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Fruit quality and morphoagronomic characterization of a Brazilian *Capsicum* germplasm collection

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ABSTRACT. *Capsicum* peppers are valued in Brazil and throughout the world for their great variety of shapes, colors, flavors, and pungency levels. Various health benefits have been attributed to chili peppers, including their antioxidant activity. Despite great efforts to characterize *Capsicum* germplasm around the world, few studies have used biochemical descriptors. We examined 11 morphoagronomic and six biochemical descriptors for the fruit to analyze 69 *Capsicum* accessions from four regions of Brazil; these belong to the Instituto Federal do Espírito Santo – Campus de Alegre Germplasm Collection. The accessions comprise five different *Capsicum* taxa: *Capsicum chinense*, *Capsicum baccatum var.*

pendulum, *Capsicum frutescens*, *Capsicum annuum var. annuum* and *Capsicum annuum var. glabriusculum*. The experiment was conducted in a completely randomized design with three replicates under field conditions. For all the traits examined, ANOVA showed a significant variability between accessions. We detected significant diversity among the *Capsicum* genotypes; the Scott-Knott's test grouped the accessions into five to 14 groups, depending on the trait. Pearson's correlation analysis revealed strong correlation between morphoagronomic (fruit length, diameter and weight) and physical and-chemical (soluble solids and moisture content) traits. Principal component analysis indicated that fruit diameter and fruit fresh mass were among the most important traits contributing to diversity among the accessions. Together these results demonstrate the success of indirect selection of useful chemical properties in *Capsicum* peppers through breeding for other easy-to-measure morphoagronomic traits. This research is also an important step for the creation of a representative collection of pepper germplasm, facilitating the conservation of this genetic resource, which suffers a continuous process of genetic erosion.

Key words: Peppers; Biochemical characterization; Bromatological characterization; Genetic resources; Solid soluble content; Titratable acidity

INTRODUCTION

The *Capsicum* genus (Solanaceae) is particularly relevant to Brazilian agribusiness since this genus included the hot and sweet peppers, which are cultivated worldwide (Razo-Mendivile et al, 2021). Currently, this genus comprises 42 species, of which five are considered domesticated because of their widespread use: *Capsicum annuum*, *Capsicum baccatum* var. *pendulum*, *Capsicum pubescens*, *Capsicum frutescens* and *Capsicum chinense*. The other 37 species are classified as semi-domesticated or wild according to Barboza et al. (2019).

Peppers have great appeal in the world market, especially in Asia, South America and in Africa, which prefer the most pungent species, and in the markets of North America and Europe that consume, mostly, species that are not pungent, such as sweet peppers (Scossa et al., 2019). All this appreciation for peppers is due to their versatility in the culinary and pharmaceutical-cosmetic industry. In cooking, they can be consumed as vegetables through their fresh fruits or as condiments and seasonings such as paprika powder (Moulin et al., 2022). When it comes to consumption in the form of vegetables, much of the diversity of peppers was directed towards the development of large, sweet-tasting fruits, with thicker pericarps and more vivid colors (Pinto et al., 2016). On the other hand, in regions where the consumption of hot peppers predominates, characteristics such as pungency, fruit size and pericarp thickness are considered. In order to produce condiments, where the fruits need to be dehydrated, peppers with lower water content and specific colors are sought (González-López et al., 2021).

In addition to the different criteria adopted by the different branches of the pepper industry, consumers are increasingly interested in tastier vegetables, containing healthier components and with higher nutritional quality (Neocleous et al., 2019). Thus, interest in foods of plant origin as a source of health-promoting phytochemicals has increased over the years, as it has already been reported that consumption of natural antioxidant compounds from fruits and vegetables is associated with the prevention of chronic diseases due to its ability to neutralize free radicals in the body (Prakash et al., 2009). Pepper fruits are an excellent source of various compounds such as carotenoids, which are responsible for the variety of their attractive colors – vitamins E, C, and the B complex, and precursors of vitamin A (Karim et al., 2021); phenolic compounds such as flavonoids and cinnamic acid derivatives with anticancer activity; carbohydrates and capsaicin – an alkaloid that gives peppers their characteristic pungency (Razo-Mendivil et al., 2021). Given all the potential that the *Capsicum* genus offers, it is essential that breeding programs explore the diversity of the genus to improve existing commercial types, which will allow the expansion of the market to new segments.

Despite the great importance of bromatological characterization, most works related to the *Capsicum* genus aim at phenotypic and molecular characterization (Moulin et al., 2015; Bianchi et al., 2020; Brilhante et al., 2021; Lahbib et al., 2021; De Oliveira et al., 2022) from germplasm collections. However, the organoleptic and bioactive properties of pepper fruits are largely due to the presence of phytochemical compounds which, in turn, still represent a gap in knowledge given the great diversity that the genus has (González-López et al., 2021). All these attributes will have a fundamental influence on how consumers perceive product quality and decide their preferences.

In this sense, considered it opportune to develop this work, which aimed to carry out the morphoagronomic and bromatological characterization of fruits of 69 accessions of *Capsicum* from four out of five Brazilian regions (Midwest, North, Northeast and Southeast).

MATERIAL AND METHODS

Evaluated genotypes, experimental design and environmental conditions

An entire collection of 69 genotypes of *Capsicum* was evaluated. These genotypes belong to the Germplasm Collection of the Instituto Federal do Espírito Santo —Campus de Alegre, located in the municipality of Alegre, Espírito Santo State, Brazil, where all the traits were accessed. These 69 accessions belong to four of five Brazilian regions (Supplementary 1) and they were classified into four species: *Capsicum chinense*, *Capsicum frutescens*, *Capsicum baccatum* var. *pendulum* and *Capsicum annuum*, according to the description proposed for the genus (Moscone et al., 2007).

For planting, three seeds from each genotype were sown in polystyrene trays with 128 cells, filled with commercial substrate. The germination of seedlings was carried out in protected conditions, in a greenhouse, with no control of humidity and temperature. The plants were irrigated once a day, in the morning. The plants were transferred to the field at the stage of four to six pairs of fully developed leaves, the seedlings were planted in in a randomized complete block scheme with three replications, totaling 207 experimental plots.

The space between rows was 1.0 m and 0.5 m between plants. After thinning only one seedling of each access was maintained in final stand.

