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Source of antibiosis to *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in mocó cotton genotypes (*Gossypium hirsutum* raça *marie galante* L. Hutch)

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Abstract

Spodoptera frugiperda (Lepidoptera: Noctuidae) cause damage in cotton plants since the plant emergence until maturation. The objective of this work was to evaluate antibiosis in mocó cotton genotypes (*Gossypium hirsutum* var *marie galante* L. Hutch) to *S. frugiperda*. Newly hatched larvae were individualized in plastic containers (100 mL) sealed with polyethylene film to prevent the insect scape, where they were fed with cotton leaves. The treatments were 27 cotton genotypes, with 40 replications for antibiosis and 20 for nutritional parameters, using a completely randomized design. The biological parameters evaluated were: larval period and viability, weight of caterpillars at ten days, pre-pupal period and viability and pupal weight at 24 hours, life cycle and total viability, adult longevity and sex ratio. Additionally, cotton genotypes were characterized by four microsatellite markers linked blue disease resistance, angular leaf spot, root-knot nematode and reniform nematode. There was high initial mortality of *S. frugiperda* larvae in genotypes BA0512, MA0423, PI0438, PI0432, CE0461, CE0507, RN0512, MA0425, MA0438, PI0429, MA0430, PI0440, PI0467, AC0602, PI0416, BA0477, MA0409 and MA0409 CE0467. The cotton genotypes presented antibiosis and SSR resistance markers for nematode and blue disease, highlighting that the germplasm of this species is an important source of multiple resistance for insect resistant genotype selection programs.

Keywords: Resistance of plants to insect, Integrated Management, Mocó Cotton, fall armyworm.
Abbreviations: SSR_Simple Sequence Repeats; Brazilian states (AC – Acre; BA – Bahia; CE – Ceará; MA – Maranhão; PI – Piauí; RN – Rio Grande do Nortes); IPM_Integrated Pest Management; CNPA_Centro Nacional de Pesquisa de Algodão; PVC_Polyvinyl chloride.

Introduction

There is a pest complex in cotton causing damage in all phenological plant stages that affects negatively the crop yield. The fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae), cause damage in cotton and several others important crops worldwide (Fernandes et al., 2002; Santos, 2001; Montezano et al., 2018) The damages of *S. frugiperda* are observed when the larvae feeds on leaves and fruiting structures, in the vegetative and reproductive stages, of the cotton development (Moreno et al., 2008; Jesus et al., 2014a).

The main tactic to control *S. frugiperda* in cotton is through the use of insecticides, which has favored the selection of resistant pests, the death of natural enemies and an increase in production costs (Degrande, 1998; Jesus et al., 2014b). Thus, chemical control should be an alternative method, used with other control tactics, enhancing pest control and preventing the death of non-target organisms beneficial to the crop (Miranda, 2010). The use of resistant varieties becomes a promising tactic in Integrated Pest Management - IPM contributing to the mitigation of the frequent use of chemical insecticides, promoting sustainable agriculture practices (Williams et al., 1983; Boiça Junior et al., 2015).

The occurrence of plant resistance is assigned to three categories: *i)* non-preference or antixenosis, when the plant exerts less attractiveness to the insect for oviposition, feeding and/or shelter; *ii*) antibiosis, when the insect feeding on the plant negatively affects biological parameters, such as: reduced viability and fecundity, prolonged larval or pupal development, reduced size or weigh or increased mortality; and *iii*) tolerance, when the plant continues to grow and reproduce despite being damaged by the insects compared to susceptible host (Painter, 1951; Seifi et al., 2013). The adoption of resistant cultivars in IPM has contributed to the sustainability of cotton production, due to reducing the use of insecticides, in addition to not polluting the environment (Seifi et al., 2013; Vendramim et al., 2019).

It is necessary to know the effects of resistant cotton cultivars on the biology of the insect pests (Mullis and Pieters, 1982). The varieties CNPA 9211-31, CNPA Precoce 1, CNPA Precoce 2 and CNPA 9211-41 showed antibiosis and/or non-preference to *Alabama argilacea* (Lepidoptera: Noctuidae) (Santos and Boiça Júnior, 2001). The cultivars NuOpal, FMT 701 and FMX 910 presented antibiosis to *A. argilacea*, since the caterpillars fed on NuOPal did not complete the first

Table 1. Larval, prepupal and pupal period (days) and viability (%) (±standard error) of *S. frugiperda* (Lepidoptera: Noctuidae) on cotton genotypes.

