Phenotypic and Molecular Characterization of Brazilian Capsicum Germplasm

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Abstract: Population growth and the high demand for food production has caused environmental degradation losses in biodiversity. The conservation of genetic resources is the most appropriate strategy for maintaining the variability of species of great importance, such as Capsicum. In this study, we describe the characterization of 69 pepper accessions of four Capsicum species from different regions of Brazil on the basis of qualitative and quantitative descriptors and ISSR markers. The 11 quantitative descriptors grouped the 69 populations into five clades. The fruit traits had the greater discrimination power. A strong correlation was detected between some pairs of quantitative descriptors: petiole and fruit length, fruit diameter and fresh fruit mass, leaf length and leaf width, canopy and plant height, stem diameter and plant height, stem diameter and canopy diameter. A negative correlation was also observed between plant height and pericarp thickness. Moreover, the molecular analysis grouped accessions into five clades. The morpho-agronomic and molecular characterization were efficient to estimate the genetic diversity between accessions, being important tools for the knowledge and use of accessions. This study is of great importance to preserve pepper germplasm and to provide data to facilitate the process of the selection in breeding programs.

Keywords: peppers; genetic resources; germplasm bank; molecular markers

1. Introduction

The genus Capsicum (Solanaceae) is home to peppers, a culture extremely relevant to Brazil and grown all over the world. The genus has about 42 described species [1]. Of these, five species are classified as domesticated: Capsicum annuum L., Capsicum baccatum L. var. pendulum, Capsicum pubescens Ruiz and Pav., Capsicum frutescens L. and Capsicum chinense Jacq. The other species are considered semi-domesticated and wild.

Pepper is considered to be one of the most important vegetables in the world. This is attributed to its versatility, since its use is used in the culinary, pharmaceutical, and cosmetics industry [2]. The most pungent varieties have great appeal in the markets of Southeast Asia, South America, and also in Africa, while sweet peppers and non-pungent varieties are more consumed in North America and Europe [3].

According to the latest data from FAO (Food and Agriculture Organization) [4], in 2019, around 38 million and 4.3 million tons of green and dry pepper were produced worldwide,
respectively, in a cultivated area of about 3.7 million hectares. Pepper consumption and production data in Brazil are still scarce, mainly because most peppers are produced by small producers and sold in popular markets and fairs. However, even though little is known about the pepper market in Brazil, they are part of the country’s wealth and culture and are a valuable asset of biodiversity [5]. A wide variety of types, names, size, colors, and flavor pungencies are found throughout Brazilian territory [6,7]. It is estimated that the country has the largest number of wild species, in addition to being considered an important center of diversity of domesticated species of the genus *Capsicum* [8,9].

With the growing global demand for food—a consequence of the increase in population—there is also an increase in the cultivated area and inadequate exploitation of the available genetic resources [10–12]. As a result, there is a great loss of biodiversity for many species, resulting from the process of genetic erosion. These anthropic actions require immediate actions to maintain the resources available for human survival. In this context, the United Nations 2030 Agenda [13] provides several guidelines for more sustainable production models that guarantee food security and the preservation of available genetic resources.

From this perspective, accessing existing genetic variability and creating measures to preserve existing genetic resources is one of the most important strategies for plant breeding programs [14,15]. This is because the success in improving a variety lies in the genetic diversity available in the studied germplasm. Therefore, in order to meet the demand of the consumer market with regard to the productivity and quality of *Capsicum*—an extremely important culture for Brazil—it is essential that breeders have access to the genetic diversity sheltered by the genus *Capsicum*. That is the reason why it is so important to implement measures to preserve local varieties, as well as studies on the collection and characterization of germplasm.

In order for one to make the accessions of a germplasm bank available for use in improving characteristics and launching new cultivars, it is essential that they be properly described [16,17]. In this sense, *Capsicum* germplasm characterization studies have been based on predictive methods that take into account agronomic, physiological, morphological, bromatological, and molecular descriptors [18–21]. The study of morphological characters associated with molecular markers has been used by many studies because they provide more accurate genetic information of the genotypes studied [22].

Many molecular markers are used to study *Capsicum* diversity, namely, RAPD (random amplification polymorphism DNA) [23–25], AFLP (amplified fragment length polymorphism) [26–28], SSR (simple sequence repeat) or microsatellites [29–32], and ISSR (inter simple sequence repeats) [8,33–36]. As the new generation sequencing techniques have become more accessible, new studies were carried out and were able to access the variations existing in *Capsicum* in greater depth. As an example, the study by D’Agostino et al. [37] determined the complete plastome nucleotide sequence of eight *Capsicum* species, allowing for the identification of sequence variations that enable the development of extremely useful and informative PCR-based markers to discriminate *Capsicum* species. More recently, Colonna et al. [38], in a study sequencing 1.8% of the genome of 11 commercially important *Capsicum* species from 51 countries, described the genomic variation in addition to finding loci associated with fruit-related phenotypes, which guarantees future studies with greater precision in the association of characteristics of interest for breeding programs.