To support the calculation of the need for fertilization in the experimental area, the soil of the experimental site was submitted to physicochemical and granulometric analysis. On this basis, a post-fertilization with 3 g of NPK 25-00-20 (Heringer SA, Manhuaçu, MG, Brazil) was applied 30 days after planting. The cultivation practices were the same as those recommended for peppers in Brazil, according to Filgueira (Filgueira et al., 2012), and irrigation was based on the daily needs of the plants.

Morphoagronomic characterization

The 69 genotypes were characterized using 11 morphoagronomic variables specific to *Capsicum*, following the descriptors for the genus proposed by Bioversity International (IPGRI, 1995), in which four are quantitative and eight qualitative. The four quantitative variables measured on samples of ripe fruit were: Fruit length (FL – cm), measured using a digital caliper in the longitudinal region of five fruits per accession; Fruit diameter (FD – mm), measured using a digital caliper in the equatorial region of five fruits per accession; Fresh fruit mass (FFM – g), determined using a precision balance by weighing five fruits; Pericarp thickness (PT – mm), determined by measuring the greatest thickness of the pulp after the cross-section of five fruits using a digital caliper. The eight qualitative variables associated with the fruit were indicated by rating scales based on the descriptors in Table 1. The presence of capsaicin in fruits was determined using Derera's method (Derera et al., 2000), which consisted of immersing a sample of fruit placenta (about 1 cm^2) in a 3 mL ammonium vanadate solution for 15 hours. The occurrence of brown patches on the placenta after this period indicated the presence of capsaicin.

Table 1. Eight qualitative descriptors used for phenotyping of 69 *Capsicum* accessions according to IPGRI (1995).

Descriptor	Phenotype
Fruit color at intermediate Stage	1 = White; 2 = Yellow; 3 = Green; 4 = Orange; 5 = Purple; 6 = Deep purple; 7 = Other;
Fruit color at mature stage	1 = White; 2 = Lemon-yellow; 3 = Light orange yellow; 4 = Orange-yellow; 5 = Light orange; 6 = Orange; 7 = Light red; 8 = Red; 9 = Dark red; 10 = Purple; 11 = Brown; 12 = Black; 13 = Other;
Fruit shape	1 = Elongate; 2 = Almost Round; 3 = Triangular; 4 = Campanulate; 5 = Block; 6 = Other
Fruit surface	1 = Smooth; 2 = Semiwrinkled; 3 = Wrinkled;
Fruit shape at blossom end	1 = Pointy; 2 = Blunt; 3 = Sunken; 4 = Pointed and sunken; 5 = Other;
Fruit shape at pedicel attachment	1= Acute; 2 = Obtuse; 3 = Truncate; 4 = Cordate; 5 = Lobate.
Fruit cross-sectional corrugation	3 = Slightly corrugated; 5 = Intermediate; 7 = Corrugated;
Capsaicin	0 = Absente; 1 = Present.

Biochemical characterization

Using 150 g of fruit at the mature stage collected from the 69 *Capsicum* accessions studied, biochemical analyzes were performed in the Genetics and Molecular Biology and Applied Chemistry Laboratories at the Ifes Campus de Alegre. The fruits were crushed using a blender and packed in plastic bags. Based on the fruit extract, the bromatogical parameters were evaluated: pH, soluble solids content (SSC – °Brix), total titratable acidity (TTA – %), moisture (MC – %) and ash content (AC – %).

The pH of the fruits was determined using a pH meter (MS Tecnopon, Piracicaba, SP, Brazil). For this, the pH meter was calibrated with a solution of pH 7 and then to pH 4. Then, an aliquot of 1 mL of juice from the initial extract was removed and added to a beaker with 20 mL of distilled water for dilution. The contents were stirred until the particles were uniformly suspended for later pH reading with the previously calibrated device. For each sample examined, the apparatus was washed with distilled water. The total soluble solids content was measured in a digital refractometer, according to the methodology described by Mattos et al. (2007).

To determine the total titratable acidity, the sample was titrated with 0.1 N sodium hydroxide solution (NaOH). 1 mL of juice was pipetted, and 20 mL of distilled water was added. Three drops of 1% phenolphthalein were added to this solution and the titration was obtained with the aid of a 25 mL burette containing NaOH. The titration with NaOH was carried out until pH 8.2, in which all citric acid, an organic acid predominant in peppers, is considered to have been titrated. The results were expressed in percentage of citric acid, obtained according to the methodology proposed by Mattos et al. (2007).

For the determination of moisture, the gravimetric method with the use of heat was used, with the aid of an oven set at 105°C, weighing from 2 to 10 g of the pepper sample in porcelain capsules. The capsules were submitted, in advance, to the oven to remove all moisture and taken to the desiccator with an interior relative humidity of around 0%. The capsules with the samples properly weighed were submitted to an oven at 105°C until constant weight (approximately 24 hours). After this procedure, the percentage moisture (MC %) was obtained.

The ash content was determined by incinerating the sample in a muffle furnace at 550°C for five hours, until light ash was obtained. Subsequently, the capsules and samples were placed in a desiccator to cool and then weighed, and the results expressed in percentage (AC %).

Data analysis

The qualitative descriptors were subjected to descriptive statistical analysis and the most frequent phenotypic class (mode) was used to define the code of each descriptor of each selected plant while the quantitative descriptors (Ph, SSC, TA, SSC/TA, MC, AC, FL, FD, FFM and PT) were subjected to analysis of variance (ANOVA) and the means with significant differences were grouped with the aid of the Scott-Knott test ($p < 0.05$). The software GENES (Cruz et al., 2013) was used to perform statistical analyses. Pearson's correlation analysis – which was based on the average of replicates between pairwise combined traits – and PCA were performed using Rstudio (R Core Team, 2023) using the Corrplot package (Wei et al., 2023). The adequacy of the correlation structure was evaluated using the Kaiser–Meyer–Olkin criterion (KMO) method and Bartlett test (Cerny et al., 1977).

