



Genetic diversity, population structure and genetic parameters of fruit traits in *Capsicum chinense*

Aline Fabiana Paladini Moreira^a, Paulo Maurício Ruas^b, Claudete de Fátima Ruas^b,
Viviane Yumi Baba^a, Willian Giordani^a, Isabella Mendonça Arruda^c, Rosana Rodrigues^d,
Leandro Simões Azeredo Gonçalves^{a,*}

^a Agronomy Department, Universidade Estadual de Londrina (UEL), Rodovia Celso Garcia Cid, Km 380, Londrina, PR 86051-900, Brazil

^b Biology Department, Universidade Estadual de Londrina (UEL), Rodovia Celso Garcia Cid, Km 380, Londrina, PR 86051-900, Brazil

^c Agronomia Department, Universidade Estadual de Maringá (UEM), Av. Colombo, n° 5.790, Maringá, PR 87020-90, Brazil

^d Plant Breeding Department, Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, RJ 28013-602, Brazil

ARTICLE INFO

Keywords:

Peppers

Germplasm bank

Mixed models

Heritability

Association analysis

ABSTRACT

The fruits of *Capsicum chinense* stand out with a wide diversity of sizes, colors, shapes, and levels of pungency. Knowledge about this variability is essential for the success in species conservation and breeding programs. This study evaluated 65 *C. chinense* accessions, from different geographic regions of Brazil, for genotypic fruit traits, correlations between fruit traits, genetic parameters and diversity, population structure, and associations of AFLP markers with fruit traits. Deviance analysis showed significant differences between the accessions for all traits. Heritability in the broad sense (h^2_b) ranged from 0.31 (soluble solids content) to 0.90 (fruit diameter). The accuracy of genotype selection was considered high (≥ 0.70) for most of the traits, except for soluble solids and dry matter contents (0.43 and 0.62, respectively). A high correlation between fresh and dry fruit weight was observed. However, these traits were negatively correlated with dry matter content. Soluble solids content was not correlated with any of the traits. The AFLP markers indicated high genetic variability among accessions. When the groups formed by molecular data and fruit traits were compared, no associations were observed, indicating that both stages of characterization are important. Structure analysis formed two groups, partially concordant with molecular hierarchical grouping. The AFLP markers were significantly associated with several fruit traits and considered interesting candidates for further studies.

1. Introduction

Pepper (*Capsicum* spp.) is one of the most important vegetables in the world due to its high versatility and wide application range in industry, cooking and for decoration (Hulse-Kemp et al., 2016; Silvar and García-González, 2017). It is a genus with wide variability, with 38 described species, of which only *C. annuum* var. *annuum* L., *C. baccatum* var. *pendulum* (Willd.) Eshbaugh, *C. chinense* Jacq., *C. frutescens* L. and *C. pubescens* Ruiz et Pav. are domesticated (Pozzobon et al., 2006; Dewitt and Bosland, 2009; García et al., 2016).

Brazil is considered one of the most important diversity centers of domesticated and wild species of *Capsicum* (Barboza et al., 2005). In this country peppers play an important role in socioeconomic, biological and cultural aspects, due to the wide applicability of fruits in traditional culinary foods and dishes, with regional diversity and sometimes regional uniqueness (Sudré et al., 2010). The wide

diversification in the use of *Capsicum* spp. fruits is probably related to the genetic diversity observed in each region of the country.

Among the domesticated species, *C. chinense* is considered the most important in Brazil, with the Amazon Basin as putative center of domestication (Pickersgill, 1971; Moses and Umaharan, 2012; Moses et al., 2014). This species has a wide variability of fruits in terms of size, color, shape, and pungency levels, and is widely used in Brazilian cuisine (Baba et al., 2016). Therefore, knowledge about the variability in this species is essential for successful conservation and its use in breeding programs.

Several studies have addressed the characterization of the genetic diversity of *C. chinense* based on phenotypic and molecular descriptors (Jarret and Berke, 2008; Finger et al., 2010; Moses and Umaharan, 2012; Bharath et al., 2013; Moses et al., 2014; Baba et al., 2016). However, genetic studies for fruit traits are scarce for this species (Sousa and Maluf, 2003; Schuelter et al., 2010; Bharath et al., 2013),

* Corresponding author.

E-mail address: leandrosag@uel.br (L.S.A. Gonçalves).

since most of them focused on *C. annuum*. In an recent evaluation of 264 *C. chinense* accessions from the Caribbean, Bharath et al. (2013) detected wide variability for fruit traits, with the broad-sense heritability (h^2_g) varying from 0.52 (pedicel length) to 0.81 (fruit diameter and weight) among accessions. In *C. annuum*, Naegele et al. (2016) observed a high h_a (> 0.90) for most of the 13 fruit traits evaluated in 116 accessions.

In studies based on quantitative traits in *Capsicum* spp., multiple QTL maps have been associated with fruit size, weight, shape, color, and metabolites (Ben-Chaim et al., 2001; Zygiel et al., 2005; Yarnes et al., 2013; Naegele et al., 2014; Han et al., 2016; Nimmakayala et al., 2016). Two major QTLs (*fs3.1* and *fs10.1* – chromosome 3 and 10, respectively) that control fruit shape variation (FS) were identified, accounting for about 60 and 40% of the phenotypic variation, respectively (Ben-Chaim et al., 2001; 2003). For fruit weight (FW), Rao et al. (2003) and Zygiel et al. (2005) identified a major QTL (*fw2.1*) located on chromosome 2. These results were corroborated by Han et al. (2016), in an evaluation of an ultra-high density map of crosses between *C. annuum* cultivars, who also identified the importance of these QTLs for the respective traits. However, Nimmakayala et al. (2016) evaluated 96 *C. annuum* accessions by associative mapping, and observed 16 SNPs strongly associated with FW, located in different genomic regions.

In these studies of genetic diversity and QTL mapping in *Capsicum* spp., several molecular markers (e.g., RAPD, AFLP, SSR, and SNPs) were applied. Among these techniques, Amplified Fragment Length Polymorphism (AFLP) is an important tool in view of its wide genome coverage, reproducibility, cost-effectiveness, and sequence information independent genotyping (Zhang et al., 2014). In this study we used AFLP markers addressed to achieve the following objectives: i) determine the genetic parameters associated with fruit traits of *C. chinense* accessions from Brazil; ii) identify correlations between these traits; iii) determine population structure and genetic diversity; and iv) identify AFLP markers associated with the fruit traits.