Genotypes	Larval[1]		Pre-pupal[1]		Pupal[1]	
	Period	Viability	Period	Viability	Period	Viability
PI0433	50.00±1.24a	25±0.00	2.33±0.33a	100±0.00	11.44±0.29a	90±0.00
BA05101	49.56±1.53a	23±0.00	1.78±0.15b	100±0.00	11.67±0.37a	100±0.00
MA0418	49.00±2.28a	13±0.00	2.00±0.00b	100±0.00	10.40±0.24b	100±0.00
BA0502	48.11±1.36a	25±0.00	2.00±0.00b	100±0.00	10.11±0.61b	90±0.00
MA0407	45.57±0.84a	23±0.00	2.00±0.00b	89±0.00	11.71±0.42a	88±0.00
BRS Buriti	42.18±1.51b	30±0.00	2.27±0.19a	100±0.00	11.27±0.66a	92±0.00
IAC25	38.36±1.55b	35±0.00	2.64±0.15a	93±0.00	9.00±0.63b	92±0.00
BRS Cedro	38.25±2.01b	25±0.00	2.50±0.27a	100±0.00	9.13±0.69b	90±0.00
F	10.256	0.8955	2.3442	0.7361	3.9878	0.2143
P	<0.001	0.5102	<0.05	0.6420	<0.01	0.9810

[1] Means followed by the same letter, within a column, do not differ significantly by the Scott–Knott test at the 5% probability.

Table 2. Larval and pupal weights (mg), life cycle (days) and total viability (%), adult longevity (days) in *S. frugiperda* (Lepidoptera: Noctuidae) fed on cotton genotypes.

Genotypes	Weight[1]		Adults[1]		[1]Life cycle	Total viability
	Larval	Pupal	Longevity	Viability		
PI0433	2.53±0.36c	13.64±0.79a	3.11±0.48	100±0.00	67.00±1.40a	22.50±0.07
BA05101	1.74±0.30d	10.08±0.64b	3.00±0.44	100±0.00	[2-2]66.00±1.50a	22.50±0.07
MA0418	1.51±0.48d	9.60±0.73b	3.00±0.32	100±0.00	64.40±2.23a	12.50±0.05
BA0502	1.56±0.16d	13.81±0.53a	3.78±0.28	100±0.00	64.00±1.41a	22.50±0.07
MA0407	3.06±0.44c	11.80± 0.33a	2.71±0.42	100±0.00	62.00±0.62a	17.50±0.06
BRS Buriti	1.40±0.17d	12.84±0.58a	3.45±0.31	100±0.00	59.18±1.77b	27.50±0.07
IAC25	3.75±0.49b	12.49±0.80a	4.00±0.45	92±0.08	54.00±1.92c	27.50±0.07
BRS Cedro	5.47±0.90a	10.86±0.82b	3.25±0.37	89 ±0.11	53.13±2.78c	20.00±0.06
F	9.895	4.667	1.1692	0.6884	8.8098	0.5814
P	<0.001	<0.001	0.3337	0.6814	<0.001	0.7710

[1] Means followed by the same letter, within a column, do not differ significantly by the Scott–Knott test at the 5% probability. [2]Insufficient number of replications for statistical analysis.

instar, and insects fed with the cotton cultivars FMT 701 and FMX 910 presented a longer larval and pupal stages (Boiça Júnior et al., 2012). The cultivar BRS Camaçari promoted the lowest weight of *S. frugiperda* larvae and pupae of (Moreno et al., 2008).

Wild genotypes and local varieties are possible sources of resistance to insects in cotton plants, (Menezes et al., 2014). The selection of wild plant resistant to insect will contribute to the genetic improvement of cotton crop, in plant breeding program as donor source of resistance against insect pests. Silva et al. (2021) evaluating antixenosis in wild cotton genotypes (*Gossypium hirsutum* var. *marie galante*) observed antixenosis on PI0440, CE0467, PI0416, BA0512 and MA0425 to *S. frugiperda*.

The objectives of this study were to identify sources of antibiosis in cotton genotypes (*Gossypium hirsutum* race *marie galante* L. Hutch) though bioassays (biologic parameters) to *S. frugiperda* and characterize the same genotypes to resistance to blue disease, angular leaf spot and nematodes) using SSR markers.

