The role of the genetic diversity of *Capsicum* spp. in the conservation of the species: Qualitative and quantitative characterization

O papel da diversidade genética de *Capsicum* spp. na conservação da espécie: Caracterização qualitativa e quantitativa

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ABSTRACT

Peppers need to be characterized for the *in situ* conservation of species that might be important for breeding purposes. In this study, we characterized the diversity of 23 pepper genotypes through eight qualitative and 12 quantitative descriptors. The pepper samples were collected from the city of Manaus and in the municipalities of Rio Preto da Eva and Iranduba in the state of Amazonas. We performed descriptive analysis, analysis of variance, Pearson's correlation analysis, and principal component analysis (PCA) and used the unweighted pair group method with arithmetic mean (UPGMA) to analyze the data. The differences between the genotypes for all descriptors evaluated were significant. Among the genotypes collected, four pepper species were identified, including *Capsicum chinense* (14 genotypes), *Capsicum frutescens* (two genotypes), *Capsicum baccatum* (one genotype), and *Capsicum annuum* (four genotypes), and two other genotypes were obtained with unidentified species. The PCA and UPGMA clustering methods could be used to efficiently differentiate between the genotypes by applying quantitative and qualitative descriptors of fruits and seeds. These methods helped to identify different genotypes (G1 and G15) and separate them into distinct groups associated with the distribution of species based on quantitative data only. Overall, our findings suggested that the genetic variability in *Capsicum* available in the State of Amazonas might be useful in breeding programs.

Index terms: Amazonas; multivariate analyses; pepper; superior genotypes.

RESUMO

As pimentas precisam ser caracterizadas para a conservação *in situ* da espécie que possam ser importantes para fins de reprodução. Neste estudo, caracterizamos a diversidade de 23 genótipos de pimenta por meio de oito descritores qualitativos e 12 quantitativos. As amostras de pimenta foram coletadas na cidade de Manaus e nos municípios de Rio Preto da Eva e Iranduba no estado do Amazonas. Realizamos análise descritiva, análise de variância, análise de correlação de Pearson e análise de componentes principais (ACP) e usamos o método de grupo de pares não ponderados com média aritmética (UPGMA) para analisar os dados. As diferenças entre os genótipos para todos os descritores avaliados foram significativas. Dentre os genótipos coletados, foram identificadas quatro espécies de pimenta, sendo elas *Capsicum chinense* (14 genótipos), *Capsicum frutescens* (dois genótipos), *Capsicum baccatum* (um genótipo) e *Capsicum annuum* (quatro genótipos), e outros dois genótipos foram obtidos com espécies não identificadas. Os métodos de agrupamento ACP e UPGMA podem ser usados para diferenciar eficientemente os genótipos por meio da aplicação de descritores quantitativos e qualitativos de frutos e sementes. Esses métodos ajudaram a identificar diferentes genótipos (G1 e G15) e separá-los em grupos distintos associados à distribuição das espécies com base apenas em dados quantitativos. No geral, nossos achados sugerem que a variabilidade genética em *Capsicum* disponível no Estado do Amazonas pode ser útil em programas de melhoramento.

Termos para indexação: Amazonas; análises multivariadas; pimenta; genótipos superiores.

INTRODUCTION

Capsicum pepper is an important vegetable and is popularly used as a spice around the world. Thus, the use of different forms of *Capsicum* is increasing (Cortez, 2022).

The FAO (Food and Agriculture Organization of the United Nations - FAO, 2021) reported global productivity of 38.3 million tons of *Capsicum* pepper in a cultivated area of 19 million hectares. The three largest producers of peppers

are China (45.3%), Mexico (8.4%), and Turkey (6.3%). In 2016, Brazil produced 28,270 tons of pepper; the highest producers were São Paulo (4,878 tons), Amazonas (3,690 tons), Pará (3,629 tons), Ceará (3,119 tons), and Goiás (2,296 tons) (Instituto Brasileiro de Geografia e Estatística - IBGE, 2017).

The *Capsicum* genus has high genetic diversity and has approximately 35 described species (Carvalho et al., 2003; Carrizo-García et al., 2013). Based on the levels of domestication for the genus, there are domesticated, semi-domesticated, and wild species. The domesticated species (*Capsicum annuum, Capsicum baccatum, Capsicum chinense, Capsicum frutescens,* and *Capsicum pubescens*) are the product of the selection of certain genetic alterations, and they are no longer able to survive under natural conditions (Ribeiro et al., 2008; De Jesus et al., 2020). These species produce fruits that differ in shapes, colors, and sizes; these fruits may have different degrees of pungency, which is determined by the concentration of capsaicinoids unique to this genus (Moscone et al., 2007; Dewitt; Bosland, 2009).

The semi-domesticated species (*C. baccatum* var. Baccatum and *C. baccatum* var. Praetermissum) were selected and cultivated by humans, but they are not fully domesticated (Carvalho et al., 2003; Ribeiro et al., 2008). These species are found in human-modified environments but are morphologically similar to the wild populations from which they originated. Wild species (*C. annuum* var. Glabriusculum*, Capsicum buforum, Capsicum campylopodium, Capsicum dusenii, Capsicum flexuosum, Capsicum mirabile, Capsicum parvifolium, Capsicum schottiamum,* and *Capsicum villosum*) are not cultivated and do not occur in environments altered by humans, and thus, they do not depend on humans (Ribeiro et al., 2008).

The genetic variability of a crop promotes the development of new cultivars with differences in fruit shape, color, and yield. Thus, maintaining diversity is important for its application as a tool in crop improvement programs (Heinrich et al., 2015). The preservation and conservation of this variability in germplasm banks and under in situ conditions allow the selection of genotypes that are valuable in different market niches, which in turn fosters agroindustry and family farming. Several studies conducted in Brazil (Neitzke et al., 2014; Heinrich et al., 2015; Cardoso et al., 2018; Fernandes; Gomes; Nogueira, 2019) and other parts of the world (Dewitt; Bosland, 2009; Zhang et al., 2016; Gu et al., 2019; Pereira-Dias et al., 2020) have shown the importance of knowing and studying the diversity of a species to better explore genetic variability in breeding, conservation, and to promote its use for the benefit of humans.