RESULTS

Phenotypic Variation and Biochemical Attributes

In the basis of the descriptive analysis, we can observe a great variation in the qualitative traits related to the fruits (Figure 1). In terms of fruit color at the intermediate stage, 53 accessions showed orange fruits (78.2%), seven accessions showed yellow fruit (10.3%), five accessions showed fruit with green coloration (7.2%), while two accessions showed dark purple fruit (2.9%) and only one showed red color (1.4%). At the mature stage, 30 accessions showed red fruits (43.5%), 24 accessions showed dark red fruits (34.8%), four accessions showed orange fruits (5.8%), three accessions showed light orange fruits (4.3%), three showed light red fruits (4.3%), two accessions showed lemon-yellow fruits (2.9%), and two accessions showed orange-yellow fruits (2.9%). The shape of the fruits also varied, with the triangular and elongated shapes being the most common. Twenty-eight accessions had the triangular shape (39.1%), and 26 accessions had the elongated shape, corresponding to 39.1% and 36.2% of the 69 accessions evaluated, respectively. The other accessions were block-shaped (11.6%), round-shaped (8.7%), and bell-shaped (4.4%) (Supplementary 2).

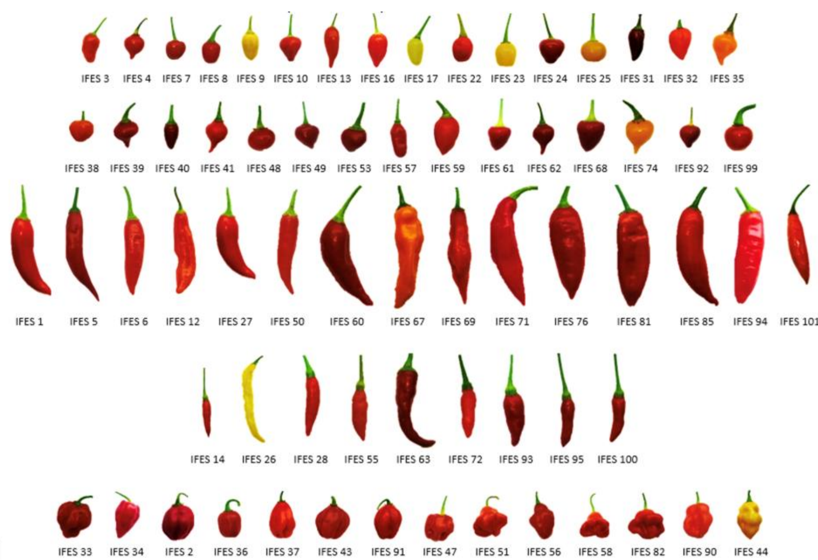

Figure 1. Phenotypic representation of fruits from 69 accessions of *Capsicum*.

Regarding the surface of the fruits, most of the studied accessions had a smooth surface (39 accessions - 56.6%); the semi-wrinkled surface was observed in 29 accessions (42%), followed by the wrinkled surface, which was present in only one accession (1.4%). Regarding the fruit shape at the blossom end, most of the fruits presented a pointed shape, a characteristic observed in 39 accessions, *i.e.*, 65% of the accessions studied. The sunken shape at the tip of the fruits was observed in 18 accessions (26.1%), followed by the blunt shape, present in ten accessions (14.5%). The pointy and sunken format was observed only in two accessions (2.9%) (Supplementary 2).

Analyzing the fruit shape at the pedicel attachment, we found that the obtuse shape was the most common (55.1%) and was observed in 38 of the 69 accessions studied, followed by the blunt shape, which was observed in 14 accessions (20.3%). The chordate form was observed in 8 accessions (11.6%), followed by the acute form (10.1%) and lobate form (2.9%), which were observed in eight and two accessions, respectively (Supplementary 2).

In terms of the fruit cross-section corrugation, more than half of the accessions – 55% – were classified as slightly corrugated (38 accessions). In the other accessions, the fruits were classified as corrugated and intermediate, in the order of 23.2% and 21.7%, respectively. Regarding pungency, the presence of capsaicin was detected in fifty-two of the sixty-nine evaluated accessions, representing 75.4%. The remaining accessions were classified as non-pungent, *i.e.*, seventeen accessions (24.6%) (Supplementary 2).

For the six biochemical parameters and the four morphoagronomic traits evaluated, ANOVA detected a highly significant difference (P < 0.01) between the genotypes studied, except for pH and FD in *Capsicum annuum*, which were significant at the 5% probability level (P < 0.05) (Table 2).

Table 2. Analysis of variance of the six biochemical and four quantitative descriptors studied for the four *Capsicum* species of the Ifes Campus de Alegre Germplasm Collection.

SV	DF	pH	SSC (°Brix)	TA (%)	SSC/TA	MC (%)	AC (%)	FL (cm)	FD (mm)	FFM (g)	PT (mm)
		***Capsicum chinense* — Mean Squares**									
Blocks	2	0.009	0.727	0.010	1.331	0.143	0.004	0.033	4.4564	0.334	0.095
Genotype	36	0.164**	18.701**	0.236**	89.169**	71.438**	0.933**	7.352**	231.483**	52.517**	1.024**
Error	72	0.005	0.244	0.006	0.754	0.763	0.014	0.025	1.631	0.066	0.028
Mean		5.34	8.27	0.63	14.80	84.15	1.16	2.94	16.21	4.13	1.85
CV (%)		1.31	5.98	12.20	5.87	1.04	10.34	5.34	7.88	6.21	8.99
		***Capsicum baccatum* var. *pendulum* — Mean Squares**									
SV	**DF**	**pH**	**SSC (°Brix)**	**TA (%)**	**SSC/TA**	**MC (%)**	**AC (%)**	**FL (cm)**	**FD (mm)**	**FFM (g)**	**PT (mm)**
Blocks	2	0.005	0.007	0.001	0.396	0.177	0.021	0.059	6.851	12.450	0.037
Genotype	22	0.210**	10.574**	0.189**	118.895**	5.915**	0.435**	21.073**	451.519**	119.029**	1.080**
Error	44	0.001	0.061	0.002	0.886	0.441	0.017	1.003	2.174	7.884	0.045
Mean		5.46	9.12	0.56	18.51	84.93	1.08	6.22	23.22	9.53	2.2
CV (%)		0.62	2.71	7.2	5.09	0.78	12.17	16.11	6.34	29.46	9.68
		***Capsicum frutescens* — Mean Squares**									
SV	**DF**	**pH**	**SSC (°Brix)**	**TA (%)**	**SSC/TA**	**MC (%)**	**AC (%)**	**FL (cm)**	**FD (mm)**	**FFM (g)**	**PT (mm)**
Blocks	2	0.001	0.074	0.001	3.832	0.1291	0.002	0.002	0.010	0.001	0.001
Genotype	6	0.324**	101.381**	0.141**	310.110**	339.079**	1.279**	0.800**	2.925**	0.072**	0.093**
Error	12	0.002	0.042	0.001	3.387	0.501	0.037	0.013	0.046	0.001	0.001
Mean		5.45	12.47	0.63	20.75	69.29	2.42	2.75	6.22	0.58	0.58
CV (%)		0.82	1.64	4.88	8.87	1.02	7.95	4.09	3.46	6.01	5.96
		***Capsicum annuum* var. *annuum* and *Capsicum annuum* var. *glabriusculum*—Mean Squares**									
SV	**DF**	**pH**	**SSC (°Brix)**	**TA (%)**	**SSC/TA**	**MC (%)**	**AC (%)**	**FL (cm)**	**FD (mm)**	**FFM (g)**	**PT (mm)**
Blocks	2	0.002	0.013	0.001	0.004	0.011	0.001	0.007	0.012	0.002	0.001
Genotype	1	0.052*	0.882**	0.099**	20.424**	24.523**	0.020**	4.335**	1.561*	0.328**	0.602**
Error	2	0.002	0.003	0.001	0.004	0.001	0.000	0.004	0.020	0.002	0.002
Mean		5.61	6.68	0.85	8.43	84.36	0.83	3.91	12.75	2.4	1.5
CV (%)		0.81	0.81	3.22	0.77	0.03	0.85	1.66	1.10	1.61	2.58