The associated use of phenotypic characteristics to molecular studies of accessions generates more information about the diversity existing in a germplasm collection, which allows for significant advances in the identification of accessions with potential for breeding programs. In addition, it provides an overview of the species’ conservation status, ensuring that strategic measures are taken to conserve the species’ genetic resources. In this study, we describe the characterization of 69 *Capsicum* accessions from different regions of Brazil, performed using qualitative and quantitative characters and ISSR molecular markers. ISSR is one of the most widely used molecular markers that are reproducible, highly polymorphic, independent of environmental influence, cost-effective, and do not require
prior sequence knowledge [39]. The results obtained contribute to the knowledge of the potential and use of accessions in breeding programs, as well as to the conservation of pepper species, a culture of great importance in the country.

2. Materials and Methods

2.1. Plant Material, Experimental Conditions, and Phenotyping

Sixty-nine Capsicum accessions from the germplasm bank of the Instituto Federal do Espírito Santo (IFES)—Campus de Alegre, located in Alegre, ES, Brazil, were evaluated. Sixty-four accessions were from four Brazilian regions (North, Northeast, Midwest, and Southeast) and five were commercial varieties (IFES 09, IFES 51, IFES 99, IFES 100, and IFES 101) from Alegre, ES (Southeast). These accessions were characterized into four species (Capsicum chinense, Capsicum baccatum var. pendulum, Capsicum frutescens, and Capsicum annuum) according to the description of Moscone et al. [40] (Supplementary Materials—Table S1).

Three seeds of each accession were sown in a 128-cell polystyrene trays containing commercial substrate. Germination and seedling development were monitored daily, and irrigation was performed once a day, usually in the morning. There was no humidity and temperature control. The seedlings were kept in greenhouse conditions until transplanting to the field, at the stage of four to six pairs of definitive leaves. In the field, the plants were arranged at a spacing of 1.0 m between rows and 0.5 m between plants. By the occurrence of thinning, only one seedling of each accession was kept.

The experiment was carried out in a complete randomized block design with four replications, totaling 276 experimental plots in the experimental area of the same institution. Physico-chemical and granulometric analysis of the soil in the experimental area was performed to calculate the need for correctives and fertilizers. Covering fertilization was performed 30 days after planting; 3 g of the NPK 25-00-20 (Heringer S.A., Manhuaçu, MG, Brasil) formulation was used. All cultural treatments used followed the recommendations for the pepper culture. The irrigation shifts vary according to the plants needing at different stages of development.

The accessions were characterized on the basis of 33 specific morpho-agronomic traits (Tables S2 and S3) for Capsicum following Bioversity International descriptors for the genus [41]. Eleven descriptors were based on quantitative traits and 22 were based on qualitative traits. Among qualitative descriptors, the presence of capsaicin in fruits was evaluated on the basis of Derera’s [42] method, where a fruit placenta sample (approximately 1 cm²) was immersed in a solution of 3 mL of ammonium vanadate for 15 h. After this period, the presence of brown spots in the placenta indicated the presence of capsaicin.

2.2. Genotyping

Samples of young leaves from the accessions were collected in bulk for extraction of genomic DNA. The extraction followed the procedure described by Doyle and Doyle [43], with modifications proposed by Daher [44]. The integrity and quantification of the genomic DNA were verified via 0.8% agarose gel using the High DNA Mass Ladder marker (100 pb) (Invitrogen, Waltham, MA, USA).

On the basis of some studies published in the literature [45–48], we selected and tested 50 ISSR primers to determine optimal amplification reaction conditions. Of this amount, 17 ISSR primers were selected for the analysis of DNA amplification reactions in this study once they resulted in profiles of well-separated fragments. The ideal amplification temperature (Tm) and cycling time and discarded ISSR markers can be find at Table S4. The PCR reactions were carried out in a thermocycler (Veriti 384-well Thermal Cycler Applied Biosystems, Invitrogen, Waltham, MA, USA). The PCR reaction was carried out in a final 25 µL containing 30 ng of template DNA (3 µL), 13.3 µL of ultrapure water, 2.5 µL of buffer + 1 × PCR MgCl₂ (1.9 mM), 2 µL of dNTP mix (0.4 mM), 0.2 µL of Taq polymerase (1 U), and 4 µL of each primer (0.08 µM) (Celbio Biotec). The PCR reactions were conducted as follows: 5 min at 94 °C for initial denaturation, followed by 45 cycles of 5 min at 94 °C,
30 s at 46.6–52 °C, 3 min at 72 °C, and a final extension of 7 min at 72 °C. The amplified fragments were separated on a 2% agarose gel, stained with 4 µL of the TIV dye, and subjected to UV light from the Minibis Pro photocomputer (Bio-imaging System, Israel, Jerusalem), evaluating the most consistent bands (Figure S1).

