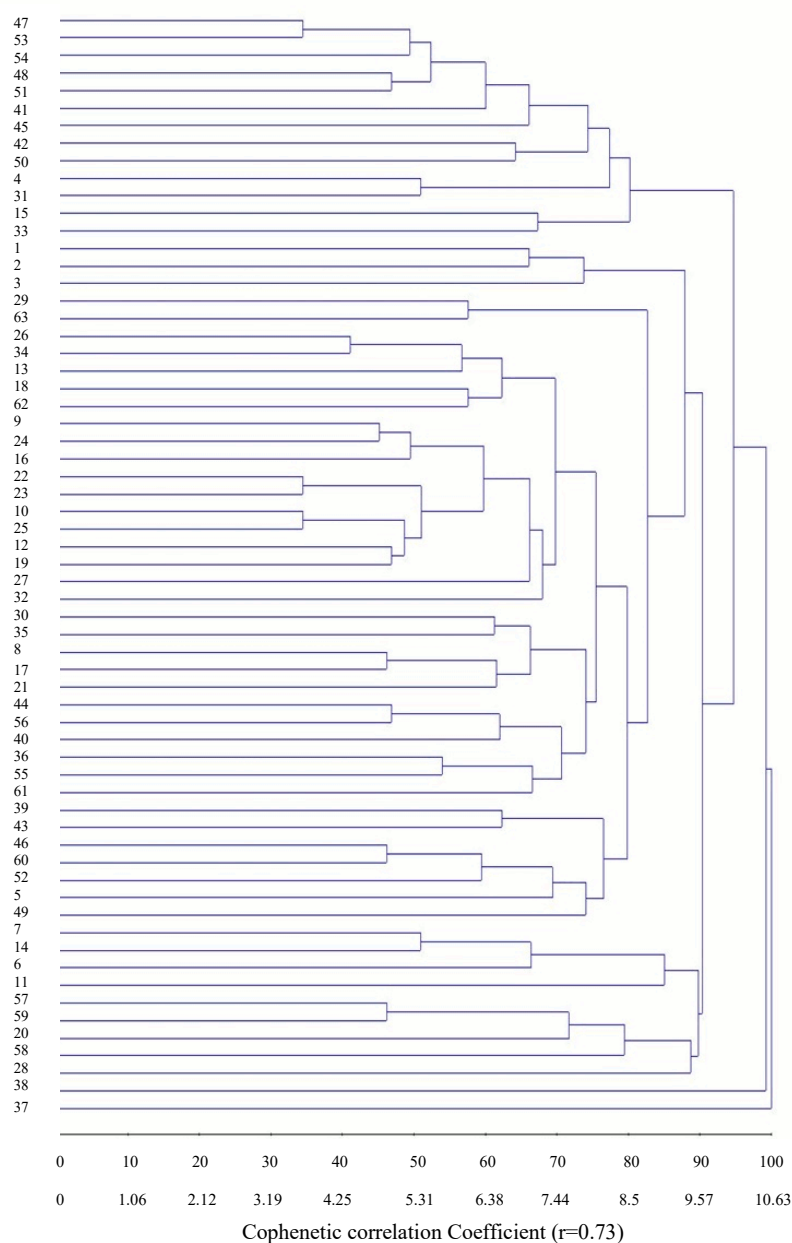


Figure 4. Dendrogram generated by the UPGMA method, using the complement arithmetic of the Jaccard coefficient for estimating qualitative variables of 63 garlic accessions grown in different locations of the state of São Paulo, Brazil (Monte Alegre do Sul - MAS; and Piracicaba - PCBA).

The genetic variability among the studied garlic accessions is evidenced by the effectiveness of clustering methods in discriminating the accessions. This was also observed by Puiatti et al. (2014), who evaluated 60 garlic accessions from the vegetable germplasm bank of the Federal University of Viçosa, Minas Gerais, Brazil, and found the formation of six genetic groups, with the isolation of one accession, using the Tocher and UPGMA methods.

Although the estimates were significant ($p < 0.01$), qualitative characteristics showed low correlation between environments (0.25). These results indicate that the divergence in each environment did not represent the same distribution of accessions, revealing that clones varied according to the environment, probably due to the genotype-environment interaction.

This probably occurs because variation among garlic cultivars is found in most environments, with noticeable differences in plant development; leaf length and width; crop cycle; budding; shape, size, and color of bulbs; and number, size, shape, and color of cloves (VIANA et al., 2016). Thus, breeders should be aware of this phenomenon when evaluating accessions from germplasm banks to avoid hasty conclusions regarding similarity when the collection is evaluated in a single environment. The need for careful selection of environments and variables is essential to obtain reliable results of diversity among accessions.

Based on the results found in the present study, evaluations of accessions to obtain new garlic cultivars should be localized, considering a decentralized selection, i.e., selection in the target environment, to enhance favorable interactions. However, evaluating trials in different locations is one of the most expensive stages in a breeding program, and these evaluations should be conducted jointly and with farmers in their own areas, to better take advantage of the potential expressed in each environment, considering the premises of participatory breeding.

CONCLUSIONS

The garlic accessions from germplasm banks in the state of São Paulo evaluated in this study present genetic variability, with emphasis on accession Roxo de Ouro Fino from the Instituto Agrônomo de Campinas (IAC), which was isolated in various groupings.

Characteristics vary according to the evaluated environment, i.e., the dissimilarity among accessions changes. The correlation between variables in the evaluated environments was low.

Breeders should consider that conclusions reached for one environment cannot be extrapolated to others when selecting garlic accessions based on dissimilarity for developing new genotypes.

Breeding programs should consider decentralized assessments to enhance the potential of each accession in the environment where it will be grown. In this sense, assumptions of participatory breeding could be an alternative for the species, countering the high cost of evaluation in multiple locations.

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