2. Material and methods

2.1. Phenotyping

We evaluated a total of 65 *C. chinense* accessions from eight Brazilian states (Mato Grosso, Maranhão, Pará, Bahia, Rio de Janeiro, Minas Gerais, Goiás, and Espírito Santo with 26, 11, 11, 6, 4, 3, 2, and 2 accessions, respectively), comprising four geographic regions of Brazil (Mid-West, North-East, North, and South-East, with 28, 17, 11, and 9 accessions, respectively), maintained in the germplasm bank of the Universidade Estadual de Londrina (UEL), Londrina, Paraná, Brazil (Table 1).

These accessions were evaluated in a field of the UEL (latitude 23°20'32" S, longitude 51°12'32" W; 550 m asl) in two growing seasons. Experiment I was carried out from September to December 2016 and, experiment II, from December–2016 to March 2017. The experiment was arranged in a randomized block design, with two replications and 12 plants per plot, in a 0.5 × 0.9 m spacing between plants and rows, respectively. The soil of the experimental area was classified as Red Latosol and the climate was subtropical, mesothermal humid (Cfb), with hot summers, rare frosts and tendency of concentrate rainfall in the summer months.

The soil chemical properties were determined as follows: $pH_{H_2O} = 6.60$, $P = 103.65 \text{ mg/dm}^3$, $K = 0.28 \text{ cmolc/dm}^3$, $Ca^{+2} = 9.0 \text{ cmolc/dm}^3$, $Mg^{+2} = 1.7 \text{ cmolc/dm}^3$, $Al^{+3} = 0.0$, $H + Al = 2.70 \text{ cmolc/dm}^3$, $CEC = 15.0 \text{ cmolc/dm}^3$, $V = 80\%$, and $OM = 2.30\%$. The soil was prepared by fertilization with 18 t ha^{-1} organic compost one week after transplanting the seedlings, inoculated with Bokashi®, applying 25 g product per plant, manually incorporated and covered with straw. A drip irrigation system was installed and weed growth was inhibited by scattering straw in and in-between the rows.

Three crops were harvested and the following agronomic

Table 1

Geographic distribution and morphological descriptors of 65 *Capsicum chinense* accessions in Brazil of gene bank of Universidade Estadual de Londrina (UEL).

ID	Origin	Morphological descriptors		
		Fruit shape	Fruit color at intermediate stage	Fruit color at mature stage
Gen_1	Mato Grosso	Elongate	Green	Red
Gen_2	Mato Grosso	Elongate	Green	Red
Gen_3	Mato Grosso	Elongate	Green	Red
Gen_4	Mato Grosso	Elongate	Green	Red
Gen_5	Mato Grosso	Triangular	Green	Red
Gen_6	Mato Grosso	Elongate	Green	Red
Gen_7	Mato Grosso	Other	Green	Orange
Gen_8	Mato Grosso	Elongate	Green	Orange
Gen_9	Mato Grosso	Almost round	Green	Lemon-yellow
Gen_10	Mato Grosso	Campanulate	Green	Dark red
Gen_11	Mato Grosso	Almost round	Green	Light red
Gen_12	Mato Grosso	Elongate	Green	Red
Gen_13	Mato Grosso	Campanulate	Green	Red
Gen_14	Mato Grosso	Other	Green	Pale orange
Gen_15	Mato Grosso	Elongate	Green	Dark red
Gen_16	Mato Grosso	Almost round	Green	Orange
Gen_17	Mato Grosso	Triangular	Purple	Light red
Gen_18	Mato Grosso	Blocky	Green	Dark red
Gen_19	Mato Grosso	Almost round	Green	Red
Gen_20	Mato Grosso	Campanulate	Purple	Light red
Gen_21	Mato Grosso	Triangular	Green	Red
Gen_22	Mato Grosso	Almost round	Green	Red
Gen_23	Mato Grosso	Elongate	Green	Orange
Gen_24	Mato Grosso	Almost round	Green	Red
Gen_25	Mato Grosso	Elongate	Green	Orange
Gen_26	Mato Grosso	Triangular	Green	Light red
Gen_27	Goiás	Almost round	Purple	Red
Gen_28	Goiás	Almost round	Green	Lemon-yellow
Gen_29	Minas Gerais	Campanulate	Green	Red
Gen_30	Minas Gerais	Campanulate	Green	Light red
Gen_31	Minas Gerais	Campanulate	Green	Dark red
Gen_32	Bahia	Triangular	Green	Red
Gen_33	Bahia	Blocky	Green	Pale Orange
Gen_34	Bahia	Campanulate	Green	Red
Gen_35	Rio de Janeiro	Almost round	Green	Orange-yellow
Gen_36	Pará	Campanulate	Green	Pale Orange
Gen_37	Bahia	Campanulate	Green	Red
Gen_38	Pará	Elongate	Green	Red
Gen_39	Pará	Blocky	Green	Red
Gen_40	Pará	Blocky	Green	Red
Gen_41	Maranhão	Blocky	Green	Red
Gen_42	Maranhão	Campanulate	Green	Red
Gen_43	Maranhão	Triangular	Green	Red
Gen_44	Maranhão	Other	Green	Red
Gen_45	Maranhão	Blocky	Green	Red
Gen_46	Maranhão	Other	Purple	Orange
Gen_47	Rio de Janeiro	Elongate	Green	Red
Gen_48	Espírito Santo	Blocky	Green	Red
Gen_49	Espírito Santo	Elongate	Green	Red
Gen_50	Maranhão	Elongate	Green	Red
Gen_51	Maranhão	Elongate	Green	Orange
Gen_52	Maranhão	Elongate	Green	Red
Gen_53	Maranhão	Elongate	Green	Red
Gen_54	Bahia	Campanulate	Green	Red
Gen_55	Bahia	Elongate	Green	Red
Gen_56	Pará	Triangular	Green	Red
Gen_57	Pará	Elongate	Green	Red
Gen_58	Pará	Campanulate	Green	Red
Gen_59	Pará	Triangular	Green	Red
Gen_60	Pará	Elongate	Green	Red
Gen_61	Pará	Triangular	Green	Red
Gen_62	Pará	Blocky	Green	Red
Gen_63	Maranhão	Blocky	Green	Red
Gen_64	Rio de Janeiro	Campanulate	Green	Red
Gen_65	Rio de Janeiro	Elongate	Green	Red

descriptors evaluated: fruit length (cm), fruit diameter (cm), pericarp thickness (mm), mean fruit weight (g), dry fruit weight (g), fruit dry matter content (%), and soluble solids content (°Brix).

2.2. Genotyping

Genomic DNA was extracted from pools of eight young plantlets (fresh leaf tissue three weeks after sowing) per accessions following to the modified protocol of Ferreira and Grattapaglia (1998), using CTAB buffer associated with isopropanol precipitation. All samples were treated with RNase (110 ng/mL). The DNA integrity was confirmed by 1% agarose gel electrophoresis.