The maintenance of genetic diversity is crucial for preserving and improving cultivated species (Olivera; Steffenson, 2009; De Jesus et al., 2020). Thus, genetic diversity needs to be evaluated using qualitative and quantitative descriptors (IPGRI, 1995; Neitzke et al., 2014) for conserving existing genetic diversity to promote its use in breeding programs. The use of biometric information improves selection and has been performed using multivariate techniques (Aguilera et al., 2019; Bianchi et al., 2020; Brilhante et al., 2021).

The attributes of fruits and seeds obtained based on internationally presented descriptors (IPGRI, 1995) can help assess the genetic diversity of the species (Neitzke et al., 2014; Bernardo et al., 2018; Fernandes; Gomes; Nogueira, 2019). In this type of study, multivariate analyses complement traditional approaches and enhance the accuracy of data interpretation (Zago et al., 2017; Cardoso et al., 2018).

Amazonas is the second largest pepper producer in Brazil and has a high diversity of species. Although these species are well-maintained by producers, the diversity needs to be measured. Thus, in this study, we characterized the genetic diversity of the genus *Capsicum* using qualitative and quantitative descriptors obtained from the fruits and seeds of peppers collected in the city of Manaus and the municipalities of Rio Preto da Eva and Iranduba in the state of Amazonas, Brazil.

MATERIAL AND METHODS

Collection area and experimental site

In total, 23 *Capsicum* spp. genotypes were collected from markets, riverine communities, and farmers located in Manaus (3° 6'10.11" S and 60° 2'45.42" W) and in the municipalities of Rio Preto da Eva (2° 41'56.19 '' S and 59° 41'43.10 '' W) in the upper Rio Negro and Iranduba (3° 16'29.71 '' S and 60° 11'1.67 '' W), located on the left bank of the Solimões River in the state of Amazonas, Brazil (Figure 1).

The collected materials were preserved and analyzed in Seed Laboratory I of the School of Agricultural Sciences of the Federal University of Amazonas (Universidade Federal do Amazonas - UFAM). The description of the collection points, the common name, and the species are shown in Table 1.

Processing of fruits and seeds

For processing fruits (Figure 2) and seeds, gloves and masks were used to protect the hands and nose from exposure to large amounts of capsaicin present in peppers,

which can cause burning, irritation, and skin burns. The processing was performed in four stages: 1) The ripe fruits were placed in a polystyrene tray, and then, six fruits of each genotype were packed in PET-type packaging (15 × 10 × 5 cm), wrapped in 15-micron stretchable PVC film, and stored in a refrigerator at 10 ±1 °C until evaluation. 2) The fruits were cut longitudinally to extract the seeds manually with a steel spoon. 3) The seeds were dried on a paper towel in a ventilated environment for one week. 4) The seeds were stored in containers labeled with numbers specific to each genotype and kept at room temperature until further use.

Quantitative and qualitative characterization of fruits

We performed the biometric characterization (quantitative traits) of ripe fruits. To elaborate, the fruit diameter (FD; mm) was measured at the center of the fruit; fruit length (FL; mm) was measured from the base to the apex; the length of the fruit peduncle (LFP; mm) was measured at the top of the fruit and the neck length was measured at the base of the fruit (NLBF; mm). All measurements were recorded using a digital caliper (accuracy of 0.01 mm). A digital scale (accuracy of 0.001 g) was used to evaluate the fruit weight (FW; g).

For qualitative evaluations, the following descriptors were used: neck at the base of the fruit (NBF): 0 - absent and 1- present; fruit surface (FS): 1- smooth, 2- semirough, 3- rough, 4- smooth with streaks, and 5- semirough with streaks; color of the immature fruit (CIF) before the ripening stage: 1- white, 2- yellow, 3- green, 4- orange, 5- purple, 6- deep purple, and 7- other; color of the ripe fruit (CRF): 1- white, 2- lime yellow, 3- pale yellow-orange, 4- orange-yellow, 5- pale orange, 6- orange, 7- light red, 8- red, 9- dark red, 10 - purple, 11 - brown, 12 - black, and 13 - other; fruit shape (FSh): 1 - elongated, 2 - rounded, 3 - triangular, 4 - campanular, 5 - rectangular, and 6 - other, pungency (P): 1 - sweet, 2 - low-spicy, 3 - medium-spicy, and 4 - high-spicy, aroma (A): 1- low, 2 - medium, and 3 - high; consistency (C): 1 - ripe firm and 2 - mature soft; the shape of the fruit apex (SFA): 1 - acuminate point; 2 - uncut; 3 - depression; 4 - acute and depressed on the side, and 5 - caudate and irregular depression (Figure 3). The descriptors used (with slight modifications) were proposed by the IPGRI (1995), Carvalho et al. (2003), and Neitzke et al. (2014). Consistency was a new descriptor for the crop described in this study. For this descriptor, fruits of equal physiological ages were considered, and consistency, measured for these two suggested classes, was related to the degree of decomposition of the fruit after reaching maturity.

Figure 1: Location of the collection points in the city of Manaus and the municipalities of Rio Preto da Eva and Iranduba in the state of Amazonas, Brazil. Image source: Google Earth Pro (2022).

Table 1: Identification of Capsicum spp. collected in the city of Manaus and the municipalities of Rio Preto da Eva and Iranduba in the state of Amazonas, Brazil.

Genotype	Place of collection [1]	Common name	Species
1	RPV	*Habanero*	*C. chinense*
2	RPV	*Carolina Reaper*	*C. chinense*
3	RPV	Cayenne/*Cayenne* [2]	*C. annuum*
4	RPV	NI	NI
5	RPV	Black Murupi	*C. chinense*
6	RPV	7 colors[3]	*C. frutescens*
7	RPV	Red goat	*C. chinense*
8	RPV	Cherry	*C. annuum*
9	RPV	NI	NI
10	RPV	Sweet pepper	*C. chinense*
11	I	Small yellow Murupi	*C. chinense*
12	I	Yellow fish eye	*C. chinense*
13	I	Malagueta	*C. frutescens*
14	RPV	Josefá	*C. annuum*
15	RPV	*Aji panca*	*C. chinense*
16	RPV	Fish eye purple	*C. chinense*
17	I	Chestnut breast	*C. baccatum*
18	RPV	Murupi orange	*C. chinense*
19	I	Red fish eye	*C. chinense*
20	M	Murupi large yellow	*C. chinense*
21	I	*Jalapeno*	*C. annuum*
22	I	Murupi small orange	*C. chinense*
23	I	Biquinho	*C. chinense*

[1] RPV: Rio Preto da Eva; I: Iranduba (Lago do Limão Community); M: Manaus; [2] Cayenne/Cayenne: hybrid; [3] 7 Colors: ornamental and NI: genotype without passport information.

