



Genetic dissimilarity for resistance to *Mononychellus tanajoa* (bondar) (Acari, Tetranychidae) among domesticated and wild *Manihot* species

Verônica de Jesus Boaventura, Rudiney Ringenberg and Carlos Alberto da Silva Ledo*

Embrapa Mandioca e Fruticultura, Parque Estação Biológica, s/n.º, 70770-901, Brasília, Distrito Federal, Brasil. *Author for correspondence.
 E-mail: carlos.ledo@embrapa.br

ABSTRACT. The aim of this study was to evaluate the genetic dissimilarity among wild and domesticated species of *Manihot* for resistance to cassava green mite during the insect life cycle. Nine accessions of wild *Manihot* species, *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *peruviana*, and *M. carthagenensis* ssp. *glaziovii*, and two clones of *M. esculenta* (Cigana Preta and Sacá) were evaluated under laboratory conditions at $25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ RH, and a 12-h photophase. Daily observations during the mite life cycle stages (larva-adult) were recorded. The data were subjected to an analysis of variance, a Scott-Knott test (5%), and Singh criterion, cluster, and principal component analyses. The larval-adult period ranged from 5.53 to 7.01 days: the longest period was observed on an *M. glaziovii* accession (GLA-19-DF) and the shortest on an *M. flabellifolia* accession (FLA-025V). The UPGMA method allowed the division of the genotypes into six groups, with the greatest distance between the FLA-025V and GLA-19-DF accessions. The first two main components explained 77.50% of the total accumulated variation. The association of the longest cycle duration of *M. tanajoa* with the lowest larval-adult viability suggests that GLA-19-DF is less favorable to mite development compared to the other accessions. Significant variability among the genotypes was observed.

Keywords: biology, cassava, biotic stress, green mite.

Dissimilaridade genética entre espécies domesticadas e silvestres de *Manihot* quanto à resistência ao *Mononychellus tanajoa* (bondar) (Acari, Tetranychidae)

RESUMO. O objetivo deste trabalho foi avaliar dissimilaridade genética entre espécies domesticadas e silvestres de *Manihot*, a partir da duração do ciclo biológico, quanto à resistência ao *Mononychellus tanajoa*. Foram avaliados nove acessos das espécies silvestres de *Manihot*: *M. esculenta* ssp. *flabellifolia*, *M. esculenta* ssp. *peruviana* e *M. carthagenensis* ssp. *glaziovii*, dois clones de *M. esculenta*: Cigana Preta e Sacá. O estudo foi conduzido em laboratório, a $25 \pm 1^\circ\text{C}$, $70 \pm 10\%$ de UR e 12 h de fotofase. Foram realizadas observações diárias sobre a duração das fases do ciclo biológico (larva-adulto). Os dados submetidos à análise de variância, teste Scott-Knott (5%), critério de Singh, análise multivariada de agrupamento e de componentes principais. O período larva-adulto variou de 5,53 a 7,01 dias. O maior período foi verificado no acesso de *M. glaziovii* (GLA-19-DF), e o menor no acesso de *M. flabellifolia* (FLA-025V). O método de UPGMA, propiciou a divisão dos genótipos em seis grupos. A maior distância foi entre o acesso FLA-025V e GLA-19-DF. As duas primeiras componentes principais explicaram 77,50% da variação total acumulada. A maior duração do ciclo de *M. tanajoa* associado a menor viabilidade larva-adulto sugere que o acesso GLA-19-DF é menos favorável ao desenvolvimento do ácaro em relação aos demais e verificou-se variabilidade genética entre os genótipos avaliados.

Palavras chave: biologia, mandioca, estresse biótico, ácaro verde.

Introduction

In Brazil, cassava is cultivated in all biomes due to the great diversity of varieties adapted to different ecosystems, resulting in a great genetic diversity among species. This diversity is due to natural selection and is favored by cross-pollination, a high degree of heterozygosity, and abrupt fruit dehiscence, leading to a plethora of new genotypes (FUKUDA; SILVA 2002).