SV—source of variation; DF—degrees of freedom; CV—coefficient of variation; pH—hydrogenonic potential; SSC—soluble solids content; TA—total titratable acidity; SSC/TA— ratio of soluble solids content to total titratable acidity; MC— moisture content; AC — ash content; FL— fruit length; FD—fruit diameter; FFM— fruit fresh mass; PT—pericarp thickness. **, *—significant at 1% and 5%, respectively, in the F test.

Regarding the biochemical parameters, the pH ranged from 4.69 to 5.94, with the lowest values being reached by accessions IFES 06 and IFES 101 – both *Capsicum baccatum* var. *pendulum*) and the largest by accessions IFES 67 (*Capsicum baccatum* var. *pendulum*), IFES 14 (*Capsicum frutescens*) and IFES 49 (*Capsicum chinense*). As for the total soluble solids content (SSC – °Brix), the values were between 5.0 and 24.3, and the accessions IFES 91, IFES 48, and IFES 53 – *C. chinense* species – were the lowest averages. The accession IFES 28 (*C. frutescens*) was the one with the highest value for the characteristic. Regarding the total titratable acidity (TA – %), which varied from 0.25 to 1.60, the accessions classified with the lowest averages belonged to three of the five species studied, namely: *C. frutescens* (IFES 100), *C. chinense* (IFES 61, IFES 91, IFES 02, IFES 47 and IFES 56), *Capsicum baccatum* var. *pendulum* (IFES 58, IFES 94, IFES 71, IFES 82 and IFES 85). The accession with the highest value for this characteristic was IFES 44, of the *C. chinense* species (Suplementary 3).

The ratio between soluble solids content and titratable acidity (SSC/TA) ranged from 4.58 (IFES 44 – *C. chinense*) to 41.58 (IFES 28 – *C. frutescens*). As for the moisture content (MC), the values ranged between 62.84 and 92.46. The accessions IFES 14, IFES 28, IFES 93 and IFES 100 – belonging to the species *C. frutescens* – were those with the lowest values for this characteristic (62.94, 63.37, 63.49 and 63.89%, in that order). The accessions IFES 72 (*C. frutescens*), IFES 37, IFES 43 and IFES 33 – all *C. chinense* – were the ones that stood out, with values of 92.46, 90.78, 90.19 and 90.18%, in that order. For the ash content, the values presented by the accessions varied between 0.43 and 3.50. The accessions with the lowest values for the traits were IFES 48, IFES 71, IFES 47, IFES 60, IFES 25, IFES 33, IFES 91, IFES 58, IFES 43, IFES 36, IFES 56 and IFES 37, with averages ranging from 0.43 and 0.70. The accessions IFES 95, IFES 14, IFES 93 and IFES 99 were those with the highest values for characteristic in the order of 3.5, 2.88, 2.64, 2.58 % (Suplementary 3).

There were also significant statistical differences among genotypes in quantitative traits. Fruit length (FL) ranged from 0.92 to 12.79 cm for accessions IFES 48 (*C. chinense*) and IFES 67 (*C. baccatum* var. *pendulum*). In addition to accession IFES 48, ten other accessions were grouped according to the Scott-Knott method as those with the lowest measures for the trait and were classified into a single group as follows: IFES 72, IFES 53, IFES 25, IFES 23, IFES 08, IFES 40, IFES 07, IFES 99, IFES 22, IFES 17, and IFES 48, of which only accession IFES 72 belongs to the species *C. frutescens*, the others to *C. chinense* (Suplementary 3).

Fruit diameter (FD) had values ranging from 4.64 to 50.76 mm. Accessions IFES 72 (4.54 cm), IFES 14 (5.59 cm), IFES 100 (5.93 cm), IFES 28 (6.25 cm), and IFES 95 (6.29 cm) of the species *C. frutescens* had the lowest values for this trait, in that order. On the other hand, accession IFES 61 (*C. chinense*) was the only one that had the highest mean value for FD (50.76 cm) in the ranking of genotypes evaluated. Based on the classification of accessions, genotypes IFES 58, IFES 82, and IFES 82 – from the species *C. baccatum* var. *pendulum* – were those with the second highest mean in the test, in the order 46.27 mm, 46.26 mm, and 46.17 mm, respectively (Supplementary 3).

The fresh fruit mass (FFM), with values ranging from 0.38 g (IFES 100 - *C. frutescens*) to 22.33 g (IFES 67 - *C. baccatum var*. *pendulum*). However, within this range, more than half of the accessions, along with IFES 100, had the lowest values for the trait. It is also noted that of the four accessions with the highest value for FFM (IFES 67, IFES 82, IFES 51, IFES 58), two of the accessions matched the highest average value for FD (IFES 82 and 51 - of *C. baccatum* var. *pendulum*) (Supplementary 3).