2.3. Data Analysis

Qualitative descriptors were subjected to descriptive statistical analysis, while quantitative descriptors were subjected to analysis of variance (ANOVA) and means values with significant differences were grouped using the Scott-Knott test ($\alpha = 0.05$). The relative contribution of quantitative morpho-agronomic traits for diversity between accessions was obtained through the Singh \cite{49} method. The estimates of Pearson’s correlation coefficients were obtained on the basis of the average of repetitions between the traits combined two by two. The divergence among accessions was obtained by the average Euclidean distance on the basis of the 33 morpho-agronomic descriptors. The values of the morpho-agronomic traits were used for UPGMA (unweighted pair group method with arithmetic mean) clustering analysis in RStudio software \cite{50}. To evaluate the consistency of the clusters, we calculated the co-phenetic correlation coefficient \cite{51}. The significance of this coefficient was verified using the Student’s $t$-test at 5% probability. Statistical analyses were performed with the software GENES \cite{52}.

ISSR results were interpreted as presence or absence of bands and expressed in a binary matrix. The dissimilarity matrix between accessions based on molecular markers was obtained using the Jaccard index and grouping performed using the UPGMA method. The correlation between the dissimilarity matrices of the morpho-agronomic descriptors and ISSR markers was obtained with the aid of the Dendextend package \cite{53} in the RStudio software (version 4.0.4) \cite{50}.

3. Results

3.1. Morpho-Agronomic Characterization

The qualitative traits stem shape, stem pubescence, and calyx pigmentation traits were considered monomorphic and were classified as angular, sparse, and present, respectively (Table S5). The intermediate growth habit was found in 82.6% of the accessions, while 14.5% had an erect growth habit and 2.9% prostrate habit.

The shape of the leaves varied between ovate (49.3%), deltoid (44.9%), and lanceolate (5.8%). The nodal anthocyanin grouped the accessions into four classes: light purple (55.1%), green (24.7%), purple (14.5%), and dark purple (5.7%).

Analyzing flowers, 53.6% had an intermediate position, 43.5% erect, and 2.9% pendant. The number of flowers per axillary varied from one (36.2%), two and three (10.2%), or more (53.6%). The anthers showed a violet color for 66.7% of the accessions and yellow with a light blue spot for 33.3%. The corolla color grouped the accessions into four classes: greenish white (55.1%), white (34.8%), greenish yellow (8.7%), and purple (1.4%). Most of the accessions (68.1%) did not present spots on the corolla. In contrast, the remaining 31.9%, referring to accessions of $C. baccatum$ var. *pendulum* exhibited a greenish yellow corolla spot. The filament color varied between white (73.9%), yellow (23.3%), green (1.4%), and violet (1.4%). Analyzing the calyx margin, we found that 75.3% of accessions had intermediate margin, 18.9% dentate margin, and 5.8% entire margin.

Analyzing the calyx constriction, we found it was present only in $C. chinense$, which represents 53.6% of all the evaluated accessions.

The fruits presented wide phenotypic variation (Figure 1). Regarding to the color of the fruits, we observed that, in the intermediate stage, the fruits presented color ranging from orange (78.2%), yellow (10.3%), green (7.2%), dark purple (2.9%), and red (1.4%). In the mature stage, 43.5% of accessions had medium red fruits, 34.8% dark red, 5.8% orange, 4.3% pale orange, 4.3% light red, 2.9% lemon yellow, and 2.9% orange-yellow (Figure 1). There was also variation in the shape of the fruits—the triangular and elongated shapes.
being the ones that occurred most at 39.1% and 36.2%, respectively, followed by blocky (11.6%), round shape (8.7%), and campanulate (4.4%).

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Most fruits showed a smooth surface (56.6%), followed by semi wrinkled (42%) and wrinkled (1.4%). For the fruit shape at blossom end, we found that 52.2% were pointed, 27.5% sunken, 17.4% blunt, and 2.9% pointed and sunken. In the analysis of fruit shape at pedicel attachment, we observed that the obtuse shape was the one that most occurred (55.1%), followed by the truncate (20.3%), acute (10.1%), cordate (11.6%), and lobate (2.9%). Regarding the corrugation of the fruit, 38 accessions—55.1%—were grouped into the slightly corrugated class, followed by very corrugated (23.2%) and intermediate (21.7%). Capsaicin was present in 75.4% of accessions and absent in the remaining 24.6%.