Quantitative and qualitative characterization of seeds

For the characterization of the seeds, several quantitative descriptors were measured. The number of seeds per fruit (NSF; unit) was determined by counting the seeds in the selected fruits; seed diameter (SD; mm) in the central region was determined from 10 seeds using a digital caliper (accuracy of 0.01 mm); the mass of one thousand seeds (MS; g) was evaluated using a digital scale (accuracy of 0.001 g).

The qualitative descriptors were measured as follows: seed size (SSi): 3 - small, 5 - intermediate, and 7 - large, seed color (SC): 1 - straw (deep yellow), 2 - brown, 3 - black, and 4 - other; seed surface (SSu): 1- smooth, 2 - rough, and 3 - wrinkled. All descriptors used were proposed by the IPGRI (1995), Carvalho et al. (2003), and Neitzke et al. (2014).

Statistical analyses

The quantitative data on fruits and seeds were analyzed by performing an analysis of variance (ANOVA) using a completely randomized design. The treatments included the 23 collected genotypes with three replicates for the quantitative data, and each plot consisted of 20 seeds or five fruits. When the differences between the means of the quantitative descriptors of fruits and seeds were significant, the Scott–Knott test was performed at the 0.05% level of significance. Pearson's correlation

coefficients between the quantitative descriptors of fruits and seeds were evaluated, and a network of correlations was obtained, in which the proximity between the nodes (traits) was proportional to the absolute correlation values between the evaluated parameters. Positive correlations were highlighted in green, while negative correlations were highlighted in red. The qualitative and quantitative datasets were analyzed by principal component analysis (PCA) and the UPGMA method for constructing a dendrogram, using the Euclidean distance combining both classes of characters. All analyses were performed using the RBio software (Bhering, 2017).

Figure 2: The characters of the fruits of 23 *Capsicum* spp. collected in the city of Manaus and the municipalities of Rio Preto da Eva and Iranduba in the state of Amazonas, Brazil.
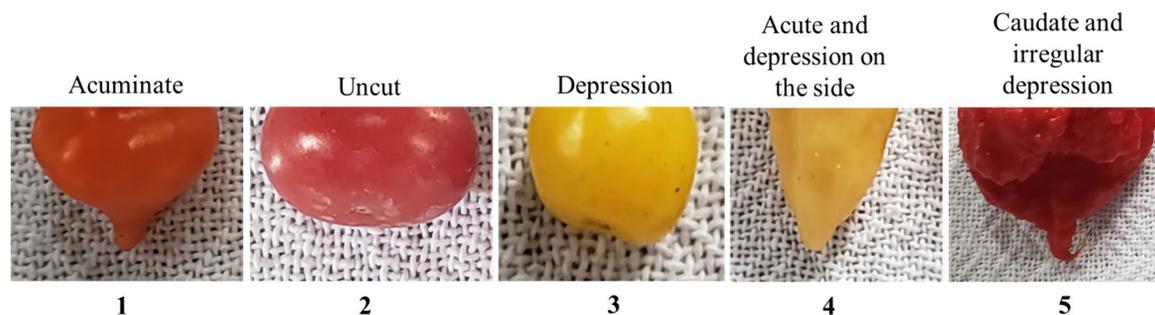
Figure 3: The apex shape of the fruit of five genotypes of pepper (*Capsicum* spp.).

RESULTS AND DISCUSSION

We obtained 23 different genotypes from the collected samples; 60.9% of the genotypes were from Rio Preto da Eva, 34.8% were from Iranduba (Lago do Limão Community), and 4.3% were from Manaus. For most genotypes, the common name and species were identified, only genotypes G4 and G9 remained unidentified (Table 1). Among the genotypes collected, four pepper species were identified: *C. chinense* (14 genotypes), *C. frutescens* (two genotypes), *C. baccatum* (one genotype), and *C. annuum* (four genotypes); however, two genotypes could not be assigned to a species (Table 1). Information on the genetic variation of peppers in the Amazonas, an extremely productive site in Brazil, can be used by breeding programs for conserving and managing genetic crop resources. The greater number of *C. chinense* genotypes matched the description of Bianchi et al. (2020), who considered it to be the most Brazilian species in the genus *Capsicum*. This species is common in the Amazon region. It was probably domesticated here and selected for different sizes, shapes, colors, and pungency of fruits (Pickersgill, 1991; Moses; Umaharan, 2012; Brilhante et al., 2021).

Phenotypic information is necessary for achieving the objectives highlighted in all breeding and conservation programs. It is also required to direct the different uses that can be assigned to the characterized genotypes. The pepper germplasm needs to be conserved to conduct any breeding project. Several studies have shown that the peppers in Brazil have a high genetic diversity (Carvalho et al., 2003; Ribeiro et al., 2008; Heinrich et al., 2015; Cardoso et al., 2018; Fernandes; Gomes; Nogueira, 2019; De Jesus et al., 2020).

Quantitative characteristics of fruits and seeds

When characterizing the fruits and seeds of pepper, eight quantitative descriptors were evaluated, and they showed highly significant differences (P < 0.001). Among the 23 genotypes collected (Table 2), the descriptors showed the presence of high diversity among the samples collected from different cities of Amazonas. The lowest and highest observed CV values were 0.18% and 17.82%, respectively; however, the precision and homogeneity of the data were low (Table 2). The CV of FL (17.82%) allowed us to infer the influence of the range of variation of the minimum (7.24 mm) and maximum values (150.69 mm). These results showed the diversity and differences among the genotypes tested (Table 2). The NSF had the lowest coefficient of variation (0.18%), which was related to fruit size, with the size ranging from 5 to 91.1 seeds.

We found that for most traits, the H² was high (H² > 97%), indicating selection potential for the genotypes studied. However, the heritability of SD was only 68%, indicating low genetic variance for this trait, which can hinder the selection process due to the influence of the environment (Table 4). Heritability indicates how much of the genetic proportion of each individual can be transmitted to the next generation (Nascimento et al., 2021). The heritability of a trait is considered to be high when the parameter is equal to or greater than 70% (Cruz; Regazzi; Carneiro, 2012).