Regarding the thickness of the pericarp (PT), it was found that the values for the traits ranged from 0.37 to 3.57 mm. The accessions with the lowest values were IFES 28

(0.66 cm), IFES 93 (0.65 cm), IFES 55 (0.65 cm), IFES 100 (0.45 cm), IFES 95 (0.39 cm), and IFES 14 (0.37 cm), from the species *C. frutescens*. The highest values were IFES 10 (3.56 cm), IFES 92 (3.52 cm), IFES 38 (3.30 cm), IFES 24 (3.28 cm), IFES 16 (3.28 cm), and IFES 51 (2.94 cm), of which only IFES 92 and IFES 38 belong to *C. chinense*, the others being *C. baccatum* var. *pendulum* (Supplementary 3).

Correlation between morphoagronomic and biochemical characteristics

Pearson's correlation analysis revealed that there were significant positive and negative correlations between the assessed traits (Figure 2). In general, there were mostly positive correlations between the physical characteristics of the fruits, ranging from 0.53 to 0.82. For example, FFM was positively correlated with FL (0.56), PT (0.53), and FD (0.82), while FD and PT had a positive correlation on the order of 0.62.

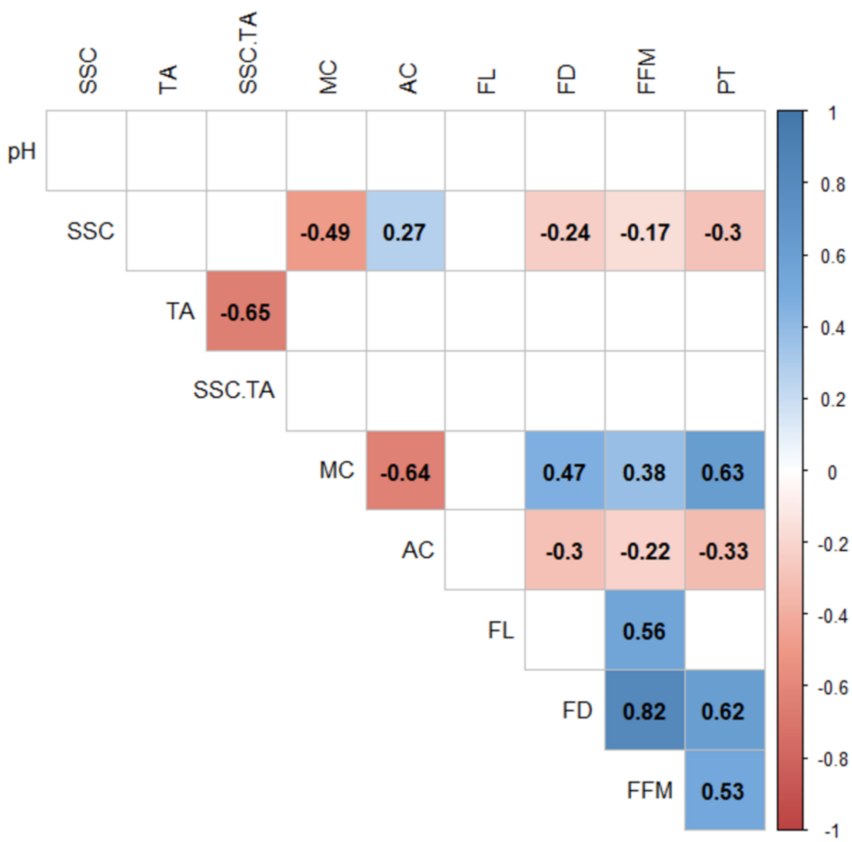

Figure 2. Pearson's correlation coefficient between physicochemical and morphoagronomic traits measured on 69 *Capsicum* accessions. Blue and red squares indicate positive and negative correlations, respectively. Empty squares indicate no significant correlation. On the right side of the correlation graph, the color of the legend indicates the correlation coefficients and the corresponding colors. pH—hydrogenonic potential; SSC—soluble solids content; TA—total titratable acidity; SSC/TA— ratio of soluble solids content to total titratable acidity; MC— moisture content; AC — ash content; FL— fruit length; FD—fruit diameter; FFM— fruit fresh mass; PT—pericarp thickness.

It is noted that between the morphoagronomic and physicochemical characteristics there was a predominance of negative correlations, even in lower magnitudes than the positive ones presented between MC and FD (0.47), FFM (0.38) and PT (0.63). In terms of negative correlations, the results show that SSC is negatively correlated with FD (-0.24), FFM (-0.17) and PT (-0.30). Similarly, AC was also negatively correlated with these characteristics, in the order of -0.30, -0.22 and -0.33. Among the physicochemical characteristics, negative correlations predominated. MC was negatively correlated with SSC (-0.49) and AC (-0.64), while AC was positively correlated with SSC (0.27).

Principal component analysis

The value obtained by the KMO statistic was 0.56. In addition, the Bartlett sphericity test was statistically significant ($p < 0.01$), indicating that the correlation matrix does not have a diagonal structure. Principal Component Analysis (PCA) indicated that the loads of the ten measured traits contributed significantly to the principal components (PCs), highlighting their relevance in determining the extent of variability among the 69 *Capsicum* accessions evaluated. The first three PCs (PC1–PC3) explained 67.20% of the total variance in the data. The scree plot of explained variance across the ten PCs showed that there was a large decrease in explained variance between PC1 and PC2, but a smaller decrease in explained variance from PC3 onwards (Figure 3).

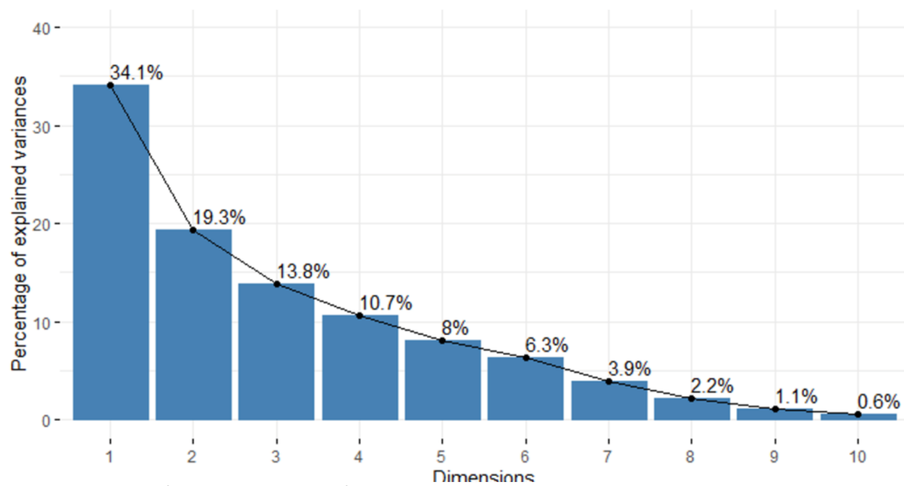

Figure 3. Scree plot of the percentage of variance explained by the 10 principal components in principal component analysis for the 69 genotypes of *Capsicum.*

In terms of the contribution of the characteristics to the variation of the data, it appears that for the first three PCs, the variables SSC/TA, TA, FFM, SSC, MC and FD were the ones that generated the most variation in the data, allowing the distinction between the accesses studied. On the other hand, the variables PT, pH, AC and FL present contributions below the average contribution, being of little relevance for distinguishing the evaluated genotypes (Figure 4).