The AFLP protocol was adapted from Vos et al. (1995) with minor modifications, with restriction and ligation being performed simultaneously. Approximately 700 ng of DNA was digested with 5 U of *EcoRI* and 1 U of *MseI* at 37 °C for 4 h, followed by adapter ligation using 2 U of T4 DNA ligase at 22 °C for 1 h and incubation at 70 °C for 10 min, for thermal inactivation of the restriction enzymes. Pre-selective amplification was performed using the primer sets *EcoRI*+A and *MseI*+C, in a final volume of 10 µL, containing 3.5 µL of 4 × diluted template. The PCR was performed at 72 °C for 2 min, followed by 20 cycles of 94 °C for 1 s, 56 °C for 30 sec and 72 °C for 2 min, with final extension at 60 °C for 30 min. Four *EcoRI*/*MseI* primer combinations (E-ACA/M-CAC, E-ACG/M-CAA, E-ACT/M-CAA and E-ACG/M-GAC), containing three selective nucleotides, were tested and the final products were observed in 7% polyacrylamide gel. Two of these primers (E-ACA/M-CAC and E-ACG/M-CAA) had proved efficient in detecting polymorphism in *C. chinense* in the study of Baba et al. (2016). Then, the *EcoRI* primers of all four combinations were labeled with the fluorescent dyes FAM, NED, VIC, and PET, respectively. Selective amplification was performed using 2.5 µL of 4 × diluted template, with 10 µL final volume, in the following thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, USA) program: initial cycle of 2 min at 94 °C; 30 s at 65 °C and 2 min at 72 °C; 8 cycles of 1 sec at 94 °C, 30 s at 64 °C and 2 min at 72 °C, decreasing 1 °C per cycle; 23 cycles of 1 s at 94 °C, 30 s at 56 °C and 2 min at 72 °C, and a final extension for 30 min at 60 °C. The resulting PCR products were separated by capillary electrophoresis using an automated ABI 3500 xL Genetic Analyzer (Applied Biosystems, USA) with GS-600 LIZ (Applied Biosystems, USA), as a molecular weight marker. The AFLP results were expressed in a binary matrix using the GeneMapper® software v.4.1 (Applied Biosystems, USA).

2.3. Data analysis

The agronomic data of the fruits were analyzed by the mixed model methodology. The statistical model was $y = Xr + Zg + Wi + e$, where y is the data vector; r the vector of replication effects (assumed as fixed) added to the general mean; g the vector of genotypic effects (assumed as random); i the vector of the effects of the genotype - environment interaction (GE) (random); and e the vector of (random) errors. X , Z and W represent the incidence matrices for the above effects. The assumed distributions and structures of means (E) and variances (Var) were:

$$E \begin{bmatrix} y \\ g \\ i \\ e \end{bmatrix} = \begin{bmatrix} Xr \\ 0 \\ 0 \\ 0 \end{bmatrix}; \text{Var} \begin{bmatrix} g \\ i \\ e \end{bmatrix} = \begin{bmatrix} I\sigma_g^2 & 0 & 0 \\ 0 & I\sigma_i^2 & 0 \\ 0 & 0 & I\sigma_e^2 \end{bmatrix}$$

The model was fitted using the mixed model equations:

$$\begin{bmatrix} X'X & X'Z & X'W \\ Z'X & Z'Z + \lambda_1 & Z'W \\ W'X & W'Z & W'W + \lambda_2 \end{bmatrix} \times \begin{bmatrix} \hat{r} \\ \hat{g} \\ \hat{i} \end{bmatrix} = \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix}$$

where $\lambda_1 = \frac{\sigma_e^2}{\sigma_g^2} = \frac{1 - h_g^2 - i^2}{h_g^2}$; $h_g^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_i^2 + \sigma_e^2}$ corresponds to the individual heritability in the broad sense of the block; $i^2 = \frac{\sigma_i^2}{\sigma_g^2 + \sigma_i^2 + \sigma_e^2}$ to the coefficient of determination of the GE interaction effects; σ_g^2 is the genotypic variance between *C. chinense* accessions; σ_i^2 the variance of the GE interaction; σ_e^2 the residual variance between plots; $r_{gloc} = \frac{\sigma_i^2}{\sigma_g^2 + \sigma_i^2} = \frac{h_g^2}{h_g^2 + i^2}$

corresponds to the genotypic correlation of genotypes across environments.

The iterative estimators of the variance components, by restricted maximum likelihood (REML) and the EM algorithm, were $\hat{\sigma}_e^2 = \frac{[y'y - P'X'y - g'Z'y - i'W'y]}{[N - r(x)]}$; $\hat{\sigma}_g^2 = \frac{[g'g + \hat{\sigma}_e^2 \text{tr}(C^2)]}{q}$; $\hat{\sigma}_i^2 = \frac{[i'i + \hat{\sigma}_e^2 \text{tr}(C^3)]}{s}$;

where: C^2 and C^3 were from $C^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} \\ C_{21} & C_{22} & C_{23} \\ C_{31} & C_{32} & C_{33} \end{bmatrix}^{-1} = \begin{bmatrix} C^{11} & C^{12} & C^{13} \\ C^{21} & C^{22} & C^{23} \\ C^{31} & C^{32} & C^{33} \end{bmatrix}$,

where C is the matrix of the coefficients of mixed model equations; tr the matrix trace operator; $r(x)$ is the rank of matrix X ; N , q , s = total number of data, number of genotypes and number of GE combinations, respectively.

By this model, the empirical BLUP predictors (eBLUP or REML/BLUP) of the interaction-free genotypic values were obtained, given by $\hat{\mu} + \hat{g}_i$, where $\hat{\mu}$ is the mean of all environments and \hat{g}_i the genotypic effect free of the GE interaction. For each environment j , the genotypic values (Vg) are predicted by $\hat{\mu}_j + \hat{g}_i + (\hat{g}e)_{ij}$, where $\hat{\mu}_j$ is the mean of environment j ; \hat{g}_i the genotypic effect of genotype i in environment j ; and $(\hat{g}e)_{ij}$ is the effect of the GE interaction in relation to genotype i .

The prediction of genotypic values by capitalizing the mean interaction (gem) in the different environments is given by

$\hat{\mu}_i + \hat{g}_i + \hat{g}e_m$, and is calculated by: $\hat{\mu} + \left(\frac{\hat{\sigma}_g^2 + \hat{\sigma}_e^2}{n} \right) \hat{g}_i$, in which $\hat{\mu}$ is the overall mean of all environments; n the number of environments, and \hat{g}_i the genotypic effect of genotype i . All mixed model analyses were performed using software Selegen (Resende, 2016).