Results

Antibiosis to S. frugiperda

The larval, pre-pupal and pupal duration of *S. frugiperda* were statistically influenced by the cotton genotypes (Table 1). However, in the remaining insects, viability was not influenced by the cotton genotypes. The larval period was longer in caterpillars fed on PI0433, BA05101, MA0418, BA0502 and MA0407 and lower on BRS Buriti, IAC 25 and BRS Cedro. The pre-pupal period of *S. frugiperda* was longer on IAC 25, BRS Cedro and BRS Buriti compared to PI0433. Insects of *S. frugiperda* that feed on MA0407, BA05101, BRS Buriti and PI0433 showed the longest pupal period compared to the others (Table 1). *S. frugiperda* completed its cycle only on PI0433, BA05101, MA0418, BA0502, MA0407, BRS Buriti, IAC 25 and BRS Cedro.

The larval and pupal weight and the larva-adult of *S. frugiperda* were statistical influenced by the cotton genotypes (Table 2). The longevity and viability of adults and the total viability did not differ statistically. *Spodoptera frugiperda* larvae feed on PI0433, BA05101, MA0418, BA0502 and MA0407 presented highest larval weight. Insects from BRS Buriti, IAC 25, BRS Cedro, MA0430, CE0467, PI0467, MA0425, MA0409, BA0477, CE0474 and PI0416 showed the lowest larval weights.

Spodoptera frugiperda pupal weight was higher on BA0502, PI0433, BRS Buriti, IAC 25 and MA0407. Also, *S. frugiperda* larva-adult cycle was longer in caterpillars fed on PI0433, BA05101, MA0408, BA0502 and MA0407 and shortest on BRS Cedro and IAC 25 (Table 2).

Table 3. Sex ratio, male and female (%) of moths of *S. frugiperda* (Lepidoptera: Noctuidae).

Genotypes	Sex ratio	
	Machos	Fêmeas
PI0433	22.00±0.15	78.00±0.15
BA05101	56.00±0.18	44.00±0.18
MA0418	67.00±0.21	33.00±0.21
BA0502	70.00±0.15	30.00±0.15
MA0407	33.00±0.17	67.00±0.17
BRS Buriti	42.00±0.15	58.00±0.15
IAC25	54.00±0.14	46.00±0.14
BRS Cedro	33.00±0.17	67.00±0.17
F	1.028	1.028
P	0.4190	0.4190

[1]Averages, followed by the same letter, do not differ statistically from each other, by the Scott-Knott test at 5% probability EPM. Mean standard error.

Table 4. Nutritional indices (± SE) of *S. frugiperda* caterpillars fed on cotton leaves. Urutaí, Goiás, 2019.

Genotypes	Nutrition indices		
	RGR (g/g/day)[1]	ECI (%)	RCR (g/g/day)
IAC25	0.08±0.03	5.63±2.12b	1.42±0.14b
RN0512	0.08±0.02	2.17±0.67b	4.11±0.39a
MA0438	0.07±0.03	13.23±3.78a	0.31±0.31c
MA0409	0.13±0.03	8.31±2.77b	1.75±0.24b
PI0429	0.14±0.02	7.35±1.79b	2.11±0.24b
PI0440	0.09±0.02	18.47±6.12a	0.59±0.11c
PI0432	0.06±0.03	6.49±3.54b	1.01±0.22c
AC0602	0.07±0.01	7.31±0.85b	0.99±0.20c
F	1.4425	2.838	21.639
P	0.2348	0.0264	<0.001

[1] Means followed by the same letter, within a column, do not differ significantly by the Scott–Knott test at the 5% probability. RGR= Relative Growth Rate, ECI= Efficiency of Conversion of Ingested food, RCR= Relative Consumption Rate.

The cotton genotypes did not influence the sex ratio of *S. frugiperda* (Table 3). The conversion efficiency of ingested food (ECI) and the relative consumption rate (RCR) were influenced by the cotton genotypes (Table 4). Insects from PI0440 and MA0438 presented higher ECI and RN0512 higher RCR presenting higher leaf intake.

