

1 **Selection of popcorn genotypes tolerant to *Spodoptera frugiperda* and key**
2 **traits related to the identification of tolerance**

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4 Amanda Tami Kuroda⁽¹⁾; Jocimar Costa Rosa⁽¹⁾; Luiz Felipe Antunes de Almeida⁽¹⁾; Giovana
5 Dal Lago Garcia⁽¹⁾; Gustavo Arana Demitto⁽¹⁾; Renata Maria Bento de Souza⁽¹⁾; Fernando Alves
6 de Albuquerque⁽¹⁾; Carlos Alberto Scapim⁽¹⁾

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8 ⁽¹⁾ Universidade Estadual de Maringá. Departamento de Agronomia. Avenida Colombo, 5790-
9 Zona 7, CEP: 87020-900 Maringa-Parana-Brazil

10 E-mails: amandakuroda@hotmail.com; joce_jcosta@hotmail.com; lfaa0597@gmail.com;
11 giovanadallago@hotmail.com; gdemitto@gmail.com; renata_d_souza@hotmail.com;
12 faalbuquerque@uem.br; cascapim@uem.br

13

14 * Corresponding author

15 Email: joce_jcosta@hotmail.com

16

17 **Authors' Contributions**

18 **Conceptualization:** Kuroda AT, Rosa JC, Scapim CA, Albuquerque FA. **Data acquisition:**
19 Kuroda AT, Almeida LFA, Garcia GDL, Demitto GA, Souza RMB. **Data analysis:** Rosa JC,
20 Scapim CA, Albuquerque FA. **Design of Methodology:** Kuroda AT, Rosa JC, Scapim CA,
21 Albuquerque FA. **Writing and editing:** Rosa JC, Scapim CA, Albuquerque FA.

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26 **Abstract**

27 The *Spodoptera frugiperda*, is one of the most deleterious pests of popcorn and the
28 identification of tolerant genotypes is determinant in breeding programs. The objective of this
29 study was to select popcorn genotypes tolerant to *S. frugiperda* and the key traits related to the
30 identification of tolerance. The popcorn varieties UEM J1, Composto Márcia, Arachida,
31 Composto Gaúcho and Zapalote Chico (resistant check) were evaluated in a completely
32 randomized design with 100 replications. The experimental unit consisted of one Petri dish,
33 containing plant material and a larva. The following traits were evaluated: larval stage duration
34 (LSt), food intake weight (IW), final larva weight (FW), mean larva weight (MW), feces (F),
35 assimilated (A) and metabolized food weight (M), relative consumption rate (RCR), relative
36 metabolic rate (RMR), relative growth rate (RGR), conversion efficiency of ingested food
37 (CEI), apparent digestibility (AD), conversion efficiency of digested food (CED) and leaf area
38 consumed (LAC). The diagnosis of multicollinearity, analysis of canonical variables, genetic
39 divergence, hierarchical clustering, factor analysis and canonical correspondence analysis were
40 carried out to perform multivariate analysis. After the multicollinearity test, the traits FW, IW,
41 RCR, AD and LAC were maintained for further analysis. Variety Arachida was considered
42 tolerant to *S. frugiperda* and can be used in the future as a source of favorable alleles to breed
43 tolerant popcorn hybrids. The traits relative consumption rate, apparent digestibility and leaf
44 area consumed were considered key traits in the identification of tolerance against *S. frugiperda*
45 in popcorn genotypes.

46

47 **Introduction**

48 The fall armyworm, *Spodoptera frugiperda*, is considered one of the most deleterious
49 pests of maize in all maize-producing regions [1]. In the tropics, it causes significant economic

50 damage because it feeds on maize from the seedling to the reproductive stage [2]. In popcorn,
51 which has been less the focus of breeding efforts than field corn, the damage tends to be more
52 severe, since no tolerant genotypes are available on the market. Considering the increasing
53 nationwide and worldwide demand for and importance of popcorn [3], the possibility of control
54 by the development of tolerant genotypes must be investigated and improved, since the number
55 of insecticide applications has increased considerably over the years [4, 5], resulting in
56 excessive costs and increased environmental risks.

57 In view of the concern to reduce or even eliminate insecticide applications and only a
58 few of studies involving *S. frugiperda* tolerance in popcorn, breeding programs ought to
59 intensify studies to the selection of tolerant popcorn genotypes, as well as to elucidate key traits
60 related to the identification of tolerance [6, 7].

61 Although some traits related to identification of *S. frugiperda* tolerance, e.g., larval stage
62 duration, final and mean larva weight, consumption rates and leaf area consumed have been
63 studied in maize breeding programs [7], they have not yet been conclusively proven and used
64 as key traits. A likely reason may be that the trials were analyzed based on a univariate and
65 inconclusive approach. The use of multivariate techniques is required, which can be an adequate
66 and more efficient tool in the analysis of tolerance-related data in popcorn [8-10].

67 Resistance is the set of physical, chemical and morphological traits that will negatively
68 affect the insect's oviposition and feeding behavior, while tolerance is the set of traits that will
69 cause plants to withstand the attack of insects without substantial reductions in productivity in
70 comparison with other susceptible genotypes [8, 9, 11].

71 In this study, the hypothesis was proposed that tolerance to *S. frugiperda* can be found
72 in popcorn germplasm from tropical regions and that multivariate analysis can discriminate the
73 main traits related to the identification of tolerance. The objective of this study was to select

74 popcorn genotypes tolerant to *S. frugiperda* and the key traits related to the identification of
75 tolerance.

76

77 **Material and methods**

78 The trial was carried out in Maringá, Paraná, Brazil, to evaluate five popcorn varieties
79 (Table 1) for tolerance to fall armyworm (*Spodoptera frugiperda*), as well as the key traits
80 related to the identification of this tolerance.

81 The popcorn genotypes used in the trial consisted of plants of the varieties UEM J1,
82 Composto Márcia, Arachida, Composto Gaúcho and Zapalote Chico, grown in a greenhouse.
83 The first four evaluated genotypes were developed by the Specialty Corn Breeding program of
84 the State University of Maringá. The variety used as check, Zapalote Chico, was introduced
85 from Central América and has tolerance against fall armyworm on the basis of antixenosis
86 (alimentary avoidance) and antibiosis (lower insect survival after feeding on host tissue) [12,
87 13].