The analysis of the mean values (grouped by the Scott–Knott test at the 0.05% significance level) (Table 3) showed that the highest number of classes for MS and NSF were 17 and 18, respectively. For MS, the G4 samples had the lowest weight of 1.41 g, while the G21 samples had the highest weight of 6.48 g. For NSF, the G11 samples had the lowest weight of 5.03 units, while the G4 and G6 samples had the highest weight of 91.03 and 90.03 units, respectively. The high-class intensity indicated by these quantitative descriptors showed the high variability of these traits for genetic improvement, which is extremely important for genetic diversity. We found that SD had the lowest number of classes (only two) (Table 3), which confirmed the low H² of this trait.

Table 2: Summary of the ANOVA for quantitative descriptors obtained from the fruits and seeds of pepper (*Capsicum* spp.).

FV[1]	FD[2] (mm)	FL (mm)	FW (g)	LFP (mm)	NLBF (mm)	SD (mm)	MS (g)	NSF (units)
					p-value			
Genotypes	***	***	***	***	***	***	***	***
CV (%)	16.09	17.82	1.16	13.34	14.96	10.76	2.5	0.18
H[2]	95.75	98.17	99.99	97.76	97.75	67.99	100	100
Minimum	4.62	7.24	0.33	21.92	2.79	1.82	1.41	5
Maximum	33.09	150.69	13.67	152.83	31.20	3.89	6.62	91.1

[1]FV: source of variation, CV: coefficient of variation, H[2]: heritability in the broad sense. [2]FD: fruit diameter, FL: fruit length, FW: fruit weight, LFP: fruit peduncle length, NLBF: neck length at the base of the fruit, SD: seed diameter, MS: mass of one thousand seeds, NSF: number of seeds per fruit. *** significance of the F test at 0.01% probability.

Table 3: Comparison of the means of the quantitative descriptors of the fruits and seeds of pepper (*Capsicum* spp.).

Genotype	-------------------------- Fruits -----------------------					-------- Seeds -------		
	FD[1] (mm)	FL (mm)	FW (g)	LFP (mm)	NLBF (mm)	SD (mm)	MS (g)	NSF (units)
1	32.56 a	47.92 d	7.03 c	64.48 d	29.52 a	3.36 a	4.22 d	44.03 e
2	22.07 b	32.10 e	3.34 e	55.63 d	17.02 c	3.02 a	3.66 g	10.03 o
3	9.53 e	58.26 c	1.03 i	79.25 c	8.30 e	3.31 a	5.77 b	36.03 g
4	13.83 d	62.20 c	5.03 d	84.72 c	16.34 c	3.63 a	1.41 q	91.03 a
5	9.09 e	42.87 d	2.67 f	74.31 c	6.45 e	3.02 a	1.97 n	14.03 n
6	9.96 e	29.05 e	2.03 g	54.25 d	12.53 d	3.16 a	2.96 i	90.03 b
7	11.05 e	11.38 f	NI	25.84 f	11.76 d	3.17 a	3.80 f	35.03 h
8	13.55 d	9.03 f	NI	29.99 f	4.97 f	3.18 a	4.65 c	7.03 q
9	11.72 d	18.93 f	1.34 h	41.72 e	10.21 d	3.44 a	3.38 h	20.03 k
10	15.47 c	38.12 e	2.03 g	51.50 e	16.59 c	3.15 a	2.25 l	45.03 d
11	9.04 e	48.21 d	NI	49.67 e	4.56 f	2.56 b	2.39 k	5.03 r
12	10.51 e	11.96 f	0.67 j	28.07 f	6.82 e	2.35 b	1.69 p	23.03 j
13	4.92 e	16.22 f	0.34 k	34.29 f	4.32 f	3.16 a	3.10 i	11.03 n
14	21.53 b	23.73 f	1.34 h	45.17 e	21.47 b	3.20 a	4.08 e	42.03 f
15	22.08 b	126.78 a	13.67 a	137.86 a	21.69 b	3.44 a	3.80 f	34.03 i
16	9.00 e	11.80 f	0.67 j	28.17 f	3.52 f	2.42 b	1.97 h	20.03 k
17	13.79 d	16.80 f	NI	30.69 f	9.57 d	3.02 a	2.82 j	36.03 g
18	7.69 e	43.90 d	0.67 j	58.29 d	5.72 f	3.18 a	3.66 g	19.03 l
19	8.85 e	10.42 f	NI	24.13 f	7.71 e	2.59 b	1.83 o	11.03 n
20	16.35 c	70.25 b	5.03 d	98.43 b	10.77 d	2.99 a	2.11 m	53.03 c
21	17.53 c	43.27 d	7.34 b	59.45 d	18.31 c	3.52 a	6.48 a	45.03 d
22	12.30 d	33.06 e	0.67 j	46.87 e	7.60 e	3.11 a	3.10 i	8.03 p
23	12.68 d	15.24 f	1.03 i	27.71 f	11.60 d	2.80 b	2.39 k	35.03 h
Groups formed	5	6	11	6	6	2	17	18

[1]FD: fruit diameter, FL: fruit length, FW: fruit weight, LFP: fruit peduncle length, NLBF: neck length at the base of the fruit, SD: seed diameter, MS: mass of one thousand seeds, NSF: number of seeds per fruit. NI: genotype without passport information. Different letters in the column represent significant differences by the Scott–Knott test at 0.05% significance.

Table 4: Qualitative descriptors of the fruits and seeds of pepper (*Capsicum* spp.).