Microsatellite markers linked to disease resistance

The four microsatellite markers linked to disease resistance genes were detected in the germplasm for *G. hisutum* r. *marie galante*. The only three alleles of the following microsatellite markers with their respective base pairs, CIR 316M-210 bp, DC 20027-202 bp and BNL3279-124 bp, are present in previously characterized genotypes, where the first occurred in 88.89%, the second in 18.52% and the third in 70.37% respectively. No resistance genes for angular leaf spot disease were characterized by the CIR 246 marker (Table 5).

The locus CIR-316 was almost monomorphic among the genotypes. Most of them presented the allele CIR316M-210 bp linked to nematode resistance, except for the genotypes PI0440, PI0429 and BA0502 where it was absent. Only one allele, CIR 246-259, was observed among the genotypes. None presented the marker linked to angular leaf spot resistance gene. PI0440, PI0467, BRS Buriti, BRS Cedro and PI0416 presented the SSR DC20027-202 bp locus, linked to the blue disease resistance gene. Finally, the genotypes that showed the marker BNL3279-124 bp linked to the reniform nematode resistance gene were: BA05101, MA0425, PI0467, IAC25, MA0409, RN0512, PI0432, CE0507, BRS BURITI, PI0429, PI0437, MA0407, CE0461, BA0477, BA0512, PI0416, MA0438, MA0418, CE0474 (Table 5).

The homologous sequences of the extension of the SSR loci linked to these genes mapped in *G. hirsutum* were accessed in the NCBI and the biological processes in the blast2GO (Table 6). Except for the gene region associated with CIR246, for which a protein product has not yet been characterized, BNL3279, DC20027 and CIR316 are associated with different protein synthesis, biological and molecular processes described only for the first two SSR loci.

Discussion

Some plants have compounds that affect the biology, development and reproduction of insects causing antibiosis. Antibiosis contributes to pest management and insect resistance to chemical insecticides, making it a promising source for plant genetic improvement research (Lara, 1991).

It was possible to observe high initial mortality of *S. frugiperda* larvae that fed on the genotypes PI0433, BA05101, MA0418, BA0502, MA0407 as well as on the commercial varieties IAC 25, BRS Buriti and BRS Cedro. This may be due to defense mechanisms present in plants providing resistance to insects, as a structural barrier (cuticular wax, thorns and trichomes) and secondary compounds (such as glucosinolates

Table 5. Allelic composition of four microsatellite markers linked to disease resistance genes in cotton genotypes.

Genotypes	Primer												
	CIR 316M			CIR 246			DC 20027		BNL 3279				
	198	**201**	**210**	**146**	259	292	200	**202**	**114**	**124**	130	136	144
PI0440	0	0	NA	0	NA	NA	0	1	0	0	0	0	0
BA05101	0	0	1	0	1	0	1	0	0	1	1	0	0
MA0425	0	0	1	0	1	0	1	0	0	1	1	0	0
PI0467	0	0	1	0	1	0	0	1	0	1	1	0	0
IAC25	0	0	1	0	1	0	1	0	0	1	1	1	0
MA0423	0	0	1	0	1	0	NA	NA	0	0	0	0	0
PI0433	0	0	1	0	1	0	1	0	0	0	0	0	0
MA0409	0	0	1	0	NA	NA	1	0	0	1	1	0	0
RN0512	0	0	1	0	1	0	1	0	0	1	0	0	0
PI0432	0	0	1	0	1	0	1	0	0	1	1	0	0
CE0507	0	0	1	0	1	0	1	0	0	1	0	0	0
BRS BURITI	0	0	1	0	1	0	0	1	0	1	1	1	0
PI0429	0	0	0	0	1	0	1	0	0	1	0	0	0
AC0602	0	0	1	0	1	0	NA	NA	0	0	0	0	0
PI0438	0	0	1	0	1	0	NA	NA	0	1	1	0	0
MA0407	0	0	1	0	1	0	1	0	0	1	1	0	0
CE0461	0	0	1	0	1	0	NA	NA	0	1	1	0	0
BA0477	0	0	1	0	1	0	NA	NA	0	1	1	0	0
BRS CEDRO	0	0	1	0	0	1	0	1	0	0	0	0	0
BA0512	0	0	1	0	NA	NA	NA	NA	0	1	1	0	0
PI0416	0	0	1	0	NA	NA	0	1	0	1	1	0	0
BA0502	0	0	NA	0	0	1	NA	NA	0	0	0	0	0
MA0430	0	0	1	0	1	0	1	0	0	0	0	0	0
MA0438	0	0	1	0	1	0	1	0	0	1	1	0	0
CE0467	0	0	1	0	1	0	1	0	0	0	0	0	0
MA0418	0	0	1	0	1	0	NA	NA	0	1	0	0	0
CE0474	0	0	1	0	1	0	NA	NA	0	1	1	1	0
Delta	1	1	0	1	0	0	0	1	1	1	0	0	0
FMX966	1	1	0	1	0	0	1	0	1	1	0	0	0
M315	0	1	1	1	0	0	1	0	1	1	0	0	0