Concerning quantitative traits, analysis of variance (ANOVA) resulted in the detection of significant phenotypic variation between the 69 accessions. In particular, great variability was observed in *Capsicum chinense* and *C. baccatum* var. *pendulum* accessions in terms of the 11 quantitative descriptors, with a significant difference between genotypes being detected by F test ($\alpha = 0.01$), except for the stem diameter (SD) and leaf length (LL), which exhibited differences at $p < 0.05$ (Table 1).
Table 1. Analysis of variance (ANOVA) of the 11 quantitative descriptors studied for each Capsicum species.

| SV       | DF | Capsicum chinense—Mean Squares | CV%       |
|----------|----|-------------------------------|-----------|
| Block 2  |    | PH  | CD   | SD  | LL  | LW  | PL  | FL  | FD  | FFM | PT  | FLD |
| 1741.84  | 2440.50 | 56.60 | 11.81 | 0.10 | 1.04 | 2.69 | 24.61 | 24.94 | 0.17 | 7.62 |       |
| Accessions 36 |    | 513.35 ** | 910.36 ** | 50.97 ** | 10.58 ** | 2.61 ** | 1.30 ** | 90.08 ** | 266.59 ** | 113.76 ** | 3.66 ** | 8.33 ** |
| Error 72  |    | 363.72 | 24.90 | 3.99 | 0.70 | 0.30 | 0.46 | 20.89 | 5.04 | 0.09 | 3.00 |       |
| CV% 24.49 |    | 30.94 | 30.04 | 24.60 | 22.36 | 20.32 | 17.19 | 22.63 | 42.31 | 15.83 | 18.67 |       |

| SV      | DF | Capsicum baccatum var. pendulum—Mean Squares | CV%       |
|---------|----|---------------------------------------------|-----------|
| Block 2 |    | PH  | CD   | SD  | LL  | LW  | PL  | FL  | FD  | FFM | PT  | FLD |
| 504.13  | 639.35 | 17.26 | 0.34 | 0.66 | 0.14 | 0.06 | 31.47 | 124,497.00 | 0.04 | 2.23 |       |
| Accessions 22 |    | 1062.58 ** | 42.51 * | 7.43 * | 1.82 ** | 2.65 ** | 21.07 ** | 427.41 ** | 119.03 ** | 1.08 ** | 12.96 ** |
| Error 44  |    | 275.89 | 18.92 | 3.89 | 0.67 | 0.42 | 1.00 | 28.51 | 7.88 | 0.05 | 5.63 |       |
| CV% 22.42 |    | 25.42 | 24.94 | 20.93 | 19.30 | 14.93 | 16.11 | 23.15 | 9.68 | 19.55 |       |

| SV      | DF | Capsicum frutescens—Mean Squares | CV%       |
|---------|----|----------------------------------|-----------|
| Block 2 |    | PH  | CD   | SD  | LL  | LW  | PL  | FL  | FD  | FFM | PT  | FLD |
| 701.29  | 1658.36 | 53.20 | 16.90 | 3.03 | 0.32 | 0.29 | 3.42 | 0.25 | 2.04 | 5.03 |       |
| Accessions 6 |    | 38.86 | 0.88 | 5.98 |       |       |       |       |       |       |       |
| Error 12 |    | 275.89 | 18.92 | 3.89 | 0.67 | 0.42 | 1.00 | 28.51 | 7.88 | 0.05 | 5.63 |       |
| CV% 23.37 |    | 24.18 | 25.06 | 23.00 | 18.20 | 21.01 | 23.07 | 22.00 | 73.89 | 161.07 | 3.78 |

| SV        | DF | Capsicum annuum var. annuum and Capsicum annuum var. glabriusculum—Mean Squares | CV%       |
|-----------|----|--------------------------------------------------------------------------------|-----------|
| Block 2   |    | PH  | CD   | SD  | LL  | LW  | PL  | FL  | FD  | FFM | PT  | FLD |
| 63.17     | 70.37 | 108.79 | 10.09 | 0.17 | 0.42 | 3.42 | 1.17 | 0.21 | 2.08 |       |
| Accessions 1 |    | 38.86 | 0.88 | 5.98 |       |       |       |       |       |       |       |
| Error 2   |    | 275.89 | 18.92 | 3.89 | 0.67 | 0.42 | 1.00 | 28.51 | 7.88 | 0.05 | 5.63 |       |
| CV% 17.79 |    | 24.18 | 25.06 | 23.00 | 18.20 | 21.01 | 23.07 | 22.00 | 73.89 | 161.07 | 3.78 |

In C. frutescens, differences between the accessions were only detected for the canopy diameter (CD), leaf width (LW), fruit length (FL), and fruit diameter (FD) descriptors at $p < 0.05$. In C. annuum, differences were only detected for plant height (PH) and flower diameter (FLD) at $p < 0.05$ (Table 1).

The Scott-Knott cluster test gathered the accessions in several groups on the basis of the different descriptors (Table S6). The characteristics related to the size of the plants were different between accessions. The plant height (PH) varied from 19.97 to 95.67 cm, and the accessions were grouped into four groups. The canopy diameter (CD) varied from 17.67 to 105.67 cm, and the stem diameter (SD) varied from 6.78 to 29.29 mm, joining the accessions in two distinct groups.

The leaf length (LL) and leaf width (LW) allowed for the accessions to be grouped into two distinct groups for each characteristic, ranging from 3.45 to 15.22 cm and 0.88 to 5.98 cm, respectively. The petiole length (PL) joined the accessions in three different groups, varying from 1.45 to 36.00 mm.