Genotype	Fruits									Seeds		
	NBF[2]	FS	CIF	CRF	FSh	SFA	P	A	C	SSi	SC	SSu
G1	0	1	3	8	4	5	4	3	1	5	1	3
G2	0	3	4	8	6	5	4	3	1	5	2	3
G3	1	1	2	8	1	1	4	1	1	7	1	1
G4	1	3	5	13	1	4	4	3	1	5	1	1
G5	1	1	6	10	1	1	4	3	1	5	1	2
G6	1	1	5	3	6	1	2	1	1	5	1	1
G7	1	1	3	9	2	3	4	3	1	5	2	1
G8	0	1	3	7	2	5	2	1	1	5	2	2
G9	0	1	3	7	6	5	3	2	1	5	1	1
G10	0	3	3	2	1	4	2	3	1	3	1	2
G11	1	3	3	2	1	1	4	3	2	5	2	3
G12	1	1	3	2	2	3	4	3	2	3	2	3
G13	1	1	3	8	6	5	4	3	2	3	1	2
G14	0	1	5	13	4	5	4	2	1	5	1	2
G15	1	3	5	8	1	1	2	2	1	5	2	1
G16	0	1	5	2	3	3	4	3	1	3	1	1
G17	NI	1	3	7	3	1	3	2	1	3	1	1
G18	1	3	5	6	1	1	4	3	1	5	3	3
G19	0	1	3	7	2	3	4	3	2	3	1	2
G20	1	3	3	2	1	1	4	3	1	5	2	2
G21	1	4	3	9	3	1	3	2	1	5	1	1
G22	1	3	3	6	3	1	4	3	1	5	1	1
G23	0	1	4	8	2	1	1	1	1	3	1	1
Number of classes	2	3	5	8	5	4	4	3	2	3	3	3

[2]NBF: neck at the base of the fruit, FS: fruit surface, CIF: color of the immature fruit, CRF: color of the ripe fruit, FSh: fruit shape, SFA: shape of the fruit apex, P: pungency, A: aroma, C: consistency, SSi: seed size, SC: seed color, SSu: seed surface. NI: genotype without passport information.

Fruit weight (FW) had 11 classes (Table 3). Among all genotypes, the G13 genotype had the lowest weight of 0.34 g, while the G15 genotype had the highest weight of 13.67 g. This characteristic is essential for breeding programs, given the high variation in the weight of the fruits across genotypes. The other measured characteristics showed six (FL, LFP, and NLBF) and five (FD) classes (Table 3). The FL ranged from 10.42 mm in G19 to 126.78 mm in G15. The LFP ranged from 24.13 mm in G19 to 137.86 mm in G15. The NLBF ranged from 3.52 mm in G16 to 29.52 mm in G1. The FD ranged from 4.92 mm in G13 to 32.56 mm in G1. The genotypes G15 and G1 differed the most, as they were always present among the groups with the highest values for four (FL, FW, LFP, and SD) and three (FD, NLBF, and SD) of the measured characteristics, respectively; these groups were formed based on the Scott–Knott test (p < 0.05) (Table 3).

The relationships between the quantitative descriptors of the fruits and seeds of pepper were determined by estimating Pearson's correlation coefficients and building a correlation network using these coefficients (Figure 4). The highest correlations were found among all fruit descriptors.

The high correlations between the characteristics measured in fruits allow us to make direct and indirect selections. Most correlations were positive, but the correlation between NSF and MS was low and negative (Figure 4).

The highest correlation occurred between LFP and FL, which are the characteristics that determine the form in which the pepper fruit is used. For example, small fruits (G8; 9.03 mm in length) are consumed more often as preserves and industrialized liquid sauces, while large fruits (G15; 126.78 mm in length) are preferably sold as dehydrated and whole flakes (pepperoni) and powder (paprika). Ribeiro et al. (2021) commented that along with the rising demand for processed products in the foreign market, the demand for new types of food products (jams, chocolates, etc.) and organic products has increased among young consumers interested in exotic flavors and healthy foods. Indirect selection for fruit diameter can also increase the number of fruits per plant (Sapucay et al., 2009). Similar to our findings, Ribeiro et al. (2008) found that fruit weight and seed diameter are related to fruit length, demonstrating that ripe fruits with thicker pulps have more seeds, whereas ripe fruits with little pulp produce fewer seeds.
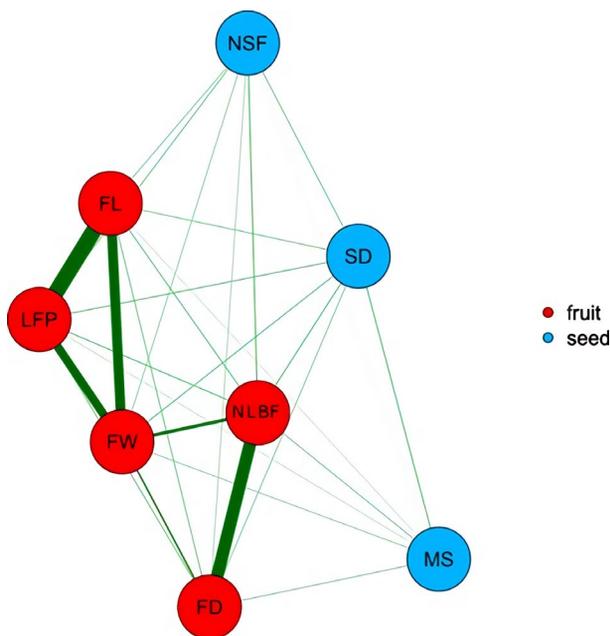
Figure 4: The correlation network was established based on the data obtained from quantitative descriptors of the fruits and seeds of pepper collected in the state of Amazonas. FD: fruit diameter, FL: fruit length, FW: fruit weight, LFP: fruit peduncle length, NLBF: neck length at the base of the fruit, SD: seed diameter, MS: thousand seed mass, NSF: number of seeds per fruit (Pearson's P > 0.60).

Qualitative traits of fruits and seeds

The results of the analysis of 12 qualitative traits of fruits and seeds recorded from 23 genotypes of peppers confirmed the presence of high genetic variability among the genotypes characterized (Table 4). Different varieties of peppers provide different tastes and flavors to food, ranging from sweet to very spicy. The qualitative descriptors consisted of two (NBF and C), three (FS, A, SSi, SC, and SSu), four (SFA and P), five (FSh and CFI), and eight (CRF) classes. (Table 4). Among the descriptors stood out genotypes of single representation among some of the classes were G21 for the fruit surface (4- smooth with striations), G5 for the fruit color in the intermediate stage (6- deep purple), and fruit color in the mature stage (10- purple), G3 for fruit color in the intermediate stage (2- yellow) and seed size (7- large), G6 for fruit color in the mature phase (3- pale yellow-orange), G23 for pungency (1- sweet), and G18 for seed color (3- black).

Among the studied genotypes, nine qualitative descriptors of fruits and three qualitative descriptors of seeds showed high variation, with at least two groups for the neck length at the base of the fruit and consistency and a maximum of eight groups for fruit color in the mature phase. We found that some attributes were unique to the genotypes G3, G5, G6, G18, G21, and G23. Only the G21 genotype had a smooth surface with streaks. Domenico et al. (2012) showed that fruits with a rough surface are unpopular in the market because the consumer perceives them as deteriorated/stale products. Therefore, the appearance of fruits is important for commercialization.