The predicted genotypic values of fruit traits were used for Pearson's correlation analysis and Ward's hierarchical cluster analysis, based on the mean standardized Euclidean distance. For these analyses we used software R (<http://www.R-project.org>) with the packages agricolae, corplot and Nbclust.

Analyses of the AFLP data were performed using the Jaccard genetic distance matrix and Ward's cluster analysis. The correlation between molecular and morphological distances was determined by the Mantel test, with 1000 permutations. These analyses were performed by software R (<http://www.R-project.org>) using the packages agricolae, ade4 and Nbclust.

Bayesian clustering was also performed for AFLP data, using software Structure V 2.3.4 (Pritchard et al., 2000), based on the method described by Evanno et al. (2005), with 100,000 interactions (Monte Carlo Markov Chain), with a burn-in of 10,000 interactions, assuming mixed clusters (admixture) and correlated allelic frequencies. Values of k ranging from 1 to 66 were tested, with 10 independent interactions for each k value. The k number was determined using Structure Harvester v0.6.92 (Earl and von Holdt, 2012) and the graphs were generated by the online interface Structure Plot 2 (Ramasamy et al., 2014).

From the information of the 645 AFLP markers, the coefficient matrix of the population structure (Q), obtained by Structure software, was constructed. The software SPAGEDI (Hardy and Vekemans, 2002) was used to generate the kinship matrix, according to the methodology proposed by Hardy (2003). The negative values between individuals were set to 0 and the matrix was formatted to a file readable by TASSEL software.

For the association of molecular markers with fruit traits, four models were used: (i) *naïve* - without control of population structure and relative kinship, (ii) GLM (General linear model) - using matrix Q , (iii) MLM (Mixed Linear Model) - using the *kinship* matrix (K) for kinship control and (iv) MLM - using matrix Q and matrix K . Population structure was always considered as a fixed effect, while matrix K was used to analyze the variance and covariance structure of the individual random effect (Yu and Buckler, 2006; Zhang et al., 2010). To improve the confidence of the 645 AFLP markers, those with a frequency lower than 5% and greater than 95% were removed, aiming to reduce the chance of identification of false positives, due to the presence of rare markers.

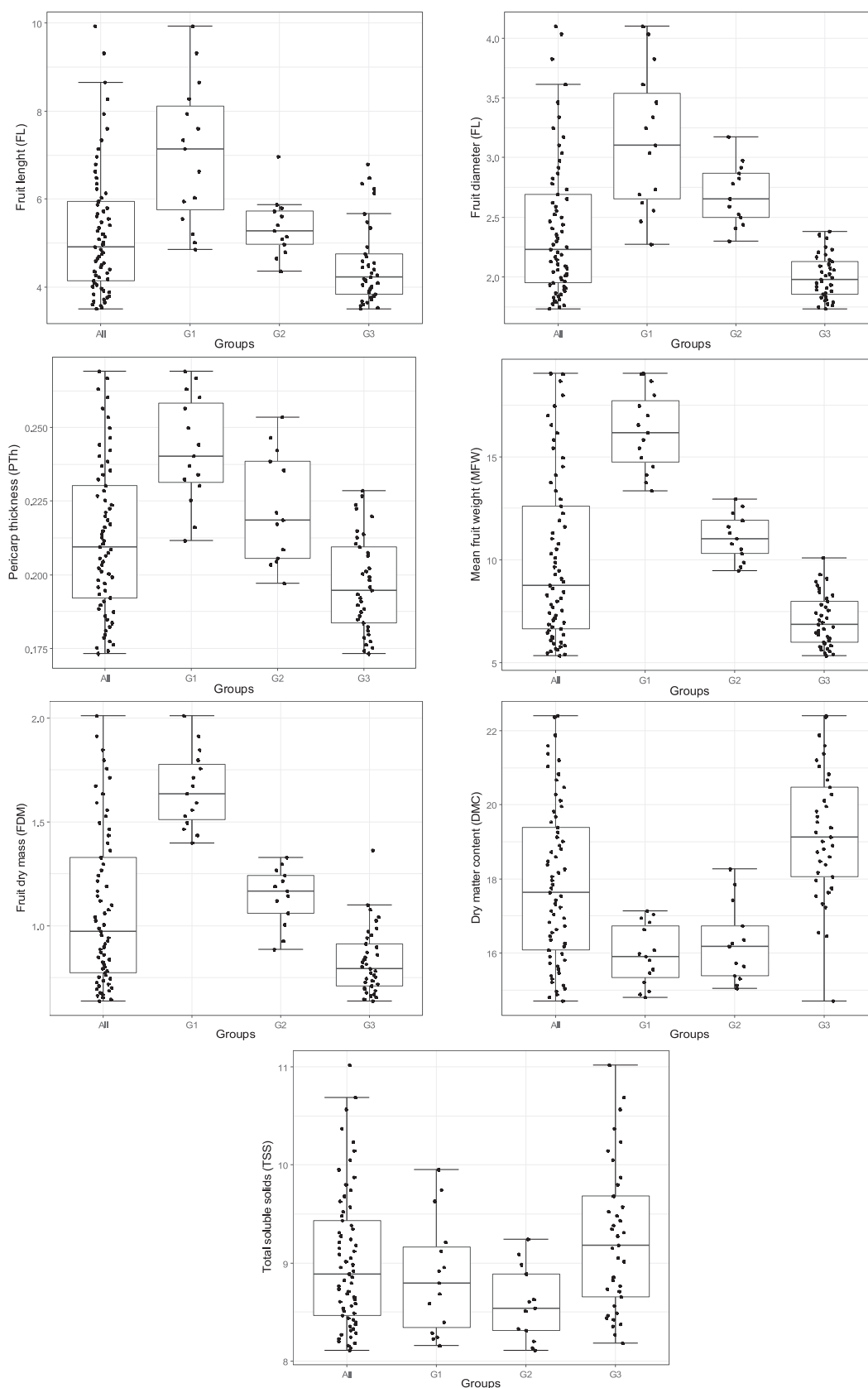


Fig. 1. Box plot for seven fruits agronomic traits of 65 *Capsicum chinense* accessions, for the three groups (G1-G3) formed by Ward's hierarchical clustering analysis.

The significance level of the association was verified considering a moderately restrictive cut-off threshold (1/number of hypotheses) (Wang et al., 2012), that is, $1/645$, so associations with a p-value below

1.55×10^{-3} were considered significant. All association analyses were performed using software TASSEL 5.2 (Bradbury et al., 2007). For the MLM, the P3D method was used.

Table 2

Estimation of variance components (REML) and genetic parameters for seven fruits agronomic traits of 65 *Capsicum chinense* accessions.