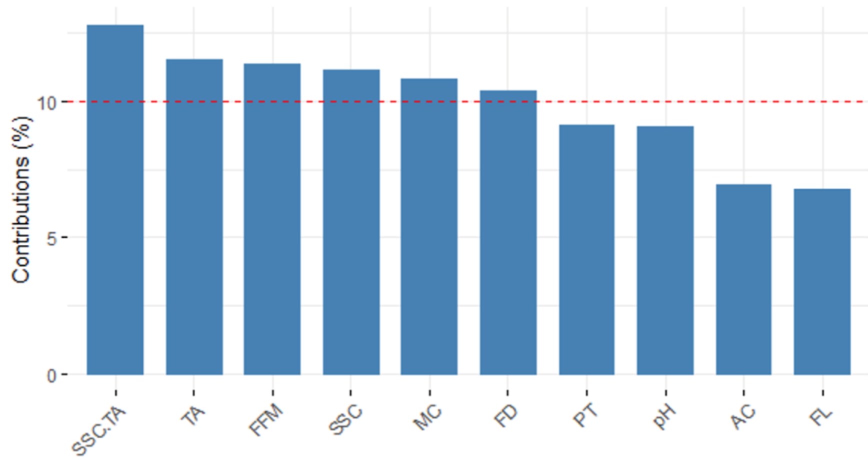

Figure 4. Percentage contributions of the 10 measured morpho-agronomic and biochemical traits to the first three components of the principal component analysis. pH—hydrogenonic potential; SSC—soluble solids content; TA—total titratable acidity; SSC/TA— ratio of soluble solids content to total titratable acidity; MC— moisture content; AC — ash content; FL— fruit length; FD—fruit diameter; FFM— fruit fresh mass; PT—pericarp thickness. The red dashed line indicates the overall mean contribution of the characteristics to PC1, PC2 and PC3.

In general, it was noted that the biochemical traits present a greater contribution to the observed variation when compared to the quantitative morphoagronomic characteristics. The cosine squared (*cos*^{2}) value for individual factors determined that various pepper genotypes contributed strongly to the variation in the first two main components – which together capitalized 53.4% of the observed variation. The IFES 28, IFES 93, IFES 95, IFES 58 and IFES 51 genotypes were the most prominent. On the other hand, the accessions with the lowest contribution to the variation were IFES 101, IFES 26, IFES 61, IFES 13 and IFES 04 (Figure 5).

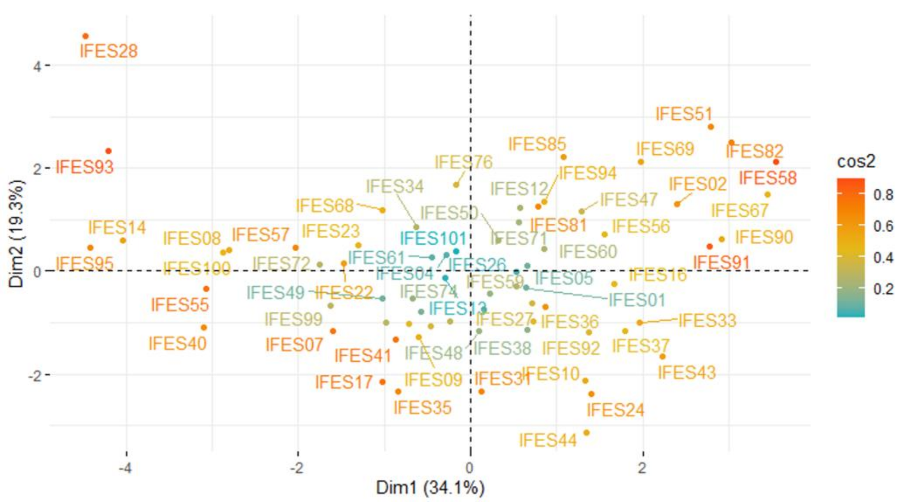

Figure 5. Contributions of 69 *Capsicum* accessions for the variation presented by the 10 measured morpho-agronomic and biochemical traits to the first two components in principal component analysis.

DISCUSSION

The variation in biochemical components

As far as plant genetic resources are concerned, the acquisition, characterization, and conservation of germplasm banks are strategies to explore and expand the genetic variability of acquired accessions and are necessary to reduce the impact of anthropogenic interventions on genetic heritage. In this sense, the creation and conservation of germplasm collections are extremely important to reduce the loss of genetic variability among commercially important species – such as *Capsicum* – that, when more exploited and domesticated, tend to suffer from genetic erosion (Hayano-Kanashiro et al., 2016). In addition, germplasm characterization studies have great potential to develop strategies with great potential for economic impact. Considering that Brazil is a secondary center for diversification of the genus, there is a broad variety of types, sizes, colors, and flavors all over the country, allowing peppers to be exploited by different market niches (Acunha et al., 2017). However, there is an increasing demand for new pepper genotypes with tastier fruits and more attractive colors that have the necessary pungency for their intended uses and contain higher concentrations of bioactive components that are important for human health.

In view of this, breeding programs face the growing challenge of developing breeding strategies for the increase in *Capsicum* fruits of important substances, such as sugars, antioxidants, and other components such as carotenoids and polyphenols, to expand opportunities for reach new market segments. For these strategies to become effective, it is necessary to fill the gap that exists regarding the conduction of studies of biochemical characterization of peppers of the genus *Capsicum*. In this sense, this study sought to conduct the biochemical and morphoagronomic characterization of fruits from 69 accessions of *Capsicum* from four Brazilian regions, to contribute to the knowledge of the diversity among these accessions and to provide information that can support the improvement of peppers for increment of important bioactive compounds.