Historically, cassava is a culture characterized by low supplies of inputs and agrochemicals with a high

tolerance to drought, which can remain in the soil until its consumption, playing an important role in feeding the population (CAMARGO, 2003). However, due to its long growing cycle, the crop is subject to attack by a wide range of feeding arthropods. It is estimated that there are over 200 mite species associated with cassava in the Americas among which the green mite *Mononychellus tanajoa* (Bondar, 1938) (Acari, Tetranychidae) is considered a major pest affecting cassava cultivation in northeastern Brazil, particularly in the semiarid region (FUKUDA, 2006).

Green mite infestations develop largely under conditions of high temperature and low relative humidity, beginning at the top of the plants, attacking the buds and young leaves. Such infestation affects the formation of leaves, which become reduced in severely attacked plants and exhibit deformation, with the shortening of internodes, potentially leading to the death of the branch apices. Losses are estimated to be 10-50%, depending on the intensity of infestation (MORAES; FLECHTMANN, 2008).

The potential use of wild species has recently been reported in studies conducted at the International Center for Tropical Agriculture (CIAT). The results of these experiments include the following: 1) interspecific hybrids of *M. esculenta* ssp. *flabellifolia* show moderate to high levels of resistance to mite, whitefly, and scale insects (BURBANO et al., 2007); 2) an interspecific hybrid between *M. esculenta* and *M. walkerae* Croizat exhibits reduced post-harvest physiological spoilage (CIAT, 2003); 3) *M. glaziovii* Muell, *M. catinae* Ule, and *M. carthaginensis* Mull. Arg. are adapted to semiarid conditions and are potential sources of genes for drought tolerance; 4) the roots of interspecific hybrids of *M. esculenta* ssp. *flabellifolia* contain a high protein content (AKINBO et al., 2012a); 5) *M. flabellifolia* accessions and interspecific crosses of *M. esculenta* ssp. *flabellifolia* exhibit different levels of resistance to the whitefly *Aleurotrachelus socialis* Bondar (CARABALI et al. 2010); and 6) resistance to *A. socialis* can be introgressed in interspecific hybrids of commercial cassava (AKINBO et al., 2012b).

Considering the characteristics of agronomical interest found in wild and domesticated cassava species, it is important to select cassava genotypes that are resistant to green mite for use in integrated mite control programs (ARGOLO et al., 2005).

This study was aimed at assessing the genetic dissimilarity among wild and domesticated *Manihot* species for resistance to *M. tanajoa* during the life cycle of the insect (larva-adult period).

Material and methods

Location of bioassays

The study was performed at the Laboratory of Entomology - Embrapa Cassava and Fruits, Cruz das Almas, Bahia State, Brazil.

Mites and *Manihot* accessions

Cassava green mite specimens (*M. tanajoa*) were obtained from colonies established in the cassava clone "Cigana Preta" maintained in a greenhouse. The wild *Manihot* genotypes are part of the Work

Collection of Wild *Manihot* Species - Embrapa Cassava and Fruits, comprising 628 accessions belonging to 28 species, and were installed in the field in 2005. This study evaluated nine accessions belonging to three wild cassava species, *M. esculenta* ssp. *flabellifolia* (FLA-025V, FLA-026V, and FLA-029V), *M. esculenta* ssp. *peruviana* (PER-006V, PER-007V, and PER-010V), and *M. carthaginensis* ssp. *glaziovii* (GLA-03-DF, GLA-10-DF, and GLA-19-DF), and clones of a domesticated species (*M. esculenta*), Cigana Preta (BGM 0116) and Sacai (BGM 0384), all from the experimental area belonging to Embrapa Cassava and Fruits. These genotypes were selected according to characteristics of interest: *M. esculenta* ssp. *flabellifolia* is the ancestor of *M. esculenta* and provides resistance to such pests as mites; *M. esculenta* ssp. *peruviana* is close to *M. flabellifolia*; *M. carthaginensis* ssp. *glaziovii* presents drought tolerance; and the *M. esculenta* clone Cigana Preta is considered susceptible and Sacai resistant to *M. tanajoa*.