The characteristics related to the fruit such as fruit length (FL) varied from 1.07 to 12.79 cm, fruit diameter (FD) varied from 3.95 to 49.50 mm, fruit fresh mass (FFM) varied from 0.38 to 30.94 g, and pericarp thickness (PT) ranged from 0.35 to 4.33 mm. On the basis of these characteristics, we grouped the accessions into nine, seven, five, and four groups, respectively. The flower diameter (FLD) joined the accessions in three groups and ranged from 5.68 to 16.46 mm.

The estimate of the relative contribution of the 11 quantitative traits in terms of the Singh [49] method indicated that fruit length (60.72%) and fruit diameter (12.71%) were the traits that contributed most for the genetic divergence among accessions followed by petiole length (8.52%) and pericarp thickness (5.49%) (Table 2). On the other hand, the
traits canopy diameter and leaf width were the ones that contributed less to the divergence between accessions, with a relative contribution of 0.75 and 0.17%, respectively.

Table 2. Relative contribution of 11 quantitative traits to genetic divergence among 69 Capsicum accessions by the Singh method.

| Trait                        | Relative Contribution (%) |
|------------------------------|----------------------------|
| Fruit length (cm)            | 60.72%                     |
| Fruit diameter (mm)          | 12.71%                     |
| Petiole length (cm)          | 8.52%                      |
| Pericarp thickness (mm)      | 5.49%                      |
| Fruit fresh mass (g)         | 3.26%                      |
| Plant height (cm)            | 3.07%                      |
| Flower diameter (mm)         | 2.31%                      |
| Leaf length (cm)             | 1.92%                      |
| Stem diameter (mm)           | 1.09%                      |
| Canopy diameter (cm)         | 0.75%                      |
| Leaf width (cm)              | 0.17%                      |

The existence of correlation between the quantitative traits was verified using Pearson’s correlation coefficient (Figure 2). A very strong correlation was found between petiole length (PL) and fruit length (FL) (0.90). Strong correlation was found between fruit diameter (FD) and fresh fruit mass (FFM) (0.85), leaf length (LL) and leaf width (LW) (0.80), plant height (PH) and canopy diameter (0.77), stem diameter and plant height (0.72), and stem diameter (SD) and canopy diameter (CD) (0.70). A moderate negative correlation was detected between plant height (PH) and pericarp thickness (PT) (−0.31).

3.2. Diversity Among Capsicum Accessions and Correlated Traits

To group accessions, we calculated dissimilarities on the basis of the squared Euclidean distances. The dendrogram obtained in the grouping of accessions allowed for the formation of five distinct groups (Figure 3a), and the cophenetic correlation coefficient was 0.82. Groups I and II allocated only one accession each. The accession allocated in group I presented the highest values for plant height, canopy diameter, and stem diameter, having elongated fruits of orange color in the intermediate stage and red when ripe.

The accession allocated in group II presented the lowest values for the traits of plant height, canopy diameter, stem diameter, and leaf length and width. Its triangular fruits were yellow in the intermediate stage and red when ripe. Group III gathered nine accessions, with the highest values for leaf length and width. In addition, they had an intermediate growth habit; filament colored in white—except for the IFES 02 accession, which had yellow filament—pigmented calyx; semi-wrinkled fruit surface; and absence of capsaicin, except for the IFES 67 and IFES 91 accessions, which showed pungent fruits.

Group IV was formed by four accessions in which the erect growth habit descriptors predominated as well as the position of the flowers. The number of flowers per node in the group’s accessions was two or three and violet in the anthers and greenish yellow corolla without the presence of spots. The filament of these accessions was yellow in color and the calyx had the presence of pigment. Its pointed fruits had a long shape with a semi-wrinkled surface and an acute base. In addition, its fruits were slightly corrugated and with the presence of capsaicin. Group V gathered the remaining 54 accessions, which, in general, had smaller plants; short petioles; and smaller and thinner fruits and with less pericarp thickness and, consequently, with less fresh mass. The growth habit of plants was intermediate, and its fruits had capsaicin.
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| Flower diameter (mm)         | 2.31                      |
| Leaf length (cm)             | 1.92                      |
| Stem diameter (mm)           | 1.09                      |
| Canopy diameter (cm)         | 0.75                      |
| Leaf width (cm)              | 0.17                      |

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Figure 2. Pearson’s correlation between the 11 descriptors used to characterize 69 Capsicum accessions. Blue and red boxes indicate positive and negative correlation, respectively. The white boxes indicate no significant correlation ($p < 0.05$). In the right side of the correlogram, the legend color shows the correlation coefficients and the corresponding colors. PH—plant height; CD—canopy diameter; SD—stem diameter; LL—leaf length; LW—leaf width; PL—petiole length; FL—fruit length; FD—fruit diameter; FFM—fruit fresh mass; PT—pericarp thickness; FLD—flower diameter.