Parameters ^{1/}	Agronomic traits ^{2/}						
	FL	FD	PTh	MFW	FDM	DMC	TSS
Vg	3.62	0.51	2.25×10^{-3}	25.81	0.23	14.82	1.45
Vint	0.05	0.01	1.30×10^{-4}	0.49	0.01	1.33	0.36
Ve	0.42	0.05	1.01×10^{-3}	3.95	0.04	10.60	2.85
Vf	4.09	0.57	3.39×10^{-3}	30.26	0.28	26.74	4.65
h ² _g	0.89	0.90	0.66	0.85	0.83	0.55	0.31
c ² _{int}	0.01	0.01	0.04	0.02	0.02	0.05	0.08
h ² _m lin	0.79	0.82	0.49	0.74	0.71	0.38	0.18
Acclin	0.89	0.90	0.70	0.86	0.84	0.62	0.43
rgloc	0.99	0.99	0.95	0.98	0.98	0.92	0.80
Mean	3.50	1.73	0.17	5.34	0.64	14.71	8.11

^{1/} Vg: genotypic variance; Vint: variance of genotype - environment interaction; Ve: residual variance; Vf: phenotypic individual variance; h²_g: Broad-sense heritability of individual plots; c²_{int}: coefficient of determination of the effects of GE interaction; h²_mlin: heritability of the genotype mean, assuming 100% survival; Acclin: accuracy of genotype selection, assuming 100% survival; rgloc: genotype correlation between performance in the various environments and overall experimental mean.

^{2/} FL: fruit length; FD: fruit diameter; PTh: pericarp thickness; MFW: mean fruit weight; FDM: fruit dry matter; DMC: dry matter content; TSS: total soluble solids.

3. Results

3.1. Deviance analysis, genetic parameters and correlation

By deviance analysis, a significant effect ($P < 0.05$) was observed between accessions for all traits. However, no significant differences were detected for GE interaction. The predicted genotypic values

identified high amplitude of variation of the agronomic fruit traits (Fig. 1). The fruit length (FL) ranged from 3.50 to 9.94 cm, while fruit diameter (FD) ranged from 1.73 to 4.10 cm. The thickness of the pericarp (PTh) varied from 1.7 to 2.7 mm.

In relation the mean fruit weight (MFW), values ranging from 5.34 to 19.05 g were recorded. The accessions with the highest MFW also exhibited the highest values of fruit dry matter (FDM), which varied from 0.64 to 2.01 g. The dry matter content (DMC) ranged from 14.71 to 22.42%, although not the largest and heaviest fruits had the highest DMC. The total soluble solids content (TSS) obtained a variation from 8.11 to 11.02°Bx.

The estimates of variance components and genetic parameters are shown in Table 2. The broad-sense heritability (h²_g) ranged from 0.31 (TSS) to 0.90 (FD). The coefficient of determination of the GE interaction effects were low (c²_{int}) (0.01–0.08), which reflected in high genotype correlation between environments (rgloc) (0.80 - 0.99). Accuracy values of genotype selection (Acclin) were considered high (≥ 0.70) for most traits, except for TSS and DMC, with values of 0.43 and 0.62, respectively.

Pearson's analysis showed a high correlation between MFW and FDM (0.97) (Fig. 2). However, MFW and FDM were negatively correlated with DMC (−0.69 and −0.56, respectively) and detected no correlation with TSS. Trait TSS was not correlated with any variable. For PTh, a positive correlation was observed with MFW and FDM (0.75 and 0.71, respectively) and a negative one with DMC (−0.60).

3.2. Morphoagronomic diversity

Ward's hierarchical cluster analysis formed three groups defined by the indices proposed by the NbClust package of the R program (<http://>

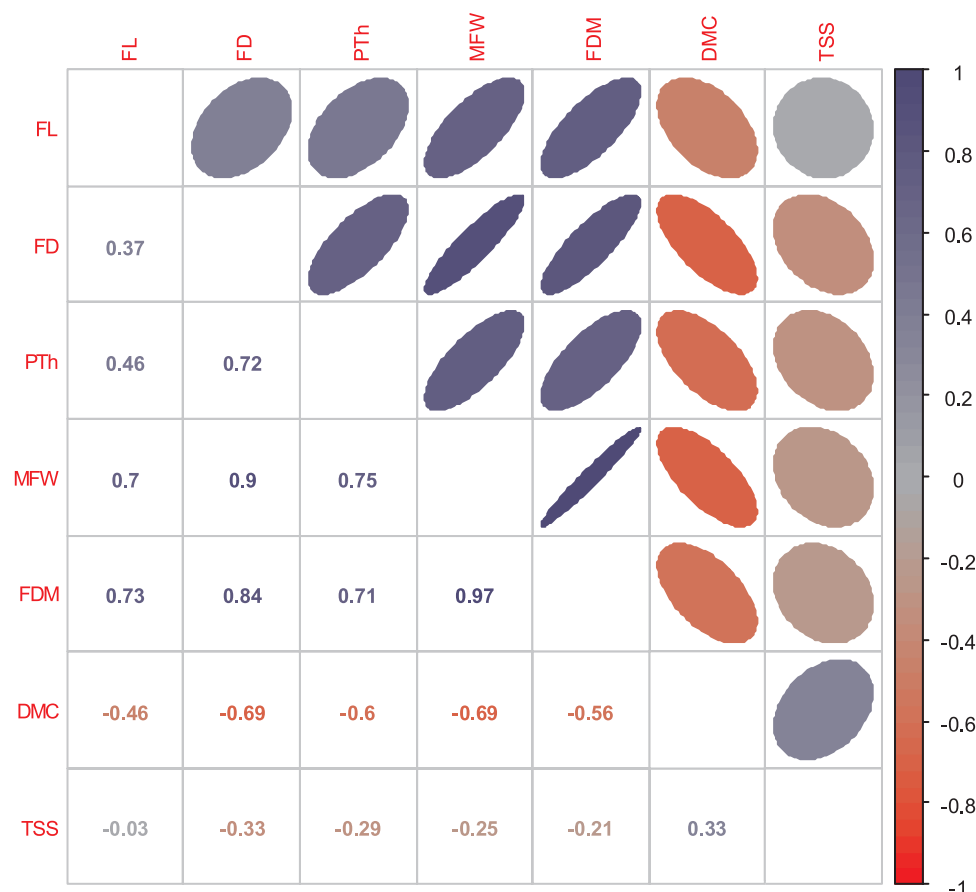


Fig. 2. Estimates of Pearson's correlation coefficients among seven fruits agronomic traits of 65 *Capsicum chinense* accessions. FL: fruit length; FD: fruit diameter; PTh: pericarp thickness; MFW: mean fruit weight; FDM: fruit dry matter; DMC: dry matter content; TSS: total soluble solids.