The fruit run before and in the mature phase was evaluated, and it showed the formation of five and eight classes, respectively. The fruit color was found to influence the physiological maturity of the seeds and pungency, for example, the orange genotypes (G2 and G23) were more pungent than the yellow fruits (G3). Additionally, the fruit development stage was found to affect pungency; the pungency was greater at the start of ripening, and this association between fruit development and pungency can be used to determine the ideal time to harvest ripe fruits. These characteristics are important indicators of productivity and can be used to generate new varieties.

The red color present in 52% of the collected genotypes is widely used as a dye in food to enhance aesthetic appeal. Pinto, De Oliveira Pinto and Donzeles (2013) found that pepper is used for coloring food items; in many cases, its color and pungency are used to modify the color and flavor of soups, sausages, cheeses, snacks, and sauces, among others. Thus, the color intensity, especially red, is highly desirable for the production of paprika powder.

However, Pino et al. (2007) found that the varieties that produced orange and brown fruits had better aroma-related chemical compositions than red fruit varieties.

In the genotypes collected, most were spicy (only G23 was sweet). Ripe fruits are spicier than immature fruits, and they are also influenced by plant stress and high temperatures that increase pungency (Dewitt; Bosland, 2009). Pungency is an important commercial attribute that occurs in fresh peppers and processed products. The active ingredient capsaicin is one of the main components that regulate the commercial quality of the fruits (Olguín-Rojas et al., 2019).

Among the qualitative descriptors, we introduced consistency as a new descriptor of the crop. It helps to quantify the degree of decomposition of the fruit after reaching maturity. Among the genotypes collected, only 17.4% had soft consistency (G11, G12, G13, and G19), and the rest had firm consistency. Consistency is important for pickling, drying, and the production of pulp used for manufacturing sauces, fresh use, processing, and freezing (Dewitt; Bosland, 2009).

Multivariate analyses based on the qualitative and quantitative traits of fruits and seeds

Based on the PCA, a biplot of two-axis scores (PC1 and PC2) was generated, which showed the positions relative to the genotypes and the qualitative and quantitative descriptors (Figure 5). The main coordinates rotated the character sets to capture most of the variability, which helped in interpreting the results to determine the genetic diversity. When analyzing the genetic divergence based on the PCA (Figure 5), the first two main coordinates of the qualitative descriptors (Figure 5a) explained 41.73% of the total variation of the character sets, contributing 22.07% to the first axis and 19.66% to the second axis. The quantitative descriptors are shown in Figure 5b. The variation in PC1 and PC2 represented 69.23% of the total variation of the traits, contributing 50.87% to the first axis and 18.36% to the second axis. The percentage of variability observed in the first two coordinates showed that the quantitative traits (Figure 5b) contributed more than the qualitative traits (Figure 5a) to the genotypes collected. The qualitative descriptors were well-dispersed (Figure 5a), indicating that the data had high variation (Table 4), and most of the genotypes correlated with a descriptor. Taking G18 and G20 associated with the descriptors NBF, FS, and SC as an example, G12 was also associated with the descriptors P, A, SSu, and C.

When considering the quantitative descriptors, the genotypes that contributed the most to these traits were located near them, which indicated the presence of high genetic variability among the genotypes (Figure 5b). The genotype G1 was associated with the descriptors MS, FD, NLBF, and SD, while G15 was associated with LFP and FL; thus, they differed from the other genotypes for these descriptors. These combinations showed the greatest heterotic potential.

While representing the species associated with each pepper genotype collected in the two PCAs generated for the qualitative (Figure 5c) and quantitative (Figure 5d) data, we found that the four species (*C. chinense*, *C. frutescens*, *C. baccatum,* and *C. annuum*) were not grouped based on the qualitative data, and their genotypes were dispersed. However, when the species were represented by performing the PCA based on quantitative data, the species *C. chinense* and *C. annuum* clustered in the representation of the genotypes, showing that these characteristics were correlated or associated with the described species (Figure 5d). Similar results were obtained in a study by Thul et al. (2009), where the genotypes of *C. annuum* were characterized. These findings suggested that taxonomic traits are not always related to agronomic traits. The genotype G4 with undescribed species was located near the cluster of *C. chinense*, which indicated its proximity to the sampled characteristics. The G9 genotype, also with undescribed species, was located close to the four species (Figure 5d).

The 20 descriptors divided into 12 qualitative and eight quantitative descriptors recorded from the fruits and seeds of peppers were used in the cluster analysis and their corresponding dissimilarity dendrogram (Figure 6). In the generated dendrogram, four groups were formed when considering the cutoff point corresponding to the mean dissimilarity between the genotypes ($\Theta = 6.5$) (Figure 6).

The G1 genotype of *C. chinense* was the most different, forming Group I, and was isolated from the others in the dendrogram (Figure 6). Group II consisted of 73.9% of the genotypes (G18, G5, G7, G22, G13, G11, G12, G19, G8, G2, G9, G14, G6, G16, G10, G17, and G23). The individuals of the four species *C. chinense, C. annuum, C. baccatum, C. frutescens,* and one of the unidentified genotypes (G9) were clustered in Group II (Figure 6). Group III had only one genotype, G15 (*C. chinense*), which had fruits with low spiciness, light burning, and a unique smoky and fruity flavor.