In terms of phenotypic diversity, the accessions studied showed great variation for the qualitative attributes evaluated, such as fruit color at distinct stages of maturation, shape, corrugation, fruit base and tip shapes and pungency level. Among the qualitative descriptors, the color of the fruit is one of the most important attributes for the commercialization of products derived from *Capsicum*. Our results show that of the sixty-nine accessions studied, about 78% presented a red coloration in the ripe fruits. The red color of pepper fruits is an important quality attribute for the processed and dehydrated raw material, as it adds value to the final product that is used by industries in the production of spices, paprika, sauces, preserves, jellies and dyes.

Pungency is also a characteristic that has great appeal for the *Capsicum* market and, in addition to being quite variable, it is directly associated with the type or group of peppers (Ribeiro et al., 2020). The botanical variety *pendulum* is represented by the morphotypes "dedo-de-moça" and "cambuci". The "dedo-de-moça" peppers have elongated and pungent fruits, while the "cambuci" peppers have fruits shaped like a bell or a jar, and are also known as "bishop's hat", or "frade's hat" and are not pungent (Martinez, 2017).

The physicochemical characteristics related to the flavor, texture and nutritional value of peppers are important attributes for the use and commercialization of fruit pulp in the elaboration of industrialized products. Lower pH values help in the conservation of the fruit after harvest, being an important characteristic related to the durability of the fruit, since the pH is a determinant characteristic in the growth potential of microorganisms capable of causing deterioration, as well as of the growth of pathogenic microorganisms (Chitarra et al., 2005; Sousa et al., 2007). In this sense, for pH, the accessions IFES 06 and IFES 101 stand out, both species *Capsicum baccatum* var. *pendulum*, with the lowest values for this trait (Supplementary 3).

The soluble solids content (SSC) is extremely important in fruits, whether for fresh consumption or for industrial processing. According to Veiga et al. (2020) the sugar content normally constitutes about 85% of the soluble solids content. Thus, for fresh consumption, it represents fruits with greater palatability, due to the degree of sweetness. The SSC comprise sugars derived from degradation of polysaccharides during ripening, and during the processing in industry, elevated levels of these constituents in the fruits mean a reduction in the addition of sugars, a shorter water evaporation time, a reduction in energy expenditure in processing, in addition to higher product yield. In turn, the titratable acidity (TA) indicates the amount of organic acids present in fruits and the level of astringency, being the main influence on the flavor of the fruits (Borges et al., 2015) The lower the titratable acidity in the fruit, the better its conservation status, which directly reflects on the quality of a final product for consumption (Sousa et al., 2007).

Based on this information and the results obtained, among the studied accessions, IFES 28 has the highest value for SSC and the fourth lowest value for TA according to the Scott-Knott mean test. Among the studied genotypes, the accessions IFES 100, IFES 61, IFES 91, IFES 02, IFES 47 and IFES 56 stand out for TA, as they presented the lowest values for this trait and being among the eight lowest values for TA. Higher TA values might be associated with the fast process of food decomposition, which may involve hydrolysis, oxidation, or fermentation (Sousa et al., 2007). However, when analyzing the SSC/TA ratio, the IFES 28 accession stands out with a value of 41.58 for this characteristic. This ratio is used as an indicator of palatability, and its increase may be associated with a more pleasant taste (Santos et al., 2009; Soethe et al., 2016) (Supplementary 3).

In this sense, even with the less expressive value of the accession IFES 28 for TA, this accession proves to be an excellent option for the *Capsicum* breeding program to obtain more palatable fruits suitable for fresh consumption. In addition, the species *C. frutescens*, known in Brazil by the morphotype “malagueta” or “chili pepper”, is widely used for the preparation of sauces because of its high pungency and is therefore of great value. Previous studies have reported a variety of bioactive phytochemicals, including flavonoids, carotenoids, phenolic compounds, and other antioxidant compounds (Olatunji et al., 2019), which have been shown to reduce the risk of certain cancers and cardiovascular diseases (Prior et al., 2000; Kaur et al., 2001; Topuz et al., 2007; Zimmer et al., 2012).

Water is the most abundant constituent in *Capsicum* fruits, and this water content has a major influence on the quality of the fruits after harvest, during the stages of storage and processing, and on the fate of the fruits. In the sixty-nine accessions evaluated in this study, moisture content ranged from 62.84 to 92.46%, with about sixty-three accessions having a value between 70 and 92%. Pepper fruits often need to be dehydrated for commercial purposes, especially when they are intended to produce spice powders, so low water contents are appreciated (González-López et al., 2021; Tripodi et al., 2019). In this sense, accessions IFES 14, IFES 28, IFES 93 and IFES 100 – of *C. frutescens* species –

were those with water content below 65%, *i.e*., dry matter content above 35%. This can be seen from the ash content, where accessions IFES 14 and IFES 93 had the second and third highest values for this trait, respectively (Supplementary 3). Ash content provides more direct information about the inorganic residues remaining in the samples after organic matter combustion and is used to analyze specific minerals in the fruit. High values of this substance, in addition to indirectly inferring moisture content, can predict the possibility of greater yield for the industry in terms of the amount of processed product and lower values, in turn, indicate greater juiciness, a factor desirable for fresh consumption (Fagundes et al., 2001).

The morphoagronomic variation among *Capsicum* genotypes

The variability between accessions found using the qualitative descriptors was confirmed by the wide variation between the accessions and the quantitative descriptors related to the fruits. In Brazil, pepper is widely distributed and used in a wide variety of ways. In this sense, consumers have a wide acceptance and use the fruit for a variety of purposes that vary according to the size, shape, color, and pungency of the fruit.

For the fruit length (FL) trait, a variation from 0.92 cm (IFES 48) to 12.79 cm (IFES 67) was observed, while the variation for fruit diameter (FD) was between 4.64 mm (IFES 72) and 50.76 mm (IFES 90). These results are in line with the work of Cardoso et al. (2018) who, evaluating a collection of 116 *Capsicum* accession, found values for fruit length ranging from 0.93 to 13.64 cm and for fruit diameter from 4 mm to 59 mm. In this sense, the variation of these two traits is very important for the different uses in the pepper trade, since the longer fruits are usually destined for the food industry to produce paprika or for their use in cosmetics and pharmaceuticals, while the smaller fruits are usually destined for fresh consumption or to produce sauces (Leite et al., 2016).