88

89 **Table 1.** Description of the genotypes selected for study.

| Code | Genotype | Origin | Grain type | Genetic base |
|------|-----------------|--------|------------|-------------------------|
| 1 | UEM J1 | LIV | Popcorn | Open-pollinated variety |
| 2 | Zapalote Chico | CIMMYT | Field corn | Open-pollinated variety |
| 3 | Composto Márcia | MABH | Popcorn | Open-pollinated variety |
| 4 | Arachida | LIV | Popcorn | Open-pollinated variety |
| 5 | Composto Gaúcho | MABH | Popcorn | Open-pollinated variety |

90 LIV: local landrace varieties, MABH: mixture of American and Brazilian hybrids.

91

92 The plants were grown in a greenhouse with automatic irrigation. Crop management
93 was carried out in accordance with the recommendations for corn culture [16]. The seeds were
94 separated and three sown in each pot, which contained soil and substrate (3:1). After sowing,
95 the pots were irrigated daily and side-dressed with urea (45% N). The other cultural treatments

96 were applied as required for full crop development, without using any other chemical product,
97 so as not to affect larva growth.

98 The corn leaves used to feed the larvae were collected when the plants were in the
99 eight-leaf (V8) stage, so that all genotypes were evaluated when the plants were in the same
100 developmental stage [6, 7, 17].

101 The insects required to initiate the trial were hatched from *S. frugiperda* eggs donated
102 by EMBRAPA Soybean, in Londrina, Paraná, and from eggs collected in corn fields on the
103 Experimental Farm of Iguatemi - Maringá. The larvae hatched from these eggs were fed an
104 artificial diet and three generations were reared for our use in the trial.

105 The laboratory trial was carried out in an air-conditioned chamber at 25°C±1, air
106 humidity of 70% ± 10 and a 12h photoperiod. Each experimental unit consisted of a sterile
107 acrylic Petri dish (diameter 9.0cm, height 1.5cm), lined with filter paper moistened with
108 distilled water to maintain the leaf turgor, containing only one larva per dish, to avoid insect
109 cannibalism. Each treatment consisted of three Petri dishes with moist filter paper and plant
110 material, to calculate the water loss.

111 The trial was conducted in a completely randomized design, with five treatments and
112 100 replications.

113 After hatching of the fourth larva generation raised on artificial diet, they were
114 distributed in separate Petri dishes. The filter paper was changed, plant material was supplied
115 to feed the larvae and the biological parameters were evaluated daily. Plant material of the same
116 genotype was continuously supplied until the end of the larval stage.

117 The traits were evaluated as proposed by [18] with changes made by [19] as follows:

118 Larval stage duration: LSt

119 Food intake weight: IW

120 Final larva weight: FW

121 Mean larva weight during T: MW

122 Feces: F;

123 Assimilated food: $A = I - F$

124 Metabolized food: $M = A - FW$

125 Relative consumption rate: $RCR = I / (MW * T)$

126 Relative metabolic rate: $RMR = M / (MW * T)$

127 Relative growth rate: $RGR = FW / (MW * T)$

128 Conversion efficiency of ingested food: $CEI = (FW/I) * 100$

129 Apparent digestibility: $AD = (H/A) * 100$

130 Conversion efficiency of digested food: $CED = FW/A * 100$

131 Leaf area consumed: $LAC = I / SDm$, where SDm: Mean surface density.

132 Food consumption and use were evaluated daily throughout the larval stage, by
133 weighing the fresh food weight, leftover food weight, feces weight and larva weight. The values
134 were measured for each larva and the mean for each repetition was calculated according to the
135 number of live larvae on the day of the evaluation. The final data were obtained from the mean
136 of the 100 replications per treatment.

137 The larva weight was measured directly by individual weighing during the whole
138 larval stage. The daily weighing of the larvae was only initiated after the 5^d after egg hatch.
139 Food intake weight (IW) was calculated indirectly, by subtracting of the statistically corrected
140 leftover food (Lc) weight from the weight of the supplied food (SF) on the day before.

141 The excreta were collected and weighed individually for each larva during the entire
142 larval stage to obtain the total weight of feces produced (F). The leaf area consumed (LAC) was
143 calculated indirectly from the relationship between food intake (I) and mean surface density
144 (SDm).

145 The assumptions of normality of residuals and homogeneity of variances were
146 evaluated by the Shapiro-Wilk and Levene tests, respectively. The data were processed
147 statistically by multivariate analysis of variance (MANOVA), using the statistical software
148 Genes [20] integrated with R software [21].

149 The Variance Inflation Factor (VIF) and the Condition Index (CI) were used as criteria
150 to assess the degree of multicollinearity between the predictive traits. Variance inflation factors
151 of > 10 are generally considered evidence of substantial multicollinearity and normally the
152 reason for the removal of certain predictors. In addition, multicollinearity is considered weak
153 when the CI is less than 100 [14, 16].

154 Genetic divergence between the genotypes was evaluated by UPGMA
155 (Unweighted Pair-Group Method using Arithmetic Averages), based on Mahalanobis'
156 distance. The groups were established according to the methodology proposed by [22]. Then,
157 canonical variable analysis was carried out with clustering by the Tocher method, factor
158 analysis and later canonical correspondence analysis [23].

159 Factor analysis was performed considering all evaluated traits [24]. The factorial loads
160 were extracted by the principal component method, and the factors established by varimax
161 rotation. In this study, factor loads above 0.90 as well as the highest values for the community
162 were considered [25]. Canonical correspondence analysis was performed as described by [26].

163 The analyses were performed using the statistical software Genes [20] and SAS [27]
164 at 1% probability.

165

166 **Results and discussion**

167 Normality ($p > 0.01$) and homogeneity of variances ($p > 0.01$) were reported for all
168 evaluated traits. The MANOVA test showed significant differences between the mean vectors
169 of the genotypes for all evaluated traits ($p < 0.01$), indicating the existence of genetic variability.

170 Multicollinearity among traits was assessed by the criteria VIF and CI. Traits with VIF
 171 and CI values greater than 10 and 100, respectively, are generally considered evidence of
 172 substantial multicollinearity among variables and makes the removal of these predictors
 173 necessary [28, 15]. The traits LSt, MW, F, A, M, RMR, RGR, CEI and CED had high VIF and
 174 CI values and were therefore eliminated. On the other hand, the traits FW, IW, RCR, AD and
 175 LAC had VIF and CI had values below 10 and 100, respectively, and were therefore maintained
 176 for subsequent analyses (Table 2).