Mite eggs and bioassays

To obtain eggs, eighty female *M. tanajoa* obtained from maintenance breeding were allowed to lay eggs in the lobes of fully developed young leaves of the tested accessions. The lobes were positioned with the top (adaxial surface) in contact with nylon foam (1 cm thick) that was moistened with distilled water and surrounded with strips of cotton wool inside trays (20 x 15 x 5 cm). The trays were sealed with transparent polyvinylchloride film (PVC), which was perforated to allow aeration. After 24 hours, the females were removed, and the eggs were allowed to develop on the lobes until hatching. After hatching, the larvae were isolated on leaf disks (2.5 cm in diameter) of each accession tested; the disks were deposited on nylon foam (1 cm thick) moistened with distilled water inside Petri dishes (14 cm in diameter x 2 cm deep) and sealed with PVC film perforated to allow aeration, according to the methodology of Noronha et al. (1995). The Petri dishes were maintained in a BOD-type incubator at $25 \pm 1^\circ\text{C}$, a relative humidity of $70 \pm 10\%$, and a photoperiod of 12 hours. Except when they were in quiescent stage (protochrysalis, deutochrysalis, and teleiochrysalis stages), the mites were transferred to new disks every two days with the aid of a stereoscopic microscope and a brush with fine bristles. The development of *M. tanajoa* was followed to adulthood, with daily observations of the larval, protochrysalis, protonymph, deutochrysalis, deutonymph, teleiochrysalis, and adult stages to verify the succession to the next stage, as characterized by the presence of exuviae. A completely randomized design with 30 replicates per

genotype was used. The first 30 hatched larvae were captured. Each plot consisted of one mite.

Statistical analysis

The data were subjected to an analysis of variance, and the means of the genotypes were grouped by the Scott-Knott test (SCOTT; KNOTT, 1974) at 5% significance using SISVAR software (FERREIRA, 2011). Cluster and principal components analyses were performed. For the cluster analysis, the mean Euclidean distance was considered as a dissimilarity measure. The hierarchical groupings from the dissimilarity matrix were obtained by the Unweighted Pair Group Method with Arithmetic Mean (Upgma) method (SNEATH; SOKAL, 1973). The Singh (1981) criterion was also used to quantify the relative contribution of the duration of the life cycle stages for the genetic dissimilarity analyses performed using the Genes (CRUZ, 2001) and Statistica 7.1 software (STATSOFT, 2005).

Results and discussion

The biological life cycle (larva-adult period) of *M. tanajoa* was grouped according to the means of the Scott-Knott test at 5% significance (Table 1). The egg stage lasted for five days and did not vary between the accessions. The larvae used in the study were of the same age. A variation from 1.07 to 1.53 days was observed for the larval stage, whereas the protonymph stage ranged from 0.96 to 1.29 days, with the formation of only one group, which did not statistically differ from *Manihot* accessions studied in the two cycle stages. In contrast, two groups were distinguished for the other stages.

For the protochrysalis stage, the variation ranged from 0.98 to 1.62 days, with significant differences between the accessions. The longest mite protochrysalis, protonymph, and larva-adult stage durations were found for the accessions of

M. peruviana species; the shortest protochrysalis stage was observed on the *M. carthaginensis* ssp. *glaziovii* accessions.

For the deutochrysalis and deutonymph phases, the variation ranged from 0.93 to 1.28 days and 0.90 to 1.27 days, respectively. The *M. peruviana* accessions and *M. esculenta* clones Cigana Preta and Sacai were among the group showing the shortest deutochrysalis stage, whereas the *M. esculenta* clones were among the group showing the longest deutonymph stage. The teleiochrysalis stage varied from 0.64 to 1.09 days, with the *M. flabellifolia* accessions and *M. esculenta* clones exhibiting the longest durations and the *M. peruviana* accessions and two *M. glaziovii* accessions exhibiting the shortest duration.

The larva-adult period ranged from 5.53 to 7.01 days. The group that showed the longest larva-adult period was formed by FLA-026V, GLA-03-DF, GLA-019-DF, PER-006V, PER-007V, PER-010V, Cigana Preta, and Sacai. The accessions that formed the group with the shortest larva-adult period were FLA-025V, GLA-10, and DF-FLA-029V. The *M. esculenta* clones were included in the same group during all stages of the mite life cycle.