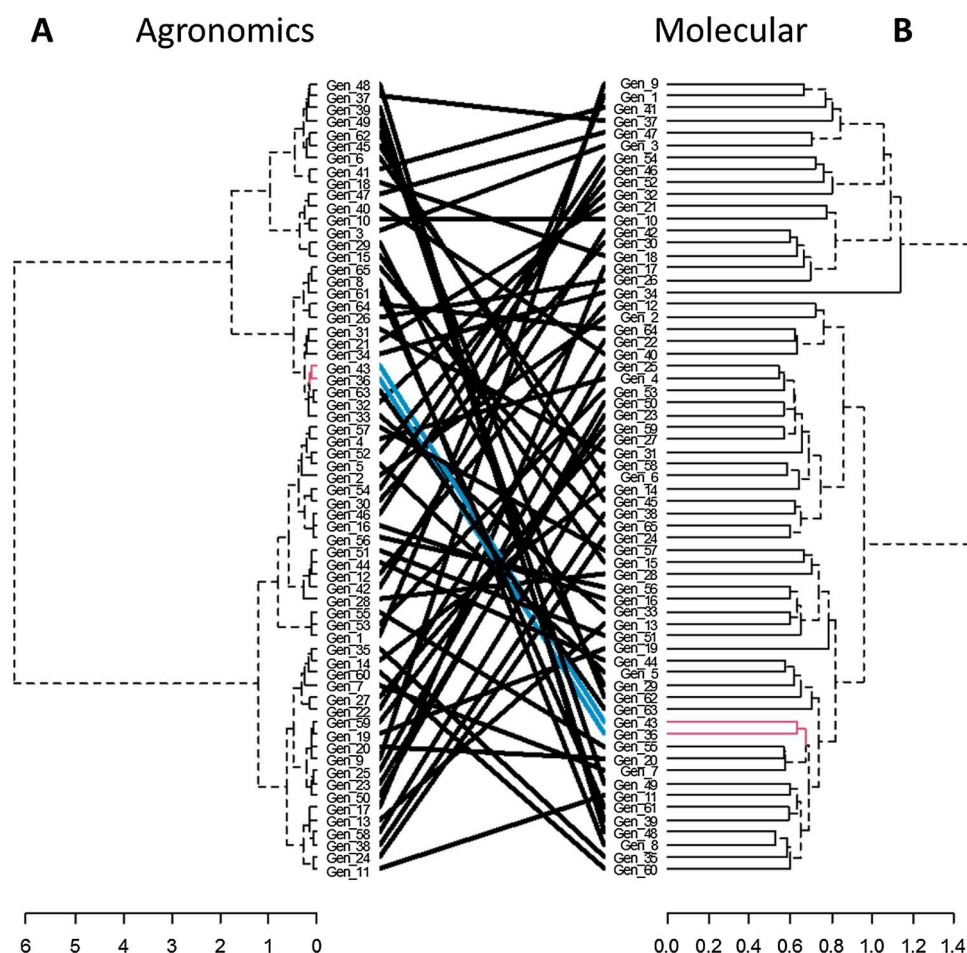


Fig. 3. Dendrogram of genetic dissimilarity obtained by Ward's method, from agronomic (A) and molecular (B) traits of 65 *Capsicum chinense* accessions.

www.r-project.org) (Fig. 3a). Group I consisted of 15 accessions, in which the highest estimates of the mean genotypic value ($\mu + g + gem$) were obtained for FL, FD, PTh, MFW, and FDM (Fig. 1). On the other hand, group III, with 38 accessions, obtained the lowest values of $\mu + g + gem$ for the same traits (FL, FD, PTh, MFW, and FDM) and the highest values for DMC and TSS. Group II, formed by 13 accessions, obtained intermediate $\mu + g + gem$ values for most of the traits.

3.3. Molecular diversity

Four AFLP primer pairs produced 695 fragments, of which 645 were polymorphic, representing 92% polymorphism. The combinations E-ACA/M-CAC, E-ACG/M-CAA, E-ACT/M-CAA, E-ACG/M-GAC produced 127, 175, 164, and 179 fragments, respectively.

The similarity coefficient used to calculate the genetic distance between the 65 *C. chinense* accessions ranged from 0.53 to 0.92, with a mean distance of 0.70 (± 0.08 SD). The distribution frequency analysis of the compared pairs of genotypes revealed a concentration of values in the classes of 0.6–0.7 and 0.7–0.8 (Fig. 4). The greatest distance was observed between accessions Gen_34 and Gen_52, while Gen_8 and Gen_48 were closest to each other.

By Ward's hierarchical clustering analysis, two groups were defined by the indices proposed by the NbClust package (Fig. 3b). Group I and II consisted of 18 and 47 accessions, respectively, in which no association with the geographical origin and agronomic fruit traits was observed. No correlation was observed either between the distance matrices of the morphoagronomic and molecular data.

Results of the simulations obtained with software Structure and the Δk value methodology (Evanno et al., 2005) revealed the association of

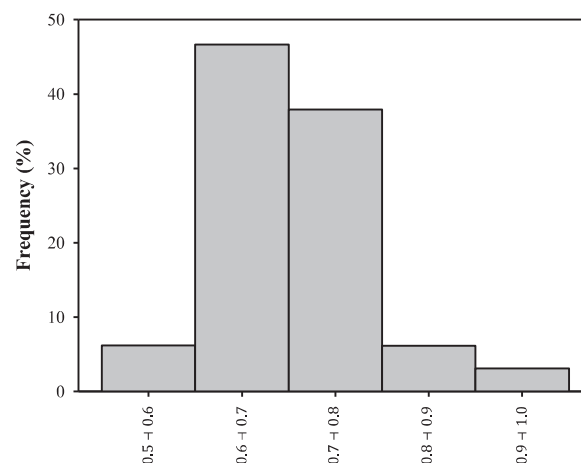


Fig. 4. Distribution of the dissimilarity frequency based on AFLP markers among the 65 *Capsicum chinense* accessions.

the accessions into two groups (Fig. 5). Also a partial agreement of Structure analysis with Ward clustering was observed, in which 9 and 48 accessions were assigned to groups I and II, respectively. Eight accessions were classified as admixture for having an adhesion coefficient lower than 0.6 for both groups.