177

178 **Table 2.** Diagnosis of multicollinearity for traits related to *Spodoptera frugiperda* tolerance in
 179 five popcorn varieties (*Zea mays* L.): final larva weight (FW), food intake weight (IW), relative
 180 consumption rate (RCR), apparent digestibility (AD) and leaf area consumed (LAC).

| Variance inflation factor (VIF) | | Condition index (CI) | |
|---------------------------------|---------------------|----------------------|-------------|
| Diagonal | Inverse element (r) | Order | Eigenvalues |
| 1 | 2.9494 | 1 | 2.0070 |
| 2 | 5.2925 | 2 | 1.9228 |
| 3 | 3.6100 | 3 | 0.8289 |
| 4 | 2.6017 | 4 | 0.1538 |
| 5 | 5.7045 | 5 | 0.0876 |
| Number of VIFs ≥ 10 | 0 | CI (max/min) | 22.9163 |

181

182 Multivariate techniques to select traits related to *S. frugiperda* tolerance in popcorn
 183 genotypes were also used by [7, 17] and as in this study, the application of multivariate analysis
 184 allowed a significant reduction in the number of traits. This raises the chances of a more
 185 effective selection, since the smaller number of traits prevents the effect of interrelationships
 186 among them, avoiding redundancy and mistakes in the process of selecting promising
 187 genotypes [8].

188 The selected traits can, at the end of this study, be described as directly related to the
 189 damage caused by *S. frugiperda* larvae and can be considered key traits for the tolerance of
 190 popcorn genotypes, in that tolerant genotypes will have a smaller leaf area consumed (LAC)

191 and a lower food intake weight (IW) and relative consumption rate (RCR), as well as less
192 apparent digestibility (AD), which will result in a lower final larva weight (FW) [29, 30].

193 In this study, the use of multivariate techniques for selection and grouping was used
194 as a way to validate both the selection of tolerant genotypes and the influence of the chosen
195 traits on the identification of tolerance.

196 In multivariate procedures, one of the most widely used techniques is hierarchical
197 clustering [6, 7, 17, 31]. By these methods, genotypes are grouped by a process that is repeated
198 at several levels, establishing a dendrogram, with no predetermined optimal number of groups.
199 For this case, [32] described different forms of representing the clustering structure based on
200 the distance between the genotype pairs, for which UPGMA is the most commonly used method
201 [17, 33, 34].

202 The relationships between the varieties UEM J1, Zapalote Chico, Composto Márcia,
203 Arachida and Composto Gaúcho can be observed in a graph of the results of the dendrogram
204 based on Mahalanobis' generalized distance, grouped by the UPGMA method (Figure 1). The
205 high cophenetic correlation coefficient ($CCC = 0.93$) indicated an optimal fit between the
206 original data and the dissimilarity matrix and high accuracy of the experimental results. The
207 dendrogram shows two main groups, making the discrimination of the tolerance and/or
208 susceptibility of each genotype group possible [1, 5, 23].

209 The first group contained the variety Zapalote Chico, considered by several authors as
210 tolerant to *S. frugiperda* [7, 13, 17], and variety Arachida. The varieties Composto Gaúcho,
211 UEM J1 and Composto Márcia were grouped in the second (Figure 1).

212

213 **Figure 1.** Dendrogram of hierarchical analysis based on Mahalanobis' generalized distance for
214 the traits grouped by the UPGMA method among five popcorn varieties. Cutline established
215 according to Mojena, (1977).

216

217 The proximity of the varieties Arachida and Zapalote Chico may suggest tolerance of
218 variety Arachida to *S. frugiperda*, since the grouping was based on the same traits for both
219 varieties. The results show (Table 3) that the varieties Arachida and Zapalote Chico had the
220 lowest values for index of leaf area consumed (LAC), food intake weight (IW) and relative
221 consumption rate (RCR), aside from the low apparent digestibility (AD) and low final larva
222 weight (FW), which indicate tolerance of these two varieties. The cultivar Zapalote Chico is so
223 far the only one treated as tolerant to *S. frugiperda*, so the identification of new tolerant cultivars
224 can be considered a great gain to the breeding since it allows to explore different sources of
225 tolerance.

226

227 **Table 3.** Means of traits related to *Spodoptera frugiperda* tolerance in five popcorn varieties
228 (*Zea mays* L.): final larva weight (FW), food intake weight (IW), relative consumption rate
229 (RCR), apparent digestibility (AD) and leaf area consumed (LAC),

| Genotypes | FW (g) | | IW (g) | | RCR (g/g) | | AD (%) | | LAC (cm ²) | |
|-----------------|--------|----|--------|----|-----------|---|---------|----|------------------------|---|
| UEM J1 | 0.5434 | ab | 2.6850 | bc | 0.0925 | b | 37.3388 | c | 209.6653 | b |
| Zapalote Chico | 0.5306 | b | 2.4318 | d | 0.0780 | d | 39.4875 | bc | 173.8532 | d |
| Composto Márcia | 0.5348 | b | 2.6810 | c | 0.0862 | c | 41.0572 | ab | 209.7829 | b |
| Arachida | 0.5547 | ab | 2.4818 | d | 0.0763 | d | 39.5563 | bc | 186.7935 | c |
| Composto Gaúcho | 0.5678 | a | 2.8366 | a | 0.1088 | a | 43.1369 | a | 222.9237 | a |

230 Means followed by the same letter in a column do not differ statistically by the Roy and Bose test at 5% probability
231

232 Moreover, the performance of variety Composto Gaúcho was the worst, and it was
233 characterized as the most susceptible to *S. frugiperda*, based on the evaluated traits, since the
234 values of FW, IW, RCR, AD and LAC were high (Table 3).

235 In this study, variety Zapalote Chico was used as check because of its known tolerance
236 of the type antixenosis, i.e., feeding avoidance [13, 35], together with variety Arachida, with
237 antixenosis tolerance as well. This type of tolerance can be inferred from the low food intake
238 rate, low relative consumption and smaller leaf area consumed. In the case of variety Zapalote
239 Chico, the final larva weight was also low, indicating possible antibiosis tolerance.

240 The dendrogram was confirmed and analyzed in more detail by canonical variable
241 analysis, grouped by Tocher's clustering (Figure 2), which showed the presence of three distinct
242 groups. Tocher's optimization is a clustering method based on the formation of groups whose
243 distances within are shorter than the distances between groups. At the end of the process, the
244 number of groups and accessions contained in each group are computed. This method was
245 applied as suggested by [32] and is an important way of determining different groups based on
246 different traits, together with the techniques of dissimilarity analysis and analysis of canonical
247 variables [6].

248 The analysis of canonical variables explained 95.12% of the total variation between
249 the five traits. When analyzing the dispersion of the scores of the first two canonical variables,
250 there was an agreement with the previous groupings, confirming the results, as well as the
251 choice of the traits used in the study of *S. frugiperda* tolerance. The first canonical variable (VC
252 1) explained 87.85% of the total variation and the second (VC 2) 25.6% (Figure 2).