[1] Alleles marked in bold are the alleles linked to the resistance gene.

isoflavonoids, terpenoids, alkaloids.), favoring the defense against herbivory (War et al., 2012).

These substances can also reduce weight reduction and increase the larval period, with lower pupal weight, as observed for MA0418 and BRS Cedro. The reduced growth of *S. frugiperda* in these genotypes was attributed to the presence of defensive compounds, such as phenolic compounds, alkaloids and proteases that can directly or indirectly affect the attack of herbivorous insects (Pechan et al., 2000; Pechan et al., 2002; Magarelli et al., 2022).

The caterpillars fed on genotypes presented a longer larval period, which may have been caused by a lower absorption of the food substrate due to the presence of chemical compounds that attribute antibiosis and/or antixenosis resistance to the insect (Silveira et al., 1997; Boiça Junior et al., 2015). Most of the genotypes studied affected the biology of *S. frugiperda*, causing low viability. These genotypes possibly present factors that manifest resistance to *S. frugiperda*, which may be of chemical or morphological origin. Jesus et al. (2014a), studying the antibiosis in different commercial cotton varieties, observed on NuOpal™ the highest level of antibiosis to *S. frugiperda*. The cotton cultivars BRS Cedro and IAC 25 promoted the shortest larva-adult cycle of *S. frugiperda* with 53.13 and 54.00 days, respectively. Furthermore, *S. frugiperda* from PI0433 genotype showed the longest larva-adult duration with 67 days and 38 and 43 days when fed on CNPA 7H or BRS 187 8H cotton genotypes. The increase in the insect cycle is a typical characteristics of antibiosis (Miranda, 2010).

Although some genotypes allow caterpillars to complete their cycle, even in a longer period, all genotypes studied provided viability lower than 50%. Campos (2008) analyzed the viability of *S. frugiperda*, obtaining a value lower than 54% in thirteen cotton varieties. Insects from Acala 90 and BRS Cedro presented high mortality.

In general, *S. frugiperda* survived in eight cotton genotypes, showing significant antibiosis effects that may be useful for plant breeding, specially to use for conventional or organic cotton producers.

When analyzing the nutritional indices, only 8 of the 27 genotypes could be evaluated, since no weight gain was obtained in *S. frugiperda* larvae when fed with leaves of the plant, thus reducing the assimilation of the food ingested by RGR indices, ECI and RCR. The highest ECI value, which represents the conversion rates of food ingested and digested into biomass, was obtained in genotype MA0438 with 13.23%. The low ECI indicates the presence of metabolic compounds that inhibit the development of *S. frugiperda.*

The caterpillars fed on MA0438, PI0440, AC0602, PI0432, IAC 25, MA0409 and PI0429 presented lower food consumption, as well as lower RCR. The reduction in larval growth is due to low efficiency of food assimilation and, thus, conversion of food into body mass (Slansky et al., 1985; Slansky, 1990). Scriber and Slansky (1981) report that the reduction in RCR is due to the presence of allelochemical and toxic compounds

in the food substrate, producing inhibitory responses to feeding by insects. Thus, these compounds inhibited the feeding of the caterpillars in addition to the high mortality.