For fruit fresh mass, the values showed a wide variation − between 0.38 (IFES 100) and 22.33 g (IFES 67). The highest values for this trait were exhibited by the fruits of the accessions of *C. baccatum* var. *pendulum* (Supplementary 3), which is typical of this species, which also includes the morphotypes 'dedo-de-moça' and 'cambuci', which fruits are valued for consumption in natura. The accessions belonging to the species *C. chinense* had the lowest values for this trait. This species is considered the most Brazilian of the domesticated *Capsicum* species and is characterized by the pungent aroma of its fruits. There are varieties of this species with extremely spicy fruits, such as the "habanero" pepper, very popular in Mexico. In Brazil, the most famous are the peppers "De Cheiro", "Bode", "Cumari do Pará" or "Cumari Amarela", "Murupi" and many others (Leite et al., 2016).

As for the thickness of the pericarp, the values ranged from 0.37 to 4.33 mm, with accessions IFES 10, IFES 92, IFES 38, IFES 24, IFES 16, and IFES 51 having the highest values (Supplementary 3). Fruits with a thicker pericarp are more valuable for the fresh market because they have a longest post-harvest durability and a more attractive appearance to customers and are more resistant to disease and parasites (Abud et al., 2018). In general, significant differences were found in all quantitative morphoagronomic traits, indicating a large genetic variation among accessions.

Correlation between biochemical and morphoagronomic parameters

The selection process to achieve genetic gains in plant breeding can lead to an increase in program costs as well as labor, depending on the number of traits analyzed. In this sense, correlation coefficients allow to make inferences about the effects of direct and indirect selection (Santana et al. 2021). In this regard, the Pearson (1920) correlation coefficient was used in this study, which is a measure of a linear relationship between two quantitative traits, where one trait can be chosen based on the other.

From the results, it can be inferred that fruits with larger diameter, thicker pericarp and higher mass have higher water content, which increases the fresh mass of the fruit. However, this increase in fresh mass implies a possible decrease in total soluble solids content, which is directly correlated with water content (-0.49) and indirectly correlated with more discrete values with fruit diameter (-0.24), fresh mass (-0.17) and pericarp thickness (-0.30). González-López et al. (2021) also reported a negative correlation between total soluble solids content and fruit moisture content of 100 *Capsicum* accessions native to the Andean region. According to the authors, the increase in traits related to fruit size and mass may have a negative effect on SSC.

Since *Capsicum* fruits are widely used in different sectors of the food industry, it is possible to promote improvements in traits depending on the objective of the breeding program's goal. In this scenario, it can be inferred from the results that smaller accessions should be used to obtain fruits with reduced water content – for example, to produce powdered condiments. For fresh fruit, indirect selection of larger fruits may result in high water content. However, selection based on fruit size traits such as diameter and length must be considered, as results show a negative association between these characteristics and the total soluble solids content, which is relatively more important.

The principal component analysis

By converting the initial traits into a smaller set of traits – the principal components – PCA reduces the dimensionality of the data, thus facilitating the selection process by reducing the set of features (Ahmed et al., 2021). Along this line, of the 10 characteristics studied, six stood out in terms of their contribution to discrimination of the accessions studied (SSC/TA, TA, FFM, SSC, MC, and FD) (Figure 4).

When characterizing *Capsicum* germplasm, characteristics associated with fruit always stand out as those that contribute most to variation among accessions, including fruit diameter. One possible justification for the greater contribution of fruit-related traits to diversity in several studies of *Capsicum* (Silva et al., 2013; Bianchi et al., 2016; Santos et al., 2019; Bianchi et al., 2020; Brilhante et al., 2021) is that the fruit is the part consumed by humans and birds and is responsible for the dispersal of pepper seeds. This leads to the hypothesis that selection and domestication processes were based organ, which has a variety of colors, shapes, sizes, pungency, flavors, and chemical compounds (Paran et al., 2007). Although the contributions of the hard-to-measure traits predominate in our work, the contribution of FFM and FD − which can be determined more quickly and with less work − should be emphasized.

The squared cosine (cos^2) indicates the importance of a genotype for a given analyzed component. The greater the distance between the center of origin of the graph and a genotype, the greater the value of $\cos^2$, *i.e.*, the greater the contribution of a genotype to the indicated PCs (Abdi et al., 2010). In general, only a few accessions presented small contribution to the variation found, showing the diversity among the evaluated genotypes (Figure 5). This fact could be related to the different origins of these materials, coming from four of the five Brazilian regions, highlighting the importance of the creation of this collection.

CONCLUSIONS

A high degree of variation was found for the qualitative, biochemical, and quantitative traits assessed in the 69 *Capsicum* accessions, demonstrating considerable diversity among the genotypes. The degree of variability found in the genotypes for all the traits make it possible to cater to various market niches for pepper consumption. Through the Pearson's correlation it was possible to observe that larger fruits with high fresh mass weight can result in direct genetic gains in water content and indirect gains in the soluble solids content, making the breeding process less time-consuming through indirect selection via easy-to-measure traits. The PCA showed that the observed variation among the 69 accessions were mostly caused by the traits diameter of the fruit and fresh fruit mass. Taken together, the results provided by the correlation analysis between traits and their contribution to genetic divergence among accession are essential for pepper breeding designed to increase fruit quality as it determines ts can be selected and the best way to transfer important attributes to varieties of commercial interest.

The accession IFES 28 (*C. frutescens*), standing out for its high values of SSC and SSC/TA and low water content, is a good candidate to be used in breeding programs aiming the production of peppers to be consumed fresh, due to its high palatability and its high post-harvesting durability. For consumptionin thr form of sauces and condiments, we highlight the accession IFES 48 (*C. chinense*), due to its small fruit size, being highly accepted in this niche of the pepper market. For industrial consumption, such as in the production of cosmetics and condiments, large fruits such as those of the IFES 67 and IFES 90 accessions (*C. baccatum* var. *pendulum*) stand out and are the most used for these purposes.

This study will also allow the construction and establishment of a collection of a paramount importance for the conservation of pepper germplasm and will provide morpho-agronomic and biochemical data to facilitate the selection process in breeding programs.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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