253 Tocher's grouping method, based on the analysis of canonical variables, grouped the
254 varieties Zapalote Chico and Arachida again. Similarly to the variety Zapalote Chico,
255 considered tolerant by several authors [7, 13, 17] and also in this study, some characteristics of
256 variety Arachida like final larva weight, food intake weight, relative consumption rate, apparent
257 digestibility and leaf area consumed also indicate tolerance. The varieties UEM J1 and
258 Composto Márcia, which, according to the means of the analyzed variables, were moderately
259 tolerant, were contained in the second group. Variety Composto Gaúcho remained isolated from
260 the other evaluated varieties, as the most susceptible to *S. frugiperda*, based on its performance
261 with regard to the analyzed traits (Table 3).

262

263 **Figure 2.** Biplot of canonical variable analysis showing the closest and most distant groups of
264 five popcorn genotypes grouped by Tocher's clustering.

265

266 Factor analysis is a multivariate statistical method that has been applied in agronomic
267 studies for a relatively short time [25]. This analysis explains the relationships observed
268 between traits and removes possible redundancies or duplications from a set of correlated
269 phenotypic data [36]. The method allows the selection of relevant traits, exploring the
270 relationships and their variations, apart from generating important information about factors
271 and genotypes [37]. In this study, the factor analysis was directed to the identification of key
272 traits related to tolerance to *S. frugiperda*.

273 By factor analysis, the traits are replaced by a smaller number of latent traits, called
274 factors. These factors group the traits, so that there is little or no variance within groups but
275 maximum variation between groups [32]. By this technique, associated with the analysis of
276 canonical variables and canonical correspondence, the traits that best discriminate genotypes
277 for a given objective, here *S. frugiperda* tolerance, can be efficiently selected.

278 In this study, the high commonality (from 0.7012 - A to 0.9912 - RCR), indicated traits
279 with a high relation to the determination of tolerance, confirming the thesis that the selected
280 traits can be considered key traits (Table 4). In the factor analysis, the first factor was
281 determinant for traits IW, FW, and RCR and the second for AD and LAC (Table 4). In this
282 study, estimates above 0.90 were considered for one factor and low estimates for the other factor
283 (Figure 3), which shows a high representativeness of the factor for the respective traits [25].
284 The estimates of the other traits were intermediate between both determined factors, which
285 despite having a certain degree of contribution to tolerance, were not as expressive as the
286 described traits. In the study of [17], a large number of traits was also reduced, and subsequently
287 divided into two factors by factor analysis.

288 According to the factor analysis, the chosen traits are directly correlated with the
289 selection of *S. frugiperda* tolerant genotypes. Thus, we proceeded to the analysis of canonical
290 correspondence.

291 **Table 4.** Factors and their factorial loads after rotation of the factor axis by the Varimax method
 292 for studied traits related to *Spodoptera frugiperda* tolerance in the composite varieties UEM J1,
 293 Zapalote Chico, Márcia, Arachida and Gaúcho.

| Variables | Factor score coefficients | | Loading factors after rotation | | Commonality |
|-----------|---------------------------|----------|--------------------------------|----------|-------------|
| | Factor 1 | Factor 2 | Factor 1 | Factor 2 | |
| LSt | 0.7481 | 0.1367 | 0.6362 | 0.4173 | 0.8784 |
| IW | 0.9849 | 0.1059 | 0.6240 | 0.8694 | 0.9813 |
| FW | 0.9745 | -0.1334 | 0.4302 | 0.9843 | 0.9195 |
| MW | 0.5249 | 0.8217 | 0.1639 | 0.8612 | 0.8508 |
| F | 0.6063 | 0.7794 | -0.1193 | 0.9803 | 0.8751 |
| A | 0.8301 | -0.5578 | 0.9820 | 0.1894 | 0.7012 |
| M | 0.8159 | -0.5627 | 0.9754 | 0.1760 | 0.9823 |
| RCR | 0.9460 | 0.1592 | 0.0645 | 0.9935 | 0.9912 |
| RMR | 0.7935 | 0.4367 | 0.5139 | 0.4968 | 0.7721 |
| RGR | 0.6952 | 0.5956 | 0.0733 | 0.9126 | 0.8381 |
| CEI | -0.8723 | -0.2911 | -0.4136 | -0.8213 | 0.8456 |
| AD | 0.1934 | 0.8863 | 0.8329 | -0.4218 | 0.9716 |
| CED | -0.8346 | 0.4910 | -0.9381 | -0.2400 | 0.9377 |
| LAC | -0.0728 | 0.9870 | -0.6063 | 0.9507 | 0.9625 |

294 LSt: larval stage duration, IW: food intake weight, FW: final larva weight, MW: mean larva weight, F: feces, A:
 295 assimilated food, M: metabolized food, RCR: relative consumption rate, RMR: relative metabolic rate, RGR:
 296 relative growth rate, CEI: conversion efficiency of food intake, AD: apparent digestibility, CED: conversion
 297 efficiency of the digested food and LAC: leaf area consumed.
 298

299 **Figure 3.** Biplot of factor analysis for traits related to tolerance to *Spodoptera frugiperda* in the
 300 composites UEM J1, Zapalote Chico, Márcia, Arachida and Gaúcho. LSt: larval stage duration,
 301 IW: food intake weight, FW: final larva weight, MW: mean larva weight, F: feces, A:
 302 assimilated food, M: metabolized food, RCR: relative consumption rate, RMR: relative
 303 metabolic rate, RGR: relative growth rate, CEI: conversion efficiency of food intake, AD:
 304 apparent digestibility, CED: conversion efficiency of the digested food and LAC: leaf area
 305 consumed.
 306

307 Canonical correspondence analysis is an exploratory technique to simplify the
 308 structure of multivariate data variability, in which the traits are arranged in contingency tables,
 309 taking correspondence measures between rows and columns of the data matrix into account.
 310 According to [38], correspondence analysis is a method to determine an association system
 311 between the elements of two or more sets, to explain the association structure of the factors in
 312 question. Thus, graphs were constructed with the principal components of the rows and
 313 columns, allowing the visualization of the relationship between the sets, where the proximity
 314 of the points referring to the row and the column indicates an association and distance indicates
 315 repulsion. According to [39], one of the great advantages of canonical correspondence analysis

316 is that relationships can be detected by this technique that would not have been perceived if the
317 analysis were based on trait pairs. In addition, it is highly flexible in the data traits, since no
318 theoretical model of probability distribution must be adopted. A rectangular matrix containing
319 non-negative data is sufficient, which in the field of breeding, makes it possible to masterfully
320 relate the effects of different traits on specific genotypes.

321 Canonical correspondence analysis explained 99.67% of the total variation between
322 the genotypes and the respective traits evaluated. The first canonical correspondence axis (CCA
323 1) explained 99.20% of the total variation and the second axis (CCA 2) accounted for 0.47%
324 (Figure 4). Most of the total variation was already explained in the first CCA, which is desirable,
325 for increasing the accuracy between the cluster and the estimated scores [38, 39].