The viability of the immature stages of the cassava green mite varied among the *Manihot* species. The survival rate ranged from 76.67 to 100%, with the lowest viability being observed on accession GLA-19-DF (76.67%) of *M. carthaginensis* ssp. *glaziovii*. The viability was greater than 80% on the accessions of *M. esculenta* ssp. *flabellifolia*, and the survival rate was greater than 90% on GLA-03-DF and GLA-10-DF. Mortality was observed during the immature mite stage on the *M. peruviana* accessions and Cigana Preta and Sacai clones (Table 1).

Table 1. Duration ($X \pm SE$) and total viability (%) of stages of the life cycle (larva-adult period) of *M. tanajoa* as a function of the *Manihot* accession at a temperature of $25 \pm 1^\circ\text{C}$, relative humidity of $70 \pm 10\%$, and photoperiod of 12 hours. Cruz das Almas, Bahia State, Brazil, 2011.

| Accession | Duration (days) of cycle stage | | | | | | | Total viability (%) |
|--------------|--------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|---------------------|
| | L | PC | PN | DC | DN | TC | LA | |
| FLA-025V | 1.13 \pm 0.01 a | 1.62 \pm 0.14 a | 1.03 \pm 0.09 a | 0.98 \pm 0.10 b | 1.04 \pm 0.08 b | 0.93 \pm 0.08 a | 5.53 \pm 0.23 b | 83.33 |
| FLA-026V | 1.26 \pm 0.09 a | 0.98 \pm 0.04 b | 1.23 \pm 0.13 a | 1.12 \pm 0.06 b | 1.27 \pm 0.09 a | 1.08 \pm 0.07 a | 6.43 \pm 0.23 a | 83.33 |
| FLA-029V | 1.13 \pm 0.08 a | 1.14 \pm 0.07 b | 1.08 \pm 0.05 a | 1.28 \pm 0.11 a | 1.13 \pm 0.08 a | 0.94 \pm 0.08 a | 6.07 \pm 0.21 b | 86.67 |
| GLA-03-DF | 1.39 \pm 0.16 a | 1.18 \pm 0.09 b | 0.98 \pm 0.08 a | 1.02 \pm 0.06 b | 1.08 \pm 0.09 a | 0.77 \pm 0.08 b | 6.32 \pm 0.20 a | 96.67 |
| GLA-10-DF | 1.39 \pm 0.14 a | 1.09 \pm 0.06 b | 0.96 \pm 0.07 a | 0.93 \pm 0.03 b | 0.93 \pm 0.04 b | 0.64 \pm 0.06 b | 5.98 \pm 0.20 b | 93.33 |
| GLA-19-DF | 1.40 \pm 0.13 a | 1.00 \pm 0.00 b | 1.29 \pm 0.11 a | 1.26 \pm 0.10 a | 1.16 \pm 0.14 a | 1.09 \pm 0.12 a | 7.01 \pm 0.26 a | 76.67 |
| PER-006V | 1.53 \pm 0.16 a | 1.41 \pm 0.10 a | 1.07 \pm 0.06 a | 1.12 \pm 0.07 b | 0.90 \pm 0.02 b | 0.68 \pm 0.06 b | 6.71 \pm 0.12 a | 100 |
| PER-007V | 1.33 \pm 0.12 a | 1.49 \pm 0.09 a | 1.04 \pm 0.05 a | 0.99 \pm 0.00 b | 0.95 \pm 0.03 b | 0.69 \pm 0.06 b | 6.51 \pm 0.08 a | 100 |
| PER-010V | 1.43 \pm 0.13 a | 1.56 \pm 0.09 a | 1.14 \pm 0.07 a | 1.06 \pm 0.05 b | 0.94 \pm 0.01 b | 0.78 \pm 0.06 b | 6.92 \pm 0.09 a | 100 |
| Cigana Preta | 1.20 \pm 0.09 a | 1.20 \pm 0.07 b | 1.08 \pm 0.05 a | 1.04 \pm 0.08 b | 1.09 \pm 0.06 a | 1.01 \pm 0.07 a | 6.62 \pm 0.20 a | 100 |
| Sacai | 1.07 \pm 0.05 a | 1.07 \pm 0.05 b | 1.23 \pm 0.08 a | 1.06 \pm 0.05 b | 1.19 \pm 0.14 a | 0.99 \pm 0.06 a | 6.62 \pm 0.17 a | 100 |
| CV (%) | 49.17 | 35.99 | 39.18 | 35.77 | 42.28 | 46.49 | 8.86 | |