3.3. ISSR Characterization and Molecular Diversity

In the ISSR analysis, 17 out of 50 primers previously tested detected polymorphisms between the accessions. Altogether, 150 fragments were generated, of which 134 were polymorphic, which indicated 90% polymorphism, considering fragments generated for all species. When analyzing the polymorphism at species level, C. chinense and C. baccatum were the ones with the best grade of polymorphism, with 88.8% each. In C. frutescens, we observed 56.6% of polymorphism, whereas in C. annuum, we observed the lowest degree of polymorph (26.9%). The polymorphism information content (PIC) values varied from 0.43 for primer ISSR 47 to 0.83 for ISSR 94. The mean value was 0.62.

The molecular characterization allowed for the formation of five groups, on the basis of the dendrogram (Figure 3b) with a cophenetic correlation coefficient of 0.76. Groups I, II, III, and IV were formed, individually, by one accession each. Group V gathered the remaining 65 accessions. The commercial accession IFES 09 was grouped alone in group III and the other four (IFES 51, IFES 99, IFES 100, and IFES 101) in group V. The entanglement value obtained comparing dendrograms was 0.59, evidencing the divergence in the distribution of the accessions in molecular and morpho-agronomic dendrograms. As observed in morpho-agronomic characterization, it can be noticed that on the basis of ISSR markers, the accessions were not grouped according to their geographic locations.
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Group IV was formed by four accessions in which the erect growth habit descriptors predominated as well as the position of the flowers. The number of flowers per node in the group’s accessions was two or three and violet in the anthers and greenish yellow corolla without the presence of spots. The filament of these accessions was yellow in color and the calyx had the presence of pigment. Its pointed fruits had a long shape with a semi-wrinkled surface and an acute base. In addition, its fruits were slightly corrugated and with the presence of capsaicin. Group V gathered the remaining 54 accessions, which, in general, had smaller plants; short petioles; and smaller and thinner fruits and with less pericarp thickness and, consequently, with less fresh mass. The growth habit of plants was intermediate, and its fruits had capsaicin.

4. Discussion

Studies related to genetic resources—such as the collection, characterization, and conservation of germplasm—of Capsicum are of great relevance for the preservation of the genetic diversity of the genus and for its use in breeding programs [37,38,54–57]. The acquisition and maintenance of germplasm banks are strategies that allow us to explore and expand the genetic variability of the acquired accessions and are fundamental to mitigate the effects of the loss of genetic variability caused by the exploitation of domesticated species [5]. Therefore, it is only possible to develop more productive varieties, resistant to diseases and reduce the impacts of genetic erosion when there is prior knowledge of the existing variability [5,58]. For these, this study aimed to characterize 69 Capsicum accessions from different regions of Brazil on the basis of qualitative and quantitative descriptors and ISSR markers.

Among the qualitative descriptors studied, three were monomorphic (stem shape, stem pubescence, and calyx pigmentation), i.e., none of them were effective in discriminating the studied accessions, and thus they can be eliminated in future studies, reducing the time consumed for the evaluation of these characteristics. Carvalho et al. [59], when studying the diversity between accessions of Capsicum frutescens, also listed these traits as monomorphic and inefficient in discriminating the accessions studied. However, our results report a great phenotypic variation for those descriptors related to flowers, and according to Baral and Bosland [60], to differentiate between the species of Capsicum, the descriptors related to inflorescence are essential, such as the position of the flower and the presence of annular constriction of the calyx, which are used to distinguish the accessions of C. frutescens and C. chinense.

Wide variation between accessions was also detected for quantitative descriptors, revealing a great diversity in characteristics related to the plant and fruits. In Brazil, peppers are widely accepted, and their use is quite diversified. Peppers are eaten fresh,
used in the preparation of sauces and condiments. In this sense, the consumer market has acceptance for the different sizes, colors, and pungency levels of the Capsicum fruits.

For the fresh mass, the values varied widely—between 0.38 (IFES 100) and 30.94 g (IFES 33). In general, the fruits of the accessions of C. baccatum var. pendulum presented the highest values for the characteristic, a common fact for this species that includes the sweet pepper variety with fruits appreciated for in natura consumption. Accessions belonging to the species C. frutescens were the ones that presented the lowest values for this characteristic. This species includes the ‘chilli-pepper’ morphotype, in Brazil ‘pimenta malagueta’, which, due to its small size and high pungency, is widely used in the production of sauces and canned peppers. The attributes of length and diameter of the fruit also showed a high variation, with values between 1.07 and 12.79 cm, and 3.95 and 49.50 mm, respectively. Similar results for fruit length and diameter have been reported by Cardoso et al. [17] who, studying the diversity between 116 Capsicum accessions, found values between 0.93 and 13.64 cm, and 4 and 59 mm, respectively. According to Leite et al. [61], longer fruits are very important for genetic improvement programs of Capsicum, mainly when they are destined for the food industry, as in the production of paprika or for use in cosmetics and pharmaceuticals.