With the genetic analysis of the genotypes, it was possible to evaluate the genetic resistance to four important cotton diseases in cotton, using microsatellite molecular markers (Lanza et al., 1999; Cassetari-Neto et al., 2001). Thinking about multiple resistance, we seek to observe whether there is any relationship between the action of the respective resistance genes to diseases and the *S. frugiperda* caterpillar. The genotypes that showed the resistance gene for the reniform nematode *Rotylenchus reniformis* also showed antibiosis to *S. frugiperda*. The molecular function of the Ren resistance gene is related to an oxidoreductase activity, which is characteristic of a hypersensitivity reaction (Jones and Dangl, 2006; Uniprot, 2019a). Studies have shown that this type of reaction can activate the plant's defense mechanisms against the nematode, which may be similar when in contact with the *S. frugiperda*. In favor of this hypothesis, Rani and Pratyusha (2013) evaluated the response of cotton plants to the herbivory of *Spodoptera litura* (Lepidoptera: Noctuidae), in which it was possible to observe a large amount of the oxidative enzymes superoxide dismutase, catalase, peroxidase, polyphenol oxidase and phenylalanine ammonia lyase (PAL) in plants infested by this pest, corroborating that "Reactive Oxygen Intermediates' (ROIs)" play an important role in activating disease resistance structures in plants and animals (Delledonne et al., 2001).

Although the genotypes that showed the blue disease resistance gene (PI0440, PI0467, BRS Buriti, BRS Cedro and PI0416), also showed antibiosis to *S. frugiperda*, we were unable to establish a relationship between them. The molecular function of this gene is related to an arginylation reaction (post-translational conjugation of arginine to the N-terminal aspartate or glutamate of a protein), which signals unwanted proteins, activating an RNA silencing mechanism important in the antiviral defense (Bortolamiol et al., 2007; Csorba et al., 2010; Fusaro et al., 2012; Incarbone and Dunoyer, 2013; Uniprot, 2019b; Li et al, 2008). However, these results demonstrate that the mocó cotton genotypes has multiple sources of resistance to different pests that attack the cotton plant.

Material and Methods

Spodoptera frugiperda rearing

The research was carried out at the Integrated Pest Management and Molecular Genetics Laboratories of the Instituto Federal Goiano, Campus Urutaí, Goiás, Brazil. Insects obtained from the Embrapa Rice and Beans Entomology Laboratory were used to develop a colony. The pupae were sexed and placed in 20 cm high x 10 cm diameter PVC cages, lined internally with paper (oviposition substrate) for emergence and mating. The adults were fed on 10% honey solution, placed in plastic containers (PET bottle cap), soaked in cotton and changed every two days. The newly hatched caterpillars were placed in 150 mL plastic pots containing an artificial diet (Kasten Junior et al., 1978). After reaching the 3rd instar caterpillars were individualized in plastic containers (50 mL) until pupal phase. The insects were reared in the laboratory at 25 ± 2 °C, 70 ± 10% RH, and 12:12 h (L:D).

Plant material

Twenty-seven cotton genotypes, belonging to the species *Gossypium hirsutum* latifolium and *G. hirsutum* race *marie galante*, obtained from the Embrapa Cotton - Germplasm Bank were used in this study (Table 7). Initially, seeds from each accession were scarified and sown in plastic bags (1 kg) with substrate (3:1:1 – soil, sand and cattle manure). Thirty days after germination (DAG) thinning was performed, keeping one plant per plastic bag. Plants with 45 DAG were transplanted to the experimental area and irrigated via central pivot under field conditions.

Biological parameters and nutritional indices of *Spodoptera frugiperda* in cotton genotypes

Newly hatched caterpillars from the F2 generation were individualized in plastic containers (100 mL) containing moistened filter paper. The caterpillars were fed with leaves of each cotton genotype which were replaced when it was consumed by the caterpillar. The larvae were kept in these containers until the pupal stage. When the insects reached the pupal stage, the supply of leaves was interrupted in the adult stage and the insect did not receive any type of food. The biological variables evaluated were: a) larval stage; period and viability of the larval stage and weight of caterpillars at ten days of age; b) pre-pupal stage: period and viability; c) pupal stage: period, weight at 24 hours of age and viability; d) total cycle: period and feasibility; e) adult phase: longevity and sex ratio (male and female).

Spodoptera frugiperda 3rd instar caterpillars from mass rearing were deprived of food for 3 h to clean the intestinal tract and weighed to determine the initial weight of the insects. Subsequently, they were individualized in Petri dishes (1.5 × 9.0 cm in diameter) coated with moistened filter paper and fed with each cotton genotype.

The food was weighed daily using a precision analytical balance and replaced when necessary until the end of the experiment. After this period, the caterpillars and the remaining leaves were separated, dried in an oven at 60 °C for 72 hours and weighed. The