The results of the principal component and dendrogram analyses by the UPGMA method were similar and showed that the G1 and G15 genotypes were the most different among all collected genotypes. The four species described in this study showed better grouping when quantitative data were considered (Figure 5d). Santo, Menezes and Carmo (2022) characterized 29 pepper accessions based

on 32 multicategorical descriptors and found that the multicategorical descriptors could adequately evaluate the diversity and showed high variability in the collection which might be used in breeding programs of *Capsicum* spp. Several studies have evaluated the combination of biometric information using multivariate analysis techniques (Aguilera et al., 2019; Bianchi et al., 2020; Brilhante et al., 2021). Multivariate analyses complement traditional approaches and allow better interpretation of the data (Zago et al., 2017; Cardoso et al., 2018).
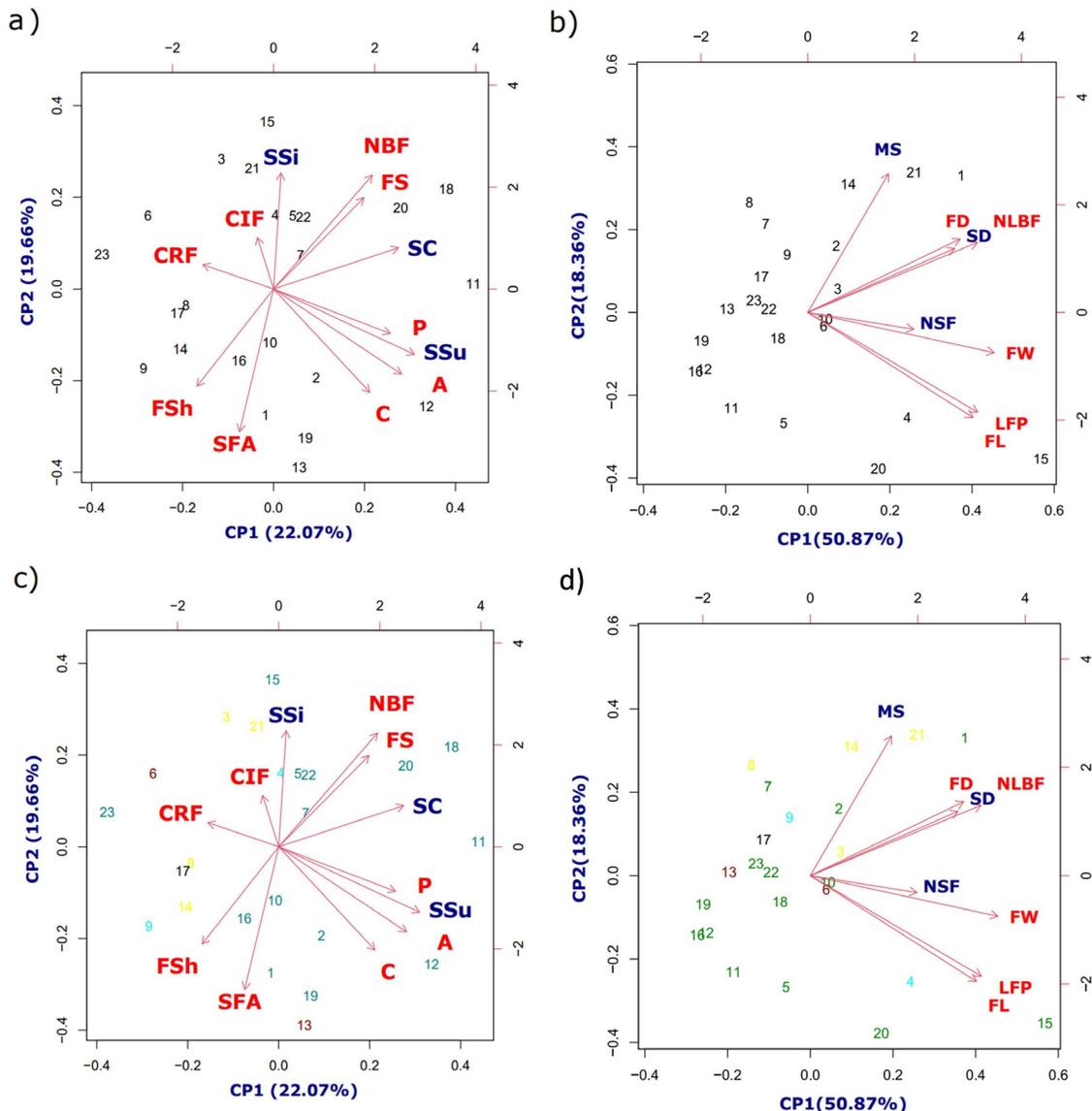
Figure 5: The results of the principal component analysis (PCA) were obtained using qualitative (a and c) and quantitative (b and d) descriptors of fruits (highlighted in red) and seeds (highlighted in blue) obtained from 23 genotypes of pepper collected in Amazonas. The distribution of genotypes is shown in different colors (c and d) for *C. chinense* (green), *C. frutescens* (brown), *C. baccatum* (black), *C. annuum* (yellow), and unknown (blue). FD: fruit diameter, FL: fruit length, FW: fruit weight, LFP: fruit peduncle length, NLBF: neck length at the base of the fruit, SD: seed diameter, MS: thousand seed mass, NSF: number of seeds per fruit, NBF: neck at the base of the fruit, FS: fruit surface, CIF: fruit color in the intermediate stage, CRF: fruit color in the ripe stage, FSh: fruit shape, SFA: shape of the fruit apex, P: pungency, A: aroma, C: consistency, SSi: seed size, SC: seed color, and SSu: seed surface.
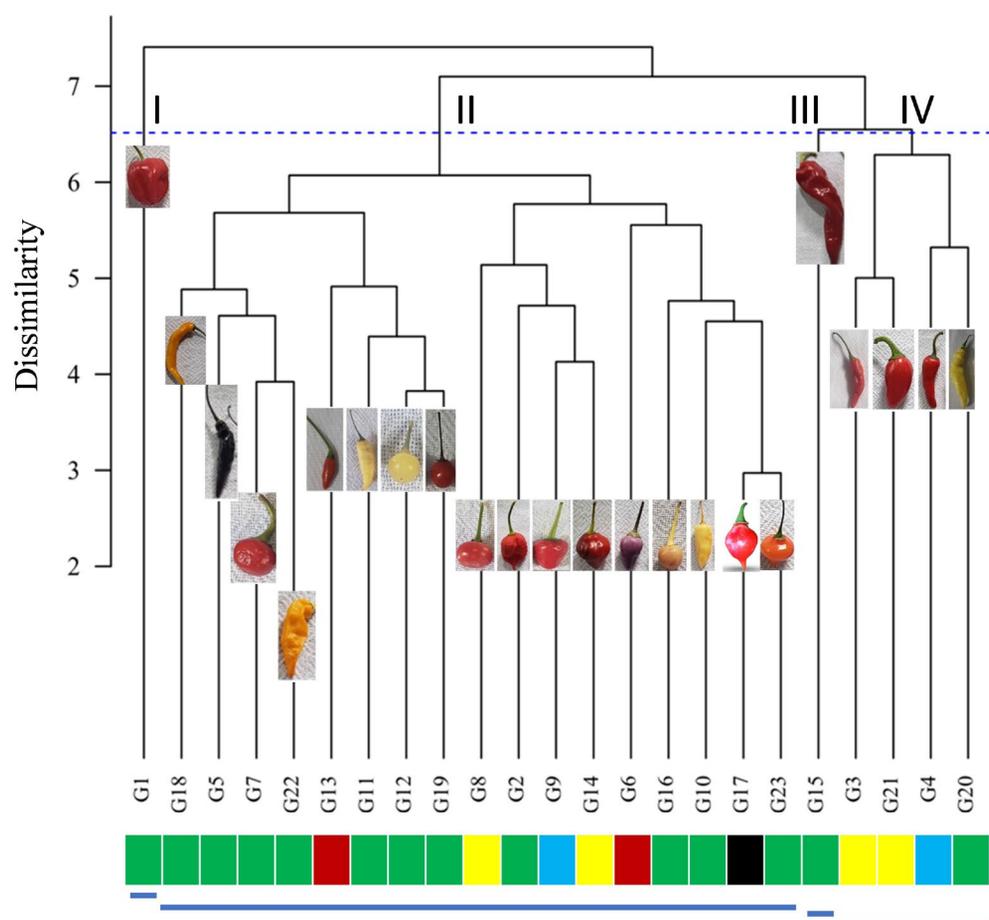
Figure 6: A dendrogram showing the dissimilarity evaluated using the UPGMA method when using information from qualitative and quantitative descriptors of fruits and seeds of 23 pepper genotypes collected in Amazonas. The distribution of the genotypes G1 to G23 and the images of their respective fruits. The bars of different colors represent the species *C. chinense* (green), *C. frutescens* (brown), *C. baccatum* (black), *C. annuum* (yellow), and the unknown species (blue) for each of the collected genotypes. The blue bars represent the groups formed.