3.4. Association analysis

Table 3 shows associations significant at 1.55×10^{-3} probability

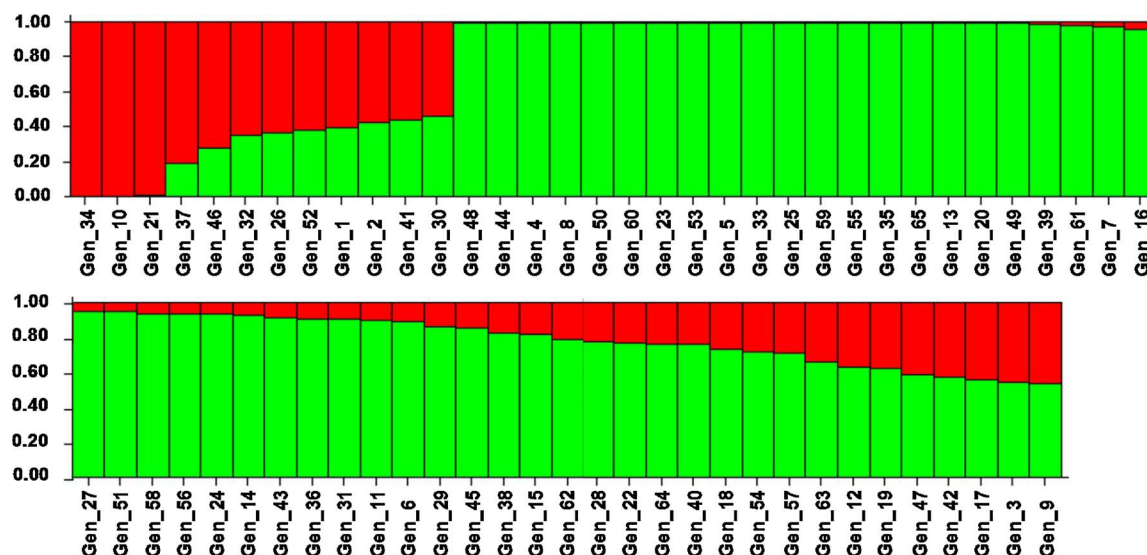


Fig. 5. Assignment of 65 *Capsicum chinense* accessions by the structure bar plots based on four AFLP primer combinations (E-ACA/M-CAC, E-ACG/M-CAA, E-ACT/M-CAA, and E-ACG/M-GAC). Two colors represent different clusters. The y-axis displays the estimated percentage membership of each accession in a determined cluster.

Table 3

Markers associated with agronomic traits by four statistical models using AFLP markers and phenotyping in 65 *Capsicum chinense* accessions.

Traits ^{2/}	Models ^{1/}											
	GLM: G + P			GLM: G + P + Q			MLM: G + P + K			MLM: G + P + Q + K		
	Marker	Size	Pvalue	Marker	Size	Pvalue	Marker	Size	Pvalue	Marker	Size	Pvalue
FL	Eacg/Mcaa154	360 pb	4.50E-05	Eact/Mcaa136	332 pb	9.82E-05	Eact/Mcaa136	332 pb	8.08E-05	Eact/Mcaa136	332 pb	4.12E-04
	Eaca/Mcac15	93 pb	5.22E-05	Eacg/Mcaa163	398 pb	3.53E-05						
	Eact/Mcaa136	332 pb	9.36E-04	Eacg/Mcaa154	360 pb	4.84E-04						
	Eacg/Mcaa163	398 pb	2.13E-04	Eaca/Mcac15	93 pb	5.04E-04						
	Eact/Mcaa135	331 pb	5.04E-04									
FD	Eaca/Mcac44	163 pb	0.00109									
	Eacg/Mcaa15	82 pb	2.37E-05	Eacg/Mcaa15	82 pb	4.95E-05						
PTh	Eacg/Mcaa14	81 pb	1.27E-04	Eacg/Mcaa14	81 pb	7.87E-05						
	Eacg/Mcaa15	82 pb	8.11E-04	Eacg/Mcaa15	82 pb	0.00126						
MFW	Eacg/Mcaa121	279 pb	0.00126									
	Eacg/Mcaa15	82 pb	2.60E-06	Eacg/Mcaa15	82 pb	6.79E-06						
	Eacg/Mcaa14	81 pb	4.59E-04	Eacg/Mcaa14	81 pb	2.35E-04						
	Eacg/Mcaa69	177 pb	0.00125									
FDM	Eacg/Mcaa15	82 pb	4.09E-05	Eacg/Mcaa15	82 pb	9.97E-05						
				Eacg/Mcaa14	81 pb	0.00125						
				Eacg/Mcaa15	82 pb	1.73E-07	Eacg/Mcaa15	82 pb	3.88E-04	Eacg/Mcaa15	82 pb	3.25E-04
DMC	Eacg/Mcaa14	81 pb	2.38E-04	Eacg/Mcaa14	81 pb	1.01E-04						
	Eacg/Mgac177	489 pb	0.00133									
	Eact/Mcaa89	220 pb	5.47E-04	Eact/Mcaa89		0.00116						

^{1/} GLM: Linear model. G: Matrix of presence/absence of markers identified by AFLP. Q: Population structure defined by STRUCTURE software. MLM: Mixed linear model. K: Kinship matrix estimated according to Hardy (2003). Significant P value according to moderately restrictive test. ^{2/} FL: fruit length; FD: fruit diameter; PTh: pericarp thickness; MFW: mean fruit weight; FDM: fruit dry matter; DMC: dry matter content; TSS: total soluble solids.

between the AFLP markers and the agronomic traits studied, for the four mathematical models tested. The naive model (GLM: G + P), derived from the phenotypic data set and molecular data, detected the highest number of significant markers for most evaluated traits. The model GLM: G + P + Q, which takes the population structure by the Structure method into account, indicated that for the traits FD and TSS, the identified markers were the same as those obtained by the naive method. For MFW, one marker was observed that was not present in the naive model, whereas for the other traits there was a reduction in the number of significant associations.

The third model, based on kinship information, i.e., the kinship matrix (MLM: G + P + K), revealed significant associations only for FL and DMC. Lastly, the model MLM, used to evaluate G + P + Q + K, identified the same markers obtained for FL and DMC.

4. Discussion

In this study, 65 *C. chinense* accessions from different geographic regions of Brazil and different varietal groups were characterized. These accessions showed wide genotype diversity in fruits, evidenced by the different shapes and sizes (Fig. 1). In fruits from the Caribbean and Brazil, Bharath et al. (2013) and Baba et al. (2016) also observed a wide diversity, respectively.

The low $\hat{\sigma}_{in}^2$ estimates indicated that the GE interaction had little influence on the phenotypic value. In this context, the accessions were predictable in relation to variations in the planting times, observed in the genotype correlation between the environments (Table 2). Based on Aclin and using the criterion proposed by Resende and Duarte (2007), the experimental accuracy was considered very high (≥ 0.90) for FD, high (≥ 0.70) for FL, PTh, MFW and FDM, moderate (≥ 0.50) for DMC,

and low for TSS. This parameter shows the accuracy of the inferences of the genotype means, informing about the correct ranking of the accessions for selection purposes and about the effectiveness of conclusions about the genotypic value of the accession, which is a correlation between the predicted and true genotypic values (Resende, 2002). A low accuracy value for the TSS variable can hamper the selection of accessions, since this variable was highly influenced by the environment.