Means followed by same letter belong to the same group by the Scott-Knott test at 5% probability. Stages: larva (L), protochrysalis (PC), protonymph (PN), deutochrysalis (DC), deutonymph (DN), teleiochrysalis (TC), larva-adult (LA). $X \pm SE$ = standard mean error.

These data show that there is genetic dissimilarity between the tested accessions, as there were significant differences in the green mite biological cycle among different genotypes of the same species.

Argolo et al. (2005) evaluated the resistance of cassava varieties (BGM 0876-Pretinha, BGM 0080-Engana Ladrão, BGM 0537-Do Céu, and BGM 0384-Sacai) selected as tolerant to *M. tanajoa* for the semiarid conditions of northeastern Brazil for varieties and found that the larva-adult period ranged from 7.39 to 9.43 days. The mite life cycle for clone Sacai was 9.28 days, which was higher than that obtained in the present work for the same clone. However, there was a difference among the individual stages, with the protonymph, deutochrysalis, and deutonymph stages being longer in our study. These data showed that the duration of each stage does not interfere with the duration of the next stage or, consequently, in the development of cassava green mites.

The present study indicates that the characteristics having the greatest influence on mite development are related to the genetics of host species because the bioassays were performed under conditions of identical temperature, relative humidity, and availability of water and nutrients. It also indicates different types of antibiosis of resistance levels of these accessions, which should be considered as an indicator of pest resistance.

Burbano et al. (2007) evaluated and compared two Brazilian wild *Manihot* species (*M. flabellifolia* and *M. peruviana*) to commercial *M. esculenta* genotypes to determine their resistance potential to *M. tanajoa* and found variable degrees of mite damage. The *M. flabellifolia* genotypes were significantly different from *M. esculenta* but similar to the *M. peruviana* genotypes, which showed lower levels of damage.

Figure 1 shows the dissimilarity dendrogram constructed based on seven quantitative descriptors: number of days for the larval, protochrysalis, protonymph, deutochrysalis, deutonymph, teleiochrysalis, and larva-adult stages).

The average group matrix that defined the number of groups was 2.6. The groups formed by the UPGMA method, as based on the average Euclidean distance matrix, allowed the division of the eleven genotypes into six groups: Group 1 (FLA-025V), Group 2 (GLA-03-DF, GLA-10-DF), Group 3 (PER-006V, PER-010V, and PER-007V), Group 4 (FLA-026V, Cigana Preta, and Sacai), Group 5 (FLA-029V), and Group 6 (GLA-19-DF) (Figure 1).

The largest distance was observed between FLA-025V and GLA-19-DF, i.e., genetically, the greatest

dissimilarity was between the genotype of Group 6 and the genotype of Group 1.

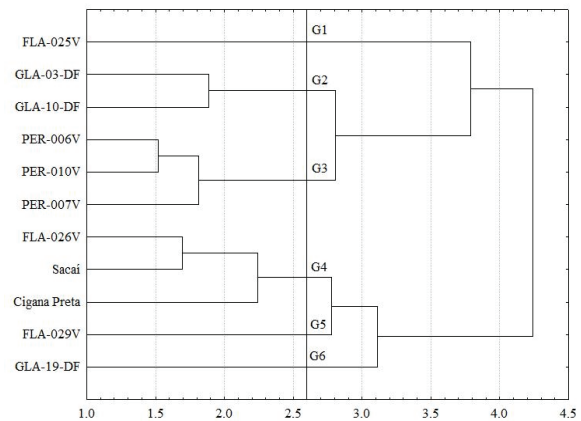


Figure 1. Dissimilarity dendrogram based on the mean Euclidean distance and UPGMA clustering method of the *M. tanajoa* life cycle (larva-adult) on 11 *Manihot* accessions.