Regarding pericarp thickness, the accessions showed values between 0.35 and 4.33 mm. According to Abud et al. [62], accessions with thicker pericarps are more important for the fresh consumer market, since thicker pericarps gives greater resistance to fruits in post-harvest treatments and guarantees an appearance more appreciated by consumers, in addition to conferring a higher degree of resistance to pathogens and parasites [22]. In general, significant differences were found for all quantitative morpho-agronomic characteristics, an indicating the presence of great genetic variation between accessions (Table S6).

By estimating the relative contribution of the descriptors to the variability between accessions on the basis of the method of Singh [38], we noticed that characteristics inherent to the fruit are the ones that most contribute to study the degree of diversity among the 69 accessions. The fruit length (60.72%) and fruit diameter (12.71%) stood out. These results corroborate previous studies, which indicated that characteristics associated with fruits had a great discriminatory power [63]. A possible explanation for the greater contribution of the characteristics related to the fruit to the diversity in the different studies of Capsicum was because the fruit is the portion consumed by humans and also by birds, being responsible for the dissemination of Capsicum seeds. This leads to the hypothesis that selection and domestication were based on this plant organ, which has a diverse range of colors, shapes, sizes, degree of pungency, and aromas [64].

When it comes to Capsicum improvement, depending on the characteristics to be improved, the use of traits correlated with the trait of interest facilitates the process of indirect gain in the selection. In this perspective, Pearson’s correlation coefficient was used in this study. It is a measure of linear association between two quantitative variables, in which one characteristic can be selected on the basis of another.

The results (Figure 2) showed that concomitant gains in fresh fruit mass (FFM) and pericarp thickness (PT) were possible when selecting genotypes on the basis of fruit diameter (FD), since there is a positive correlation between fruit diameter and the first two traits (FFM and PT). Studying 40 accessions of C. baccatum, Rêgo et al. [63] also found a positive correlation between pericarp thickness and fresh fruit mass, which corroborated the results also reported by Leite et al. [61] and, more recently, Bianchi et al. [22]. Studies of the association between easy-to-measure characteristics and those related to productivity and quality are crucial for breeding programs, since they contribute to the selection of superior plants with little time consumption [58,60,65,66].

The associations between the characteristics related to the plant’s architecture also help in improving Capsicum for ornamental purposes. A major factor for the use of pepper trees in the floriculture market is the plant’s growth capacity in pots [67]. For this, characteristics such as short stature and leaf density are essential. Our results show a strong
positive correlation between leaf length and width (0.80), between plant height and canopy diameter (0.77) and stem diameter (0.72); therefore, the selection of smaller plants or with less leaf length/width would result in reductions in the other aspects related to the good architecture of the plant in the pots.

The description of the diversity between the accessions based on the morpho-agronomic descriptors allowed for the formation of five groups. It is possible to infer that the dissimilarity matrix revealed data adjusted to the dendrograms. Such adjustment can be confirmed by the high value of the cophenetic correlation coefficient, which according to Rohlf and Fisher [68] must present values above 0.8. Thus, the results were satisfactory and revealed the conformity between the graphic representations of the distances and their original matrices. A good adjustment between the graphical representation of the distances and their original matrix makes it possible to make inferences through visual assessment [67].

Accessions could not be grouped on the basis of their geographic origin since genotypes from the same location were gathered in different groups. Other studies also reported the lack of correlation between geographic location and accession grouping [16,17,58,69]. As in this study, Finger et al. [70], when evaluating the diversity between 49 accessions of C. chinense of Brazil, did not show a relationship between the geographic distance and the estimated diversity. The authors reported that this may have been a reflection of genetic drift and the selection of plants in different environments, rather than geographic location. Another possibility was the occurrence of cross-pollination at a rate that varies in Capsicum from 0.5 to 70%, contributing to the genetic contamination of the seeds.

Characterization through molecular markers is an effective strategy when associated with phenotyping studies based on multi-categorical descriptors. However, when it comes to SSR primers, these DNA markers are species-specific, and in the case of our work, four pepper species of Capsicum (Capsicum annuum, Capsicum baccatum var. pendulum, Capsicum chinense Jacq., and Capsicum frutescens) were used and only for the species Capsicum annuum we have a good number of SSR primers already described. In this sense, countless tests of transferability of SSR primers to other species are necessary, which requires a lot of time, manpower, and financial resources. We reiterate that for the species Capsicum baccatum var. pendulum, we do not have any SSR primers described in the literature yet. In many studies, ISSR primers have been used to study genetic diversity [71–73], being considered quite efficient to discriminate diversity in germplasm of unique or many species. The efficacy of the markers quantified by PIC values showed that the ISSR markers were effective in assessing the genetic relatedness in the accessions. Generally, the PIC values were greater than 0.5, a value considered satisfactory according to Mandal [71].