The variables TSS and DMC had the lowest h_g^2 values (0.31 and 0.55, respectively) (Table 2). Geleta and Labuschagne (2006) evaluating the combining ability and heritability of *C. annuum* hybrids, verified that the TSS had a low value of h_g^2 (0.15), being that additive and non-additive effects are involved in the genetic control of these trait. Studying the inheritance of agronomic traits in *C. baccatum*, Bento et al. (2016) reported a h_g^2 of 0.38 for TSS, with a predominance of non-additive effects. However, Rêgo et al. (2011) evaluated 40 *C. baccatum* landraces, and found h_g^2 of 0.93 and 0.87 for TSS and DMC, respectively. For the other traits, the h_g^2 values agreed with those obtained by Rêgo et al. (2011) and Naegele et al. (2016), except for PTh, with a lower value. In an assessment of 264 *C. chinense* accessions, Bharath et al. (2013) found h_g^2 values of 0.73, 0.81 and 0.81 for FL, FD and MFW, respectively.

The negative correlation of fruit weight (fresh and dry) with DMC was also verified by Lannes et al. (2007), in an evaluation of 49 *C. chinense* accessions and reported correlations of -0.77 and -0.65 , respectively. Therefore, accessions with smaller fruits are more suitable for the production of dehydrated products. The accessions Gen_14, Gen_35, Gen_24, Gen_13, and Gen_11 revealed the highest values for DMC (22.42, 22.38, 21.89, 21.59, and 21.38%, respectively), while the highest values of FDM were observed for Gen_18, Gen_41, Gen_47, Gen_39, and Gen_40 (2.01, 1.91, 1.85, 1.80, and 1.76, respectively).

The accessions with heaviest fruits had a thicker pericarp, which is an important piece of information in the selection of accessions for fresh fruit sale. Fruits with a thicker pericarp are more resistant to wounds during post-harvest handling and have a fresher appearance for consumers than fruits with a thinner pericarp (Lannes et al., 2007). Positive correlation values between PTh x MFW were also mentioned by Lannes et al. (2007) and Rêgo et al. (2011) (0.79 and 0.52, respectively). The accessions with highest PTh values were Gen_37, Gen_45, Gen_6, Gen_48, and Gen_62 (0.275, 0.273, 0.269, 0.265, and 0.263 mm, respectively).

The absence of correlation between TSS and the other fruit traits (FL, FD, PTh, MFW, FDM, and DMC) agreed partly with Rêgo et al. (2011), except for TSS x DMC and TSS x PTh which were found to be significant (0.49 and -0.48 , respectively). Lannes et al. (2007) also observed significance between TSS x DMC and TSS x PTh (0.69 and -0.70 , respectively). In an analysis of the F_3 population of a cross between two *C. annuum* genotypes, Ben-Chaim et al. (2001) found that TSS was not correlated with any of the traits, except for PTh in 1997 (-0.34).

In the hierarchical grouping of fruit traits, fruit weight was one of the main discriminating traits of the groups (Fig. 1). In general, the accessions could not be grouped based on the geographic origin, since the accessions of different geographic areas were classified in different groups. This result was also corroborated by Finger et al. (2010) and Baba et al. (2016).

The AFLP markers identified high levels of genetic variability among accessions of *C. chinense* (Fig. 4). The polymorphism was higher than that described by Aktas et al. (2009), Castañón-Najera et al. (2011) and Islam et al. (2016), who also used AFLP markers in *Capsicum* spp. This difference may be related to the diversity of the accessions as well as the efficiency of the AFLP primer combination in detect polymorphism. In grouping analysis based on AFLP data, there was no association between molecular groups and geographic origin of the accessions. This can probably be attributed to the rapid seed dissemination by birds over short distances and between different

regions, seed exchange between farmers and free fruit transport between the different regions of Brazil (Baba et al., 2016).

When comparing the groups formed by the agronomic data of fruits and molecular, an absence of association was also observed, indicating that both characterization stages are important for a better understanding of the differentiation among *C. chinense* accessions. Several studies with *Capsicum* spp. pointed out the importance of phenotypic and molecular characterization for a better understanding of the genetic variability (Costa et al., 2006; Baba et al., 2016; Leite et al., 2016; Rivera et al., 2016). According to Leite et al. (2016), when using accessions of germplasm banks as source of genetic variability in breeding programs, the choice of precursor lines should be based on genotyping and phenotyping data, to meet the expectations of breeders in terms of high genetic value, heterosis and genetic variability.

The results of the association analysis showed that when the population structure and kinship information were used, there was a reduction in the number of significant associations for most of the traits. According to Achleitner et al. (2008) the greatest challenge in association analysis is to separate the role of population structure from the role of genetic linkage because of the phenotype-marker associations. The authors claim that the differences in results between the models can illustrate the relative importance of different parts of the population structure represented by different models, and the incorporation of the Q and K matrices to improve the correction of the population structure.

Considering all models, it was noted that marker Eacg/Mcaa15 was associated with more than one trait (FD, PTh, MFW, and FDM). Marker Eacg/Mcaa14 was also related to FD, MFW and FDM. The effect of these markers on these traits was reinforced by the positive correlation observed between the traits. According to Ben-Chaim et al. (2001), the traits FD and MFW shared common QTLs that were positively correlated. In addition, the authors explained that the association between traits depend on complex physiological processes, involved in fruit development and also suggested that this physiological relationship increases the probability that the co-location of these QTLs is a result of pleiotropy. In *C. baccatum*, Moulin et al. (2015) identified two common QTLs for FD, MFW and FDM and one for FD and MFW. In *C. annuum*, Han et al. (2016) also found a common QTL for FD and MFW. In both studies, a positive correlation was observed between FD and MFW.

The markers Eacg/Mcaa14 and Eacg/Mcaa15 were also detected in association with trait DMC (Table 3), which was significantly correlated with the others. However, the sign of the effect due to the presence of the fragments was contrary to that observed for the other traits. In this study, AFLP markers were used for association analysis, although, for an effective use in breeding programs, they must be validated and converted into Sequence Characterized Amplified Regions (SCAR) markers, which is considered more reproducible and less work-intensive allowing their use for large-scale screening (Wei et al., 2009). In this way, this conversion becomes an interesting alternative for their use in marker-assisted selection programs.

5. Conclusions

The studied fruit traits and AFLP molecular markers detected high levels of genetic variability among the 65 *C. chinense* accessions, potentiating their use in pepper breeding programs. Several AFLP markers, significantly associated with several traits, were identified and considered interesting candidates for further studies.

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