The *M. flabellifolia* genotypes showed different behavior for the duration of the mite life cycle, and each accession was classified into a different group. However, there was similarity between the accessions in each group, indicating genotypic and/or phenotypic proximity among them, whereas dissimilarity was observed between the groups.

The feature that most contributed to the assessment of the genetic diversity according to the Singh (1981) criterion was the larva-adult period, at 57.30%.

Alves et al. (2011) assessed 14 accessions of six wild *Manihot* species with regard to biological aspects of *M. tanajoa* and found that the life cycle (larva-adult period) ranged from 5.76 to 8.18 days. The accessions of wild *M. anomala* species formed the group showing the longest mite development period, whereas *M. peruviana* and *M. flabellifolia* accessions showed shorter larva-adult periods.

The first two principal components explained 77.50% of the total accumulated variation. This value is considered satisfactory because the principal components explained approximately 80% of the variance found in the set of characters analyzed, enabling the formation of groups among the accessions and the construction of a scatter plot based on the diversity observed (Figure 1 and 2).

The first principal component explained 52.50% of the total variance, and the phases that most contributed to explaining this variability were the teleiochrysalis, deutonymph, and protonymph stages, as these variables showed higher weighting coefficients. The second principal component explained 25.00%, with the larval-adult and larva stages contributing the most (Figure 2).

The species distribution in the scatter plot (Figure 2) confirmed the greatest distance observed in the dendrogram (Figure 1) between FLA-025V and the other accessions. This result allowed the identification of the greatest genetic dissimilarity among individuals, in addition to grouping two *M. flabellifolia* accessions with *M. esculenta* and confirming the proximity of these accessions.

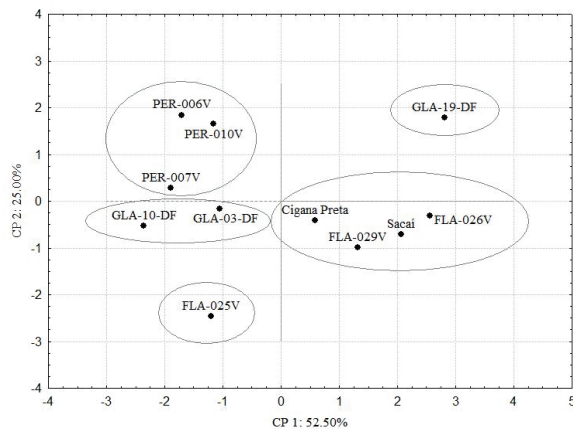


Figure 2. Graphic dispersion of the scores of 11 *Manihot* accessions in relation to principal components 1 and 2.

To verify the cassava ancestry, Carvalho (2005) studied 27 *Manihot esculenta* ssp. *flabellifolia* populations using two types of genetic markers (the glyceraldehyde 3-phosphate dehydrogenase [G3PDH] gene and five microsatellite loci) and confirmed that cassava was originally domesticated from *M. esculenta* ssp. *flabellifolia*.

There is complementarity between the two multivariate techniques used. In the cluster analysis (Figure 1) and principal component analysis (Figure 2), the GLA-19-DF and FLA-025V accessions showed no similarity with the other accessions, as they were allocated to different groups. A similar result was found for the *M. peruviana* and *M. carthaginensis* ssp. *glaziovii* accessions (GLA-10-DF and GLA-03-DF).

Conclusion

The time in days of a biological cycle phase (larva-adult period) has little influence on the duration of subsequent stages.

The longest larval to adult duration and lowest viability of the immature stage of *M. tanajoa* suggest that the GLA-19-DF accession is less susceptible to mite development when compared to the other genotypes tested.

The different behavior of the mite life cycle on accessions of the same species indicates that genetic

differences exist between accessions of the same *Manihot* species.

The accessions of *M. esculenta* ssp. *peruviana* species are genetically close.

There is genetic variability among wild and domesticated *Manihot* species with regard to resistance to *Mononychellus tanajoa*.

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