Group IV consisted of four genotypes (G3, G21, G4, and G20), which included the species *C. annuum, C. chinense,* and NI (unidentified genotype) (Figure 6). Among the groups formed, Groups I and III consisted of a single representative, i.e., G1 and G15, respectively, which were the most different among the collected genotypes. This finding was confirmed by the results of the PCA (Figure 5b). The G1 genotype had the largest fruit diameter (32.56 mm), the longest neck at the base of the fruit (29.52 mm), and the largest seed diameter (3.36 mm). The G15 genotype had a greater fruit length (126.78 mm), fruit weight (13.67 g), and fruit peduncle length (137.86 mm) and one of the largest seed diameters. (3.44 mm) (Table 3). Devi, Singh and Singh (2017) used 41 morphological characters (both qualitative and quantitative) based on the IPGRI Descriptor for chili

and found that bigger chili cultivars can be successfully differentiated from small and medium size chilies. Similar findings were reported by Wang and Bosland (2006), who found that fruit size strongly affected variation in chilies.

Peppers are sold in a *fresh* or dehydrated form (flakes with seeds) or as processed foods (sweets, jams, sauces, and in dried or powdered form). To cater to the needs of different consumers and product manufacturers, different forms and qualities of fruits and seeds of pepper need to be investigated and improved. When the fruit length and the dry matter content are high, the yield of the dried or powdered fruits increases (Silva et al., 2021). Our findings showed that most traits of pepper have high variability, which enables the selection of individuals with contrasting traits, and thus, promotes the formation of new

combinations of crosses. Similar results were found in studies by Cardoso et al. (2018) and De Jesus et al. (2020). These characteristics can minimize the use of sources conserved by small producers in regions such as those in Manaus. In the north of Brazil, peppers are commonly consumed in the form of gravy mixed with tucupi broth (extracted from cassava) and in preserves with vinegar, oil, or whey (Reifschneider et al., 2015). Characteristics such as the thousand seed mass and the number of seeds per fruit are relevant for commercialization since farmers can make profits by selling desirable seeds (Rufino; Penteado, 2006). We conducted the principal component analysis and dendrogram analysis by the UPGMA method and found differences among the collected genotypes.

The diversity in our collected samples allowed us to select genotypes for various characteristics of interest (e.g., color, type of fruit, level of pungency, etc.). Similarly, Ferrão et al. (2011) used multivariate techniques to evaluate morpho-agronomic traits in *Capsicum* peppers and identified the best genotypes for producing canned peppers. This type of approach provides useful information for collection management. However, the traits that show non-additive inheritance have some limitations because they are highly influenced by the environment (Burle; Olivera, 2010). However, in this study, the quantitative traits showed high additive inheritance, which allowed us to select genotypes based on these traits and generate gains in selection.

Characterization based on qualitative and quantitative descriptors is an effective strategy. Our findings suggested that genotypes G1 and G15 can be crossed with genotypes from Groups II and IV (Figure 6) to expand the genetic base of *Capsicum* spp., increase heterozygosity, and introduce useful genes in commercial cultivars of interest. These genotypes have important agronomic characteristics and are conserved by riparian farmers in the state of Amazonas. Determining the genetic diversity of the collected genotypes of pepper can contribute to the knowledge of the diversity of the genus in Brazil. This diversity might be useful in conducting breeding programs and further studies on the genetic resources of *Capsicum* spp.

CONCLUSIONS

High genetic variability was found in fruits and seeds of 23 pepper genotypes based on 12 qualitative and eight quantitative descriptors. The evaluation of collected and evaluated germplasm under a multivariate statistical approach provided a unique opportunity to select superior genotypes (G1 and G15) and to identify quantitative

characters associated with *C. chinense* and *C. annuum* species. This genetically heterotic germplasm can be used to develop new cultivars.

AUTHOR CONTRIBUTION

Conceptual Idea: Sampaio, A.P.; Aguilera, J.G.; Methodology design: Sampaio, A.P.; Aguilera, J.G., Mendes, A.M.S.; Data collection: Sampaio, A.P.; Aguilera, J.G.; Data analysis and interpretation: Sampaio, A.P.; Aguilera, J.G., Mendes, A.M.S.; Argentel-Martines, L.; Zuffo, A.M.; Teodoro, P.E.; and Writing and editing: Sampaio, A.P.; Aguilera, J.G., Mendes, A.M.S.; Argentel-Martines, L.; Zuffo, A.M.; Teodoro, P.E.

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