Our results show that the molecular markers indicated the presence of 90% of polymorphism considering all accessions studied. Thul et al. [24], in a molecular characterization of six Capsicum species with ISSR markers, found 98.7% of polymorphism. In contrast, Olatunji et al. [35], studying the diversity among four Capsicum species with ISSR markers, found 14 polymorphic loci out of the 75 amplified, which represented only 18.6% of polymorphism. These results show the efficiency of the markers used in our work to detect diversity in the germplasm collection evaluated. When analyzing each species, we found the fragments generated by ISSR markers in C. chinense and C. baccatum showed a great level of polymorphism. More recently, Bianchi et al. [22], studying 55 accessions of C. chinense, found 47% polymorphism for the 97 fragments produced by the ISSR markers used in their study with C. chinense accessions.

The correlation between the groups formed by the morphological and molecular characterization was considered low, with an agreement rate of only 41. Results like this are very common in studies of Capsicum diversity when molecular markers and phenotypic description of germplasm are associated [22,66,74]. One possible reason to this is the fact that the markers used in this study may be not associated with the evaluated characteristics and because they also amplify coding and non-coding regions of the genome. Since the phenotype is the result of coding regions, there is a low correlation with the bands generated
by the molecular markers. Similar to phenotypic characterization, ISSR markers did not group accessions according to their geographic location. The absence of an association between geographic origin and molecular markers is probably caused by the exchange of seeds between producers in nearby regions as well as unrestricted transport of fruits and seeds between different regions of Brazil [16,17].

However, even though the morpho-agronomic and molecular characterization did not allow us to separate the accessions by region and both did not present similarity in the formed clusters, we assumed the importance of these two methods to discriminate the diversity of a germplasm collection, guaranteeing access to the wide variability of the genetic resources to plant breeding programs.

5. Conclusions

A high level of variation was detected for the qualitative and quantitative descriptors. The characteristics associated with the fruit were those that showed greater relevance in the discrimination of accessions and correlation among some important traits related to fruits, and plant architecture is an important tool to facilitate the next studies for pepper breeding for many branches of the pepper market.

The morpho-agronomic and molecular characterization were efficient to estimate the genetic diversity between accessions, showing significant divergence, being important tools for the knowledge and use of accessions in breeding programs.

This study is going to allow for the establishment of a collection of great importance to preserve pepper germplasm and to provide morpho-agronomical data to facilitate the process of the selection in the breeding programs. The shown characterization would be useful for a possible next genome-wide association study.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11050854/s1, Figure S1: Representative sample of 2% agarose gel. DNA amplified fragments using ISSR marker 106. M—molecular weight marker. Table S1: Accession identification of 69 Capsicum accessions from Instituto Federal do Espírito Santo—Campus de Alegre germplasm collection. Table S2: Twenty-two qualitative descriptors used to phenotype 69 Capsicum accessions according to IPGRI (1995). Table S3: Eleven quantitative descriptors used to phenotype 69 Capsicum accessions according to IPGRI (1995). Table S4: Information about 17 ISSR primers used in the genotyping of 69 Capsicum accessions from Instituto Federal do Espírito Santo—Campus de Alegre germplasm collection and 33 discarded primers. Table S5: Descriptive analysis of 22 qualitative descriptor of 69 Capsicum populations from Instituto Federal do Espírito Santo—Campus de Alegre germplasm collection. Table S6: Grouping of 11 quantitative means of 69 capsicum accessions by Scott-Knott method at 5% probability.

Author Contributions: Conceptualization, B.D.G.B., A.C.S.J. and M.M.M.; methodology, B.D.G.B., T.d.O.S., PH.A.D.S., S.H.K., J.D.S.N., L.H.R., F.V.V., R.N.d.A., R.R., A.C.S.J. and M.M.M.; software, B.D.G.B., T.d.O.S., PH.A.D.S., S.H.K., J.D.S.N. and M.M.M.; validation, B.D.G.B., T.d.O.S., PH.A.D.S., S.H.K., J.D.S.N. and M.M.M.; formal analysis, B.D.G.B., T.d.O.S., PH.A.D.S., S.H.K., J.D.S.N., L.H.R., F.V.V., R.N.d.A. and M.M.M.; investigation, B.D.G.B., R.R. and M.M.M.; resources, B.D.G.B., R.R. and M.M.M.; data curation, B.D.G.B., R.R. and M.M.M.; writing—original draft preparation, B.D.G.B., T.d.O.S., PH.A.D.S., R.R., A.C.S.J. and M.M.M.; writing—review and editing, B.D.G.B., T.d.O.S., PH.A.D.S., S.H.K., R.R., A.C.S.J. and M.M.M.; visualization, B.D.G.B., R.R. and M.M.M.; supervision, B.D.G.B., A.C.S.J. and M.M.M.; funding acquisition, B.D.G.B. and M.M.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financed in part by Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo—Campus de Alegre and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES/Brasil) with the scholarship of the first author—finance code 001.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.