326

327 **Figure 4.** Biplot of canonical correspondence analysis showing the relationship between the
328 five popcorn genotypes and the main explanatory traits of tolerance to *Spodoptera frugiperda*.
329 FW: final larva weight, IW: food intake weight, RCR: relative consumption rate, AD: apparent
330 digestibility, LAC: leaf area consumed.

331

332 The biplot of canonical correspondence analysis shows that the traits IW, FW and AD
333 were determinant for the tolerance expressed by the varieties Zapalote Chico and Arachida, in
334 which variable AD had a greater influence, due to its spatial angular proximity in the graph
335 with the said varieties. For the varieties Composto Gaúcho and Composto Márcia, the traits that
336 most contributed to the determination of tolerance or susceptibility were RCR and LAC. For
337 variety UEM J1, the influence of variable LAC was the highest.

338 In general, the selected traits were efficient in discriminating the genotypes regarding
339 tolerance and susceptibility to *S. frugiperda* by the applied analyses. The identification of key
340 traits in the description of tolerant genotypes will, in future studies, allow greater emphasis on
341 specific traits and consequently a more effective selection regarding tolerance in popcorn
342 genotypes.

343 **Conclusions**

344 Variety Arachida was identified tolerant to *S. frugiperda* and can be used as a source
345 of favorable alleles for the future development of tolerant popcorn hybrids.

346 The traits relative consumption rate (RCR), apparent digestibility (AD) and leaf area
347 consumed (LAC) were efficient and considered key traits for the identification of *S. frugiperda*
348 tolerant genotypes.

349

350 **References**

351 1. Juárez ML, Murua MG, Garcia MG, Ontivero M, Vera MT, Vilardi JC, Groot AT,
352 Castagnaro AP, Gastaminza G, Willink E. Host association of *Spodoptera frugiperda*
353 (Lepidoptera: Noctuidae) corn and rice strains in Argentina, Brazil, and Paraguay. Journal of
354 Economic Entomology. 2012; 105:573-582. <https://doi.org/10.1603/EC11184>

355 2. Ribeiro LP, Dequech STB, Camera C, Sturza VS, Poncio S, Vendramim JD. Vertical and
356 temporal distribution of *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) egg
357 masses, parasitized and non-parasitized, on maize plants. Maydica. 2014; 59:315-320.

358 3. Dawande R. Popcorn Market by Type (Microwave Popcorn and Ready-to-eat Popcorn) and
359 End User (Household and Commercial) - Global Opportunity Analysis and Industry Forecast.
360 2018; 2017-2023. Portland: Allied Market Research.

361 4. Farias JR, Andow DA, Horikoshi RJ, Sorgatto RJ, Fresia P, Santos AC. Field-evolved
362 resistance to Cry1F maize by *Spodoptera frugiperda* (Lepidoptera: noctuidae) in Brazil. Crop
363 Protection. 2014; 64, 150-158. <https://doi.org/10.1016/j.cropro.2014.06.019>

364 5. Omoto C, Bernardi O, Salmeron E, Sorgatto RJ, Dourado PM, Crivellari A, Carvalho RA,
365 Willse A, Martinelli S, Head GP. Field-evolved resistance to Cry1Ab maize by *Spodoptera*

- 366 *frugiperda* in Brazil. Pest Management Science. 2016; 72:1727-1736.
367 <https://doi.org/10.1002/ps.4201>
- 368 6. Paiva LA, Corrêa F, Silva CLT, Moura TL, Silva FC, Araújo MS, Jesus FG. Resistance of
369 corn genotypes to fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). African
370 Journal of Biotechnology. 2016; 15:1877-1882. <https://doi.org/10.5897/AJB2016.15399>
- 371 7. Oliveira NC, Suzukawa AK, Pereira CB, Santos HV, Hanel A, Albuquerque FA, Scapim
372 CA. Popcorn genotypes resistance to fall armyworm. Ciência Rural. 2018; 48:1-6
373 <https://doi.org/10.1590/0103-8478cr20170378>
- 374 8. Aaliya K, Qamar Z, Nasir IA, Ali Q, Farooq AM, Husnain T. Transformation, evaluation of
375 *gt* gene and multivariate genetic analysis for morpho- physiological and yield attributing traits
376 in *Zea mays*. Genetika. 2016; 48:423-433. <https://doi.org/10.2298/GENSR1601423A>
- 377 9. Veturi Y, Kump K, Walsh E, Ott O, Poland J, Kolkman JM, Balint-Kurti PJ, Holland JB,
378 Wisser RJ. Multivariate mixed linear model analysis of longitudinal data: An information-rich
379 statistical technique for analyzing plant disease resistance. Phytopathology. 2012; 102:1016-
380 1025. <https://doi.org/10.1094/PHYTO-10-11-0268>
- 381 10. Wisser RJ, Kolkman JM, Patzoldt ME, Holland JB, Yu J, Krakowsky M, Nelson RJ, Balint-
382 Kurti PJ. Multivariate analysis of maize disease resistances suggests a pleiotropic genetic basis
383 and implicates a GST gene. Proceedings of the National Academy of Sciences of the United
384 States of America. 2011. 108:7339-7344. <https://doi.org/10.1073/pnas.1011739108>
- 385 11. Souza BHS, Boiça-Júnior AL, Silva AG, Rodrigues, NEL. 2012. Não preferência para
386 alimentação e aspectos biológicos de *Spodoptera eridania* em cultivares de feijão-caupi. Revista
387 Caatinga 2012; 25:31-37.

- 388 **12.** Silveira LCP, Vendramim JD, Rossetto CJ. Efeito de genótipos de milho pipoca no
389 desenvolvimento de *Spodoptera frugiperda* (J.E. Smith). Anais da Sociedade Entomológica do
390 Brasil. 1997; 26:291-298. <http://dx.doi.org/10.1590/S0301-80591997000200010>
- 391 **13.** Viana PA, Potenza MR. Non-preference and antibiosis of fall armyworm for selected corn
392 genotypes. Bragantia. 2000; 59:27-33. <http://dx.doi.org/10.1590/S0006-87052000000100006>.
- 393 **14.** Montgomery DC, Peck EA. Introduction to linear regression analysis. New York: John
394 Wiley. 1981.
- 395 **15.** Prunier JG, Colyn M, Legendre X, Nimon KF, Flamand MC. Multicollinearity in spatial
396 genetics: separating the wheat from the chaff using commonality analyses. Molecular Ecology.
397 2015; 24:263-283. <https://doi.org/10.1111/mec.13029>
- 398 **16.** Vendramim JD, Nishikawa MAN. Melhoramento para tolerância a insetos. In: Nass LL,
399 Valois ACC, Melo IS, Valadaris-Inglis MC. Recursos genéticos e melhoramento: plantas.
400 Rondonópolis: Fundação MT. C. 2001; 24:737-781.
- 401 **17.** Sanches RE, Suzukawa AK, Contreras-Soto RI, Rizzardi DA, Kuki MC, Zeffa DM,
402 Albuquerque FA, Scapim CA. Multivariate analysis reveals key traits of fall armyworm
403 resistance in tropical popcorn genotypes. Bragantia. 2019; 78:175-182.
404 <http://dx.doi.org/10.1590/1678-4499.20180230>
- 405 **18.** Waldbauer GP. The consumption and utilization of food by insects. Advances in Insect
406 Physiology. 1968; 5:229-288. [https://doi.org/10.1016/S0065-2806\(08\)60230-1](https://doi.org/10.1016/S0065-2806(08)60230-1)
- 407 **19.** Scriber JM, Slansky Jr F. The nutritional ecology of immature insects. Annual Review of
408 Entomology. 1981; 26:183-211. <https://doi.org/10.1146/annurev.en.26.010181.001151>
- 409 **20.** Cruz CD. Genes Software-extended and integrated with the R, Matlab and Selegen. Acta
410 Scientiarum. Agronomy. 2016; 38:547-552. <https://doi.org/10.4025/actasciagron.v38i4.32629>

- 411 **21.** R Core Team. R: A language and environment for statistical computing. R Foundation for
412 Statistical Computing, Vienna, Austria. 2015. <https://www.R-project.org/>.
- 413 **22.** Mojena R. Hierarchical grouping methods and stopping rules: An evaluation. The Computer
414 Journal. 1977; 20:359-363. <https://doi.org/10.1093/comjnl/20.4.359>
- 415 **23.** Friendly M, Sigal M. Graphical methods for multivariate linear models in psychological
416 research: An R tutorial. The Quantitative Methods for Psychology. 2017; 13:20-45.
417 <https://doi.org/10.20982/tqmp.13.1.p020>
- 418 **24.** Johnson RA, Wichern DW. Applied Multivariate Statistical Analysis. Prentice Hall,
419 Englewood Cliffs. 1992, 816p.
- 420 **25.** Keith TZ, Reynolds MR. Using confirmatory factor analysis to aid in understanding the
421 constructs measured by intelligence tests. In: Flanagan DP, Mc Donough EM (Eds.),
422 Contemporary intellectual assessment: Theories, tests and issues. The Guilford Press. 2018, p.
423 853–900.
- 424 **26.** Greenacre MJ. Theory and applications of correspondence analysis. Academic Press,
425 London. 1984.
- 426 **27.** SAS Institute Inc. SAS version 9.3 User’s Guide. SAS Institute, Cary, NC. 2011.
- 427 **28.** O’Brien RM. A caution regarding rules of thumb for variance inflation factors. Quality &
428 Quantity. 2007; 41:673-690. <https://doi.org/10.1007/s11135-006-9018-6>
- 429 **29.** Rezende MAA, Cruz I, Della Lucia TMC. Consumo foliar de milho e desenvolvimento de
430 lagartas de *Spodoptera frugiperda* (Smith) parasitadas por *Chelonus insularis* (Cresson)
431 (Hymenoptera: Braconidae). Anais da Sociedade Entomológica do Brasil, Itabuna. 1994;
432 23:473-478.

- 433 **30.** Ríos-Díez JD, Saldamando-Benjumea CI. Susceptibility of *Spodoptera frugiperda*
434 (Lepidoptera: Noctuidae) Strains From Central Colombia to Two Insecticides, Methomyl and
435 Lambda-Cyhalothrin: A Study of the Genetic Basis of Resistance. *Journal of Economic*
436 *Entomology*. 2011; 104:1698-1705. <https://doi.org/10.1603/EC11079>
- 437 **31.** Kaiser HF. The varimax criterion for analytic rotation in factor analysis. *Psychometrika*.
438 1958; 23:187-200. <https://doi.org/10.1007/BF02289233>
- 439 **32.** Cruz CD, Carneiro PCS, Regazzi AJ. Modelos biométricos aplicados ao melhoramento
440 genético. 3ed. Viçosa: Ed. da UFV. 2014, 668p.
- 441 **33.** Sathua SK, Shahi JP, Mahato A, Gayatonde V, Kumar P. Molecular diversity analysis of
442 maize (*Zea mays* L.) inbreds using SSR markers. *Electronic Journal of Plant Breeding*. 2018;
443 9:1122-1129. <https://doi.org/10.5958/0975-928X.2018.00140.0>
- 444 **34.** Vivodík M, Petrovičová L, Balážová Ž, Gálová Z. Genetic diversity of maize accessions
445 (*Zea Mays* L.) Cultivated from europe using microsatellites markers. *Agrobiodiversity for*
446 *improving nutrition, Health and Life Quality*. 2017; 1:524-528.
447 <https://doi.org/10.15414/agrobiodiversity.2017.2585-8246.524-528>
- 448 **35.** Boiça Júnior AL, Galli AL, Bortoli SA, Rodrigues JR, Lara FM. Comparação entre vinte e
449 quatro genótipos de milho infestados por *Spodoptera frugiperda* (JE Smith, 1797)
450 (Lepidoptera: Noctuidae). *Anais da Sociedade Entomológica do Brasil, Londrina*. 1993;
451 22:131-137.
- 452 **36.** Cattell RB. A biometrics invited paper. Factor analysis: an introduction to essentials. I. The
453 purpose and underlying models. *Biometrics*. 1965; 21:190-215.
454 <https://doi.org/10.2307/2528364>

- 455 **37.** Vile D, Pervent M, Belluau M, Vasseur F, Bresson J, Muller B, Granier C, Simonneau T.
456 Arabidopsis growth under prolonged high temperature and water deficit: independent or
457 interactive effects? *Plant Cell and Environment*. 2012; 35:702-718.
458 <https://doi.org/10.1111/j.1365-3040.2011.02445.x>
- 459 **38.** Ter Braak CJF. The analysis of vegetation-environment relationships by canonical
460 correspondence analysis. *Theory and Models in Vegetation Science*. 1987; 1:69-77.
461 https://doi.org/10.1007/978-94-009-4061-1_7
- 462 **39.** Nyfjäll M. Aspects on correspondence analysis plots under complex survey sampling
463 designs. Research Report, Department of Information Science, division of Statistics, Uppsala
464 University, 2. 2002. <https://urn:nbn:se:uu:diva-42867>

Dendrogram

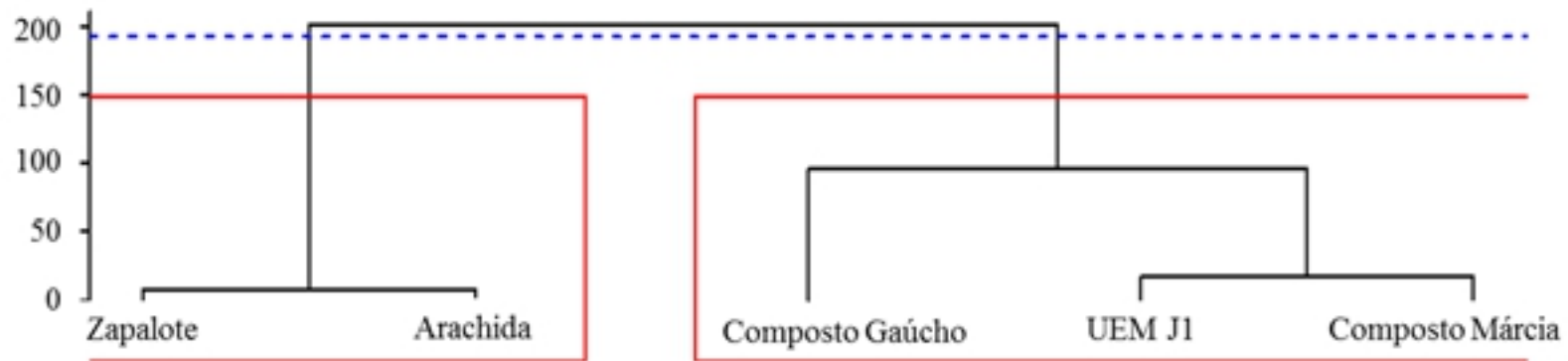


Figure 1

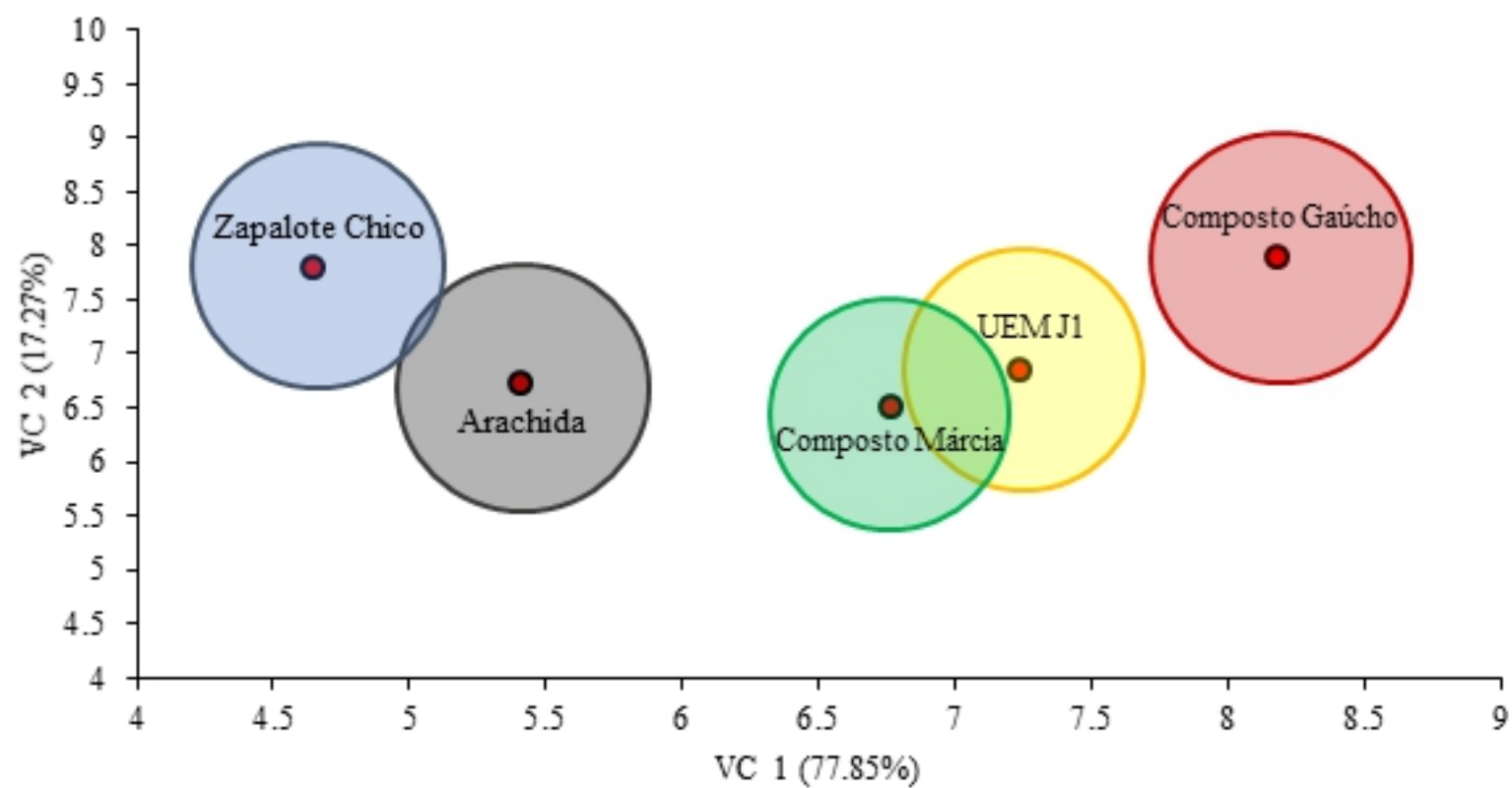


Figure 2

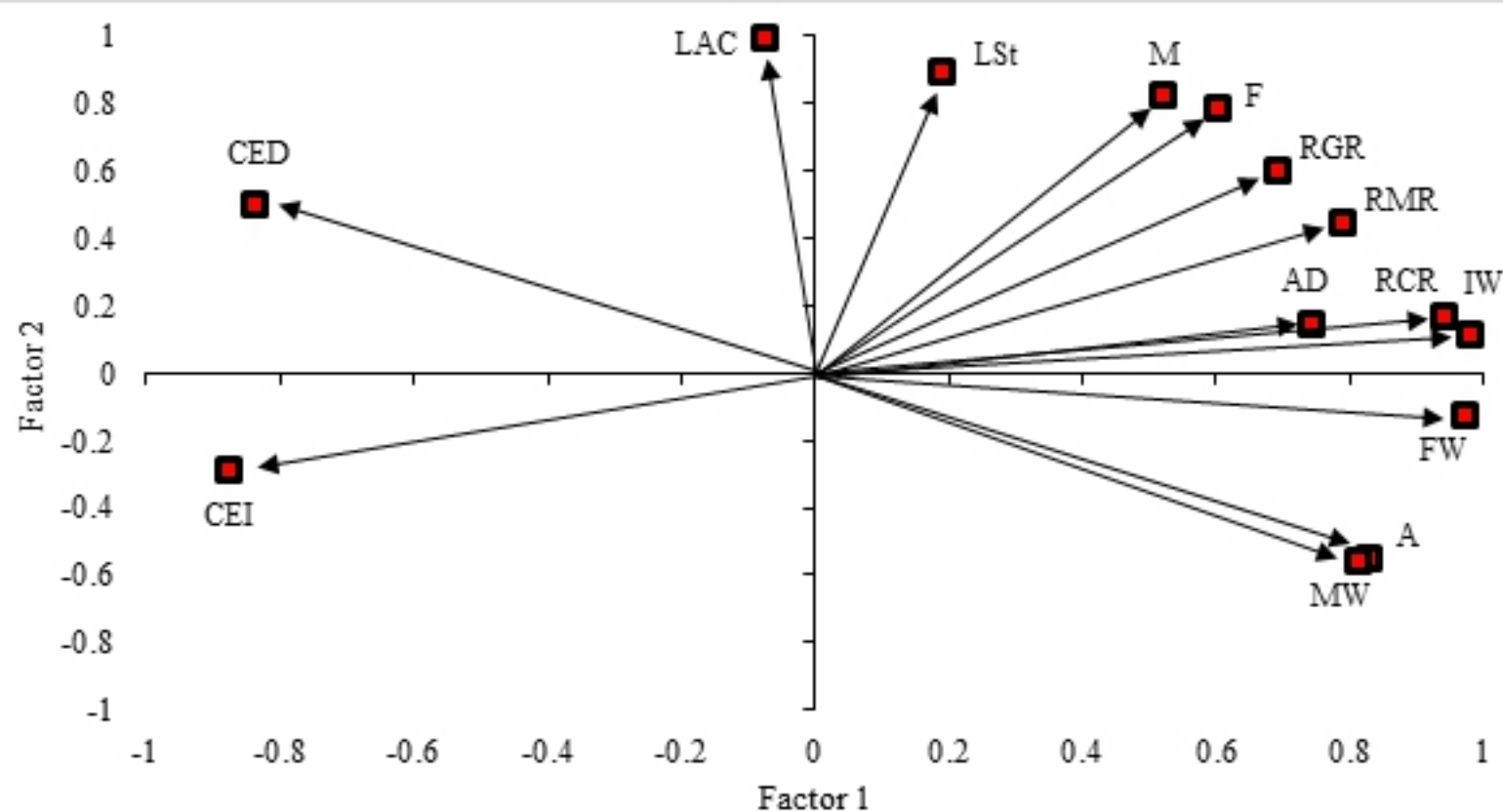


Figure 3

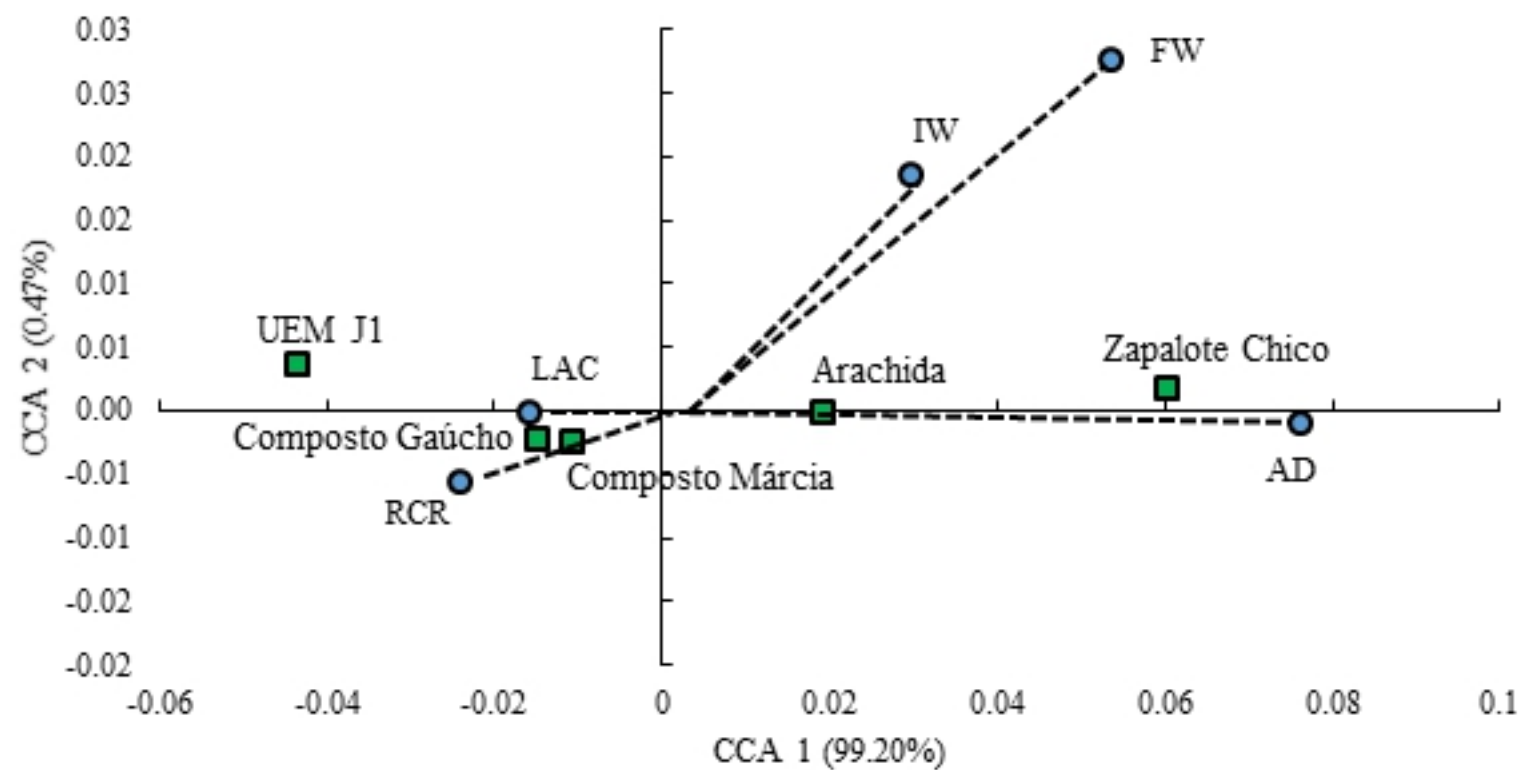


Figure 4