



Bragantia

ISSN: 0006-8705

ISSN: 1678-4499

Instituto Agronômico de Campinas

Sanches, Rafael Egea; Suzukawa, Andréia Kazumi; Contreras-Soto, Rodrigo Iván; Rizzardi, Diego Ary; Kuki, Mauricio Carlos; Zeffa, Douglas Mariani; Albuquerque, Fernando Alves de; Scapim, Carlos Alberto
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Bragantia, vol. 78, no. 2, -June, 2019, pp. 175-182
Instituto Agronômico de Campinas

DOI: 10.1590/1678-4499.20180230

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Multivariate analysis reveals key traits of fall armyworm resistance in tropical popcorn genotypes

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ABSTRACT: In the present study it is hypothesized that the germplasm of popcorn of tropical regions shows resistance to FAW, and the multivariate analysis can characterize the main traits of this resistance. Thus, the aim of this study was to identify key traits used to select fall armyworm resistance in popcorn by using factor and cluster analyses. Sixteen biological traits were evaluated at larval and pupae stages: total number of armyworm, time larval period, final mass of caterpillars, total mass of caterpillars, mean mass of caterpillars, pupae period, mass of pupae and some indices used to evaluate the food consumed by the caterpillars. Multicollinearity diagnosis, factor and canonical analysis, and the genetic divergence among the genotypes were performed to implement multivariate analysis. The groups were

established according to Mojena (1977). After multicollinearity test, only five traits were retained for further analysis. The factor analysis divided the five traits into two factors: the first factor included time larval period, metabolized food and mass of pupae; the second was composed of total number of armyworm and stool mass. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) grouped the eighteen genotypes in three clusters. The present study provides insights of popcorn resistance to fall armyworm, and the multivariate analytical approach used here is directly applicable to any species and set of traits exhibiting correlation.

Key words: *Spodoptera frugiperda*, Zapalote Chico, larval period, factor analysis, Manova.

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Received: June 20, 2018 – Accepted: Nov. 12, 2018



INTRODUCTION

Spodoptera frugiperda (J.E. Smith), or fall armyworm (FAW), is considered one of the main pests of maize, and can also cause damages to other crops such as rice, alfalfa, and sorghum (Juárez et al. 2012). In tropical areas, it occurs in all maize producing regions and feeds on plants from the vegetative to reproductive stages (Ribeiro et al. 2014). Consequently, damages can be up to 57% of reductions in grain yield if left uncontrolled (Cruz et al. 1999).

Biological and chemical methods are strategies to control FAW, however, plant resistance is especially effective in tropical regions where there is a necessity to reduce environmental damages caused by misuse of pesticides (Cunha et al. 2008; Crubelati-Mulati et al. 2014). Although transgenic is a control alternative of great importance currently, as it facilitates crop management and reduces the use of highly toxic insecticides, studies have shown that FAW evolved resistance to Cry1F-maize (Farias et al. 2014) and CryAb-maize in Brazil (Omoto et al. 2016). In temperate and tropical maize, there are few reports of non-transgenic plants with *S. frugiperda* resistance. Some reports are the genotypes Mp496, Mp707, Zapalote Chico, CMS 23 and CMS 24 (Wiseman and Widstom 1986). Non-preference feed was found in variety Zapalote Chico (Wiseman and Widstom 1986, Viana and Potenza 2000). Twenty tropical and temperate maize inbred lines were evaluated to FAW resistance, and three of them, that proceeded from tropical germplasm, showed superior performance to FAW resistance. In addition, the work emphasizes that tropical germplasm is an important source of resistance to fall armyworm and ear armyworm in maize (Ni et al. 2014). Although these studies clarify that genotypes can present resistance to FAW, few studies have been carried out to analyse popcorn genotypes (Crubelati-Mulati et al. 2014; Oliveira et al. 2018).

The mechanisms or traits used to evaluate the resistance to FAW in popcorn crop are too complex to be explained only under univariate analysis because most of these traits are biologically correlated (Wiseman and Davis 1990). Although some morphological traits related with FAW damage have been studied in maize breeding programs (Paiva et al. 2016), they have not yet been actually used, probably because performance experiments of maize have been analysed using univariate analysis.

Multivariate analysis might be a suitable approach to analyse data of resistance to FAW in maize. In fact, different statistical procedures have been used in modeling morpho-physiological and yield traits, as well as plant disease resistance, including principal component analysis and factor analysis (Aaliya et al. 2016), and also other multivariate procedures (Veturi et al. 2012; Wisser et al. 2011). The principal component analysis is a multivariate statistical method, which reduce the dimension of multivariate data by removing inter-correlation among variables. Each principal component is a linear combination of the original variables that represent a multidimensional relationship plotted on two or three principal axes (Hayman 1967). Factor analysis aims to explain observed relations among numerous variables by removing redundancy or duplication traits from a set of correlated phenotypic variables (Cattell 1965). These statistical methods can easily select important traits and reduce the data size to explore the relationships between traits, their variations and also show their relationships with the environmental factors (Vile et al. 2012).

In the present study it is hypothesized that the germplasm of popcorn of tropical regions shows resistance to FAW, and the multivariate analysis can characterize the main traits of this resistance. Thus, the aim of this study was to identify key traits used to select fall armyworm resistance in popcorn by using factor and cluster analyses.

MATERIAL AND METHODS

Experimental design

The experiment was carried out in Maringá, Paraná State, Brazil. Leaves of 18 popcorn genotypes obtained from the Specialty Maize Breeding Program from State University of Maringá were used for the evaluation and characterization of fall armyworm resistance (Table 1). A completely randomized design was used in the experiment with 18 treatments (genotypes) and 30 replicates; each caterpillar was considered a replicate.

Climate and soil conditions

The seeds of genotypes were sown in polyethylene cups with a mixture of soil and substrate (rate 3:1) with adequate fertilization for popcorn crop. The cups were kept

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Table 1. Description of the genotypes selected for the fall armyworm (FAW) bioassay.

Code	Genotype	Origin	Genetic base
1	IAC-125	IAC	Hybrid Topcross
2	ARZM 07 049	CIMMYT	Variety
3	SE 013	UEM	Variety
4	SAM1	South America/USA	Variety
5	URUG 298 roxo	CIMMYT	Variety
6	ARZM 05 083	CIMMYT	Variety
7	PARA 170	CIMMYT	Variety
8	UNB-2U C4	UENF	Variety
9	CHZM 13 0134	CIMMYT	Variety
10	PR-023	UEM	Variety
11	ARZM 13 050	CIMMYT	Variety
12	URUG 298 amarelo	CIMMYT	Variety
13	BOYA 462	CIMMYT	Variety
14	BOZM 260	CIMMYT	Variety
15	PARA 172	CIMMYT	Variety
16	PA 091	UEM	Variety
17	BRS 1030	EMBRAPA	Simples hybrid
18	Zapalote Chico	CIMMYT	Variety

¹South American Mushroom

in a greenhouse under controlled conditions (temperature of 25 °C, relativity humidity of 70% and photoperiodic of 12/12 hours). Leaves of each popcorn genotype were collected at the V6 and V8 phenological stages (six to eight fully extended leaves) to feed the caterpillars.

Fall armyworm rearing

Eggs of *S. frugiperda* were obtained from PROMIP company and the larvae were transferred to transparent acrylic plates (9.0 cm in diameter 1.5 cm in height), lined with filter paper to maintain humidity. The caterpillars were fed with sections of leaves taken from the middle third of each popcorn genotype at the V6 and V8 physiological stage. The food was renewed daily and the leaf area provided altered during the development of the caterpillars, in order to avoid the insufficiency of food.

Traits measured

The biological traits were evaluated at larval and pupae stages. In larval stage, total number of armyworm (TA), time larval period (TLP; days), final mass of caterpillars (FMC; grams), total mass of caterpillars (TMC; grams) and

mean mass of caterpillars (MMC; grams) were evaluated. In pupae stage, pupae period (PP; days) and mass at 24 hours after pupation (MP; grams) were evaluated. The caterpillars were weighted individually throughout the larval period. The stool of caterpillars was collected and weighted daily during all the larval period to obtain stool mass (SM), which was measured from the fifth day of FAW life. The FMC was measured from the fifth day of life until the final day of the larval phase. The MMC was calculated as: $MMC = \sum DMC / TLP$, where DMC is the daily mass of caterpillars and TLP is the time period larval.

The leaf consumption of the genotypes was evaluated daily throughout the larval phase. The determination of the leaf area consumed was obtained by the difference of the leaf area offered and the leaf area consumed after 24 hours. For measurements, the CI-202 Portable Laser Leaf Area Meter was used.

The indexes used to evaluate the food consumed by the caterpillars was based on area and mass of leaves, mass of caterpillars and stool mass (Scriber and Slansky Júnior 1981):

- Relative consumption rate:

$$RCR = I / (DMC \times TLP)$$

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- Relative growth rate:
 $RGR = FMC \times (MMC \times TLP)$
- Food assimilated:
 $FA = I - SM$
- Metabolized food:
 $MF = FA - FMC$
- Efficiency of conversion of ingested food:
 $ECI = (FMC / I) \times 100$
- Digested feed conversion efficiency:
 $DCI = (FMC / FA) \times 100$
- Apparent digestibility:
 $AD = (FA / I) \times 100$.

where I is the mass of food ingested by the caterpillars.

The superficial density was estimated as: $SD = M/A$, where M is the leaf mass and A is the leaf area.

Statistical analysis

The normality and homogeneity of variances assumptions were tested throughout Univariate Analysis by using the procedures PROC UNIVARIATE (Shapiro-Wilk test) and PROC GLM (Levene Test) of SAS, respectively (SAS Institute 2011). The data were analysed statistically using a multivariate analysis of variance (MANOVA) to test the hypothesis of differences between variance vectors of each genotypes. This analysis was conducted using the GLM procedure with the option MANOVA in SAS software (SAS Institute 2011).

The Variance Inflation Factor (VIF) and the Condition Index (CI) were used as criterion to evaluate the degree of multicollinearity among the variables (predictors). VIF values > 10 are usually considered as evidence for substantial multicollinearity and often justify the removal of certain predictors. In addition, multicollinearity is considered weak when CI is less than 100 (Montgomery and Peck 1981; Prunier et al. 2015). Then, canonical and factor analysis (FA) were performed (Friendly and Sigal 2017). The Kaiser criterion was used to select the main components for factor analysis, whose eigenvalues were above unity since they generate components with relevant amount of the original information (Kaiser 1958).

The genetic divergence among the genotypes was evaluated based on Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method, obtained with the Mahalanobis distance. The groups were established by

using a horizontal cut-off value of the distance means for each group formed (Mojena 1977). The analysis was performed by using the software Genes (Cruz 2016) and R (<https://www.r-project.org>) statistical software throughout the vegan package (Oksanen et al. 2013).

RESULTS AND DISCUSSION

Normality (p-value > 0.01) and homogeneity of variances (p-value > 0.01) were reported for all the evaluated traits. The test of significance based MANOVA analysis showed significant differences among genotypes for all the traits evaluated (p-value < 0.001), indicating the existence of significant genetic variability.

As mentioned above, the multicollinearity among the traits was evaluated using the VIF and CI criteria; traits that showed VIF and CI values higher than 10 and 100, respectively, are usually considered as evidence for substantial multicollinearity among variables and often justify the removal of certain predictors (O'Brien 2007; Prunier et al. 2015). The traits that showed high VIF and CI values were: FMC, TMC, MMC, PP, RCR, RGR, FA, ECI, AD, DCI and SD, consequently, these traits were eliminated. On the other hand, the traits: TA, TLP, SM, MF and MP showed VIF and CI values lower than 10 and 100, respectively, thus these traits were maintained for subsequent analyses (Table 2).

The maintained traits (TA, TLP, SM, MF and MP) were used in factor analysis. Factor analysis, through the commonality values, represents the proportion of variance in the dependent variable(s) that can be jointly explained by two or more predictors together, making factor analysis particularly well suited to select important traits (O'Brien, 2007). At the present study, commonality values varied between 0.767 (MF) and 0.948 (TA); the higher commonality values that help into the selection of the most important traits were: TA (0.948), SM (0.940) and MP (0.833) (Table 3).

The current study demonstrated that multivariate analysis associated three traits (TLP, MF and MP; Table 3) which contributed with a large variation to fall armyworm resistance in popcorn and avoided the need to handle a large number of independent variables to improve selection. The first factor combines three variables on which it is intended to generate the selection, facilitating the interpretation of

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Table 2. Diagnostic of multicollinearity for time larval period (TLP), metabolized food (MF), mass of pupae (MP), total number of armyworm (TA) and stool mass (SM) in five traits related to *Spodoptera frugiperda* resistance in 18 genotypes of popcorn maize (*Zea mays* L.).

Variation inflation factor		Condition index	
Diagonal	Element of inverse (r)	Order	Eigenvalues
1	8.092	1	2.575
2	3.097	2	1.848
3	7.529	3	0.295
4	2.772	4	0.218
5	3.165	5	0.063
Number of VIF's \geq abs 10		CI (max/min)	40.57

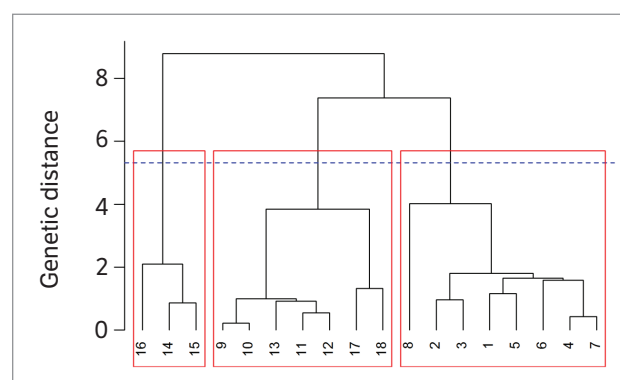
Table 3. Factors and their factor loadings after rotation of the factorial axis using the Varimax method for studied traits related to *Spodoptera frugiperda* resistance in 18 genotypes of popcorn maize (*Zea mays* L.).

Variables	Factor score coefficients		Factors loading after rotation		Commonality
	Factor 1	Factor 2	Factor 1	Factor 2	
Total of armyworm	-0.402	-0.886	-0.200	0.953	0.948
Time larval period	-0.884	-0.069	-0.878	0.124	0.787
Stool mass	-0.079	0.966	0.132	0.961	0.940
Metabolized food	0.853	0.195	0.876	0.005	0.767
Mass of pupae	0.882	0.232	0.912	0.035	0.833

the results, since, instead of simultaneously interpreting sixteen variables, the improvement can focus on three traits.

The relationships among the 18 genotypes can be observed graphically through the UPGMA results (Fig. 1). The high value of the cophenetic coefficient of correlation ($CCC = 0.73$) indicates the great adjustment for the algorithm used into UPGMA in the similarity matrix. The dendrogram and canonical results showed three main groups, and it may help to discriminate the possible mechanism of resistance and/or susceptibility of each group of genotypes. The UPGMA graph were confirmed through the canonical biplot, which showed the closest and most distant groups and its relationship with the variables that most contribute with the separation of these genotypes (groups). Specifically, the canonical analysis resulted in two canonical variables (CV) that help to identify the resistant and susceptible popcorn genotypes to FAW. The canonical biplot showed that two components explained 83.5% of the total variation among traits and popcorn genotypes. The first CV (Can1) was assigned 45.2% and the second CV (Can2) was assigned 38.3% of total variation. The first component, i.e., the abscissa axis, grouped three popcorn genotypes which may be considered as resistant to FAW (14, 15 and 16); the second group included seven genotypes (9, 10, 11, 12, 13, 17 and 18) and the third group included eight genotypes (1, 2, 3, 4, 5, 6, 7 and 8) (Figs. 1 and 2).

Our results reveal the importance for primary key traits into the selection of genotypes of popcorn with resistance to FAW. The TLP and weakly the MP and SM explained the major difference among the genotypes in the first CV axis. Major contribution for separation of genotypes in the second CV axis are due to TA as well as weakly the MF (Fig. 2). The canonical analysis, as well as the UPGMA results, showed that the genotypes BOZM 260 (14), PARA 172 (15) and PA 091 (16) were separated from the other genotypes because showed higher TLP

**Figure 1.** Dendrogram of hierarchical cluster analysis based on five morphological traits using UPGMA method among the genotypes of popcorn (1) IAC-125, (2) ARZM 07 049, (3) SE 013, (4) SAM, (5) URUG 298 roxo, (6) ARZM 05 083, (7) PARA 170, (8) UNB-2U C4, (9) CHZM 13 0134, (10) PR-023, (11) ARZM 13 050, (12) URUG 298 amarelo, (13) BOYA 462, (14) BOZM 260, (15) PARA 172, (16) PA 091, (17) BRS 1030, and (18) Zapalote Chico.

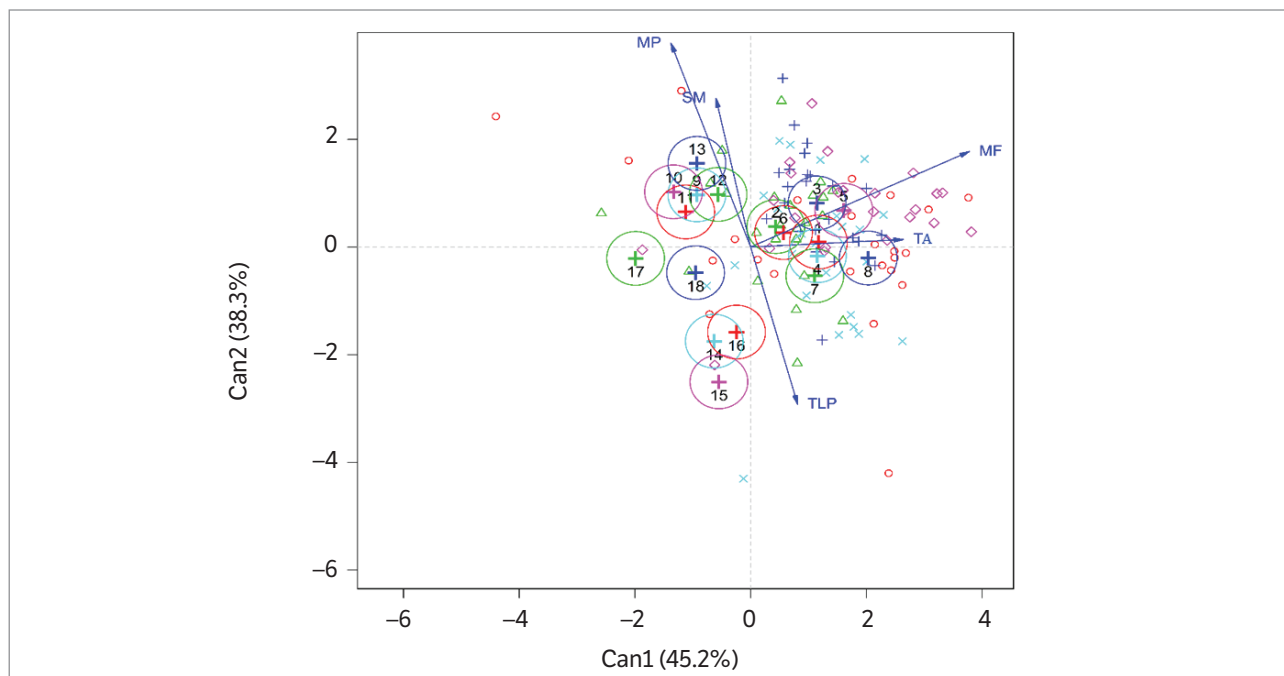


Figure 2. Biplot of the Canonical Analysis showing the closest and the most distant groups of the 18 genotypes of popcorn maize (*Zea mays* L.). (1) IAC-125, (2) ARZM 07 049, (3) SE 013, (4) SAM, (5) URUG 298 roxo, (6) ARZM 05 083, (7) PARA 170, (8) UNB-2U C4, (9) CHZM 13 0134, (10) PR-023, (11) ARZM 13 050, (12) URUG 298 amarelo, (13) BOYA 462, (14) BOZM 260, (15) PARA 172, (16) PA 091, (17) BRS 1030 and (18) Zapalote Chico. TA: Total of armyworm; TLP: Time larval period; SM: Stool mass; MF: Metabolized food; MP: Mass of pupae.

(19; 19.9 and 17.3 days, respectively). Duration of the TPL may change according to the nutritional quality of the feed provided to the caterpillar, with a tendency of prolonging its cycle if the genotype is a bad host (Cunha et al. 2008). Additionally, the low values of MP and MF allow us to suppose that the genotypes BOZM 260 (14), PARA 172 (15) and PA 091 (16) are not suitable as food source for *S. frugiperda*, characterizing the presence of antibiosis type resistance of these genotypes, which harms the development of insects, suggesting that these genotypes are resistant to FAW.

At the present study, Zapalote Chico (18) showed a TPL of 17.5 days, however was grouped in a different cluster of BOZM 260 (14), PARA 172 (15) and PA 091 (16) genotypes. Probably because Zapalote Chico was less preferred for *S. frugiperda* indicating non-preference mechanism of resistance (Viana and Potenza, 2000). On the other hand, in the BOZM 260 (14), PARA 172 (15) and PA 091 (16) genotypes resistance mechanism, occurs an antibiosis resistance, when the negative effects of a resistant plant affect the biology of the insect pest utilizing the plant as a host (Smith 2005). The effects of an antibiosis resistance may range from mild to lethal and are the result of chemical or morphological plant defenses.

Our findings demonstrated that multivariate analysis: Factor and Canonical analysis are an effective selection criterion into popcorn resistance to fall armyworm in a popcorn breeding program as supported by the genetic results of other authors (Wisser et al. 2011; Oliveira et al. 2018). The multivariate analytical approach used here is directly applicable to any species and set of traits exhibiting correlation. Moreover, making groups or clusters of popcorn genotypes is an efficient tool to minimize the plant pool during selection process.

CONCLUSION

The key traits that contributed with a large variation to fall armyworm resistance were time larval period (TLP), metabolized food (MF) and stool mass (SM) of insect at 24 hours after pupation. The genotypes with the greatest potential as a source of resistance to the fall armyworm were BOZM 260, PA 091 and PARA 172, because they presented prolongation of the larval period, and were effectively grouped with both UPGMA and canonical results.

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AUTHORS' CONTRIBUTION

Conceptualization, Scapim C.A., Albuquerque F.A. and Sanches R.E.; Investigation, Sanches R.E., Contreras-Soto R.I., Rizzardi D.A., Kuki M.C. and Suzukawa A.K.; Writing - Original Draft, Contreras-Soto R.I., Suzukawa A.K. and Rizzardi D.A.; Writing - Review and Editing, Suzukawa A.K., Contreras-Soto R.I., Scapim C.A. and Zeffa D.M.; Resources, Scapim C.A. and Albuquerque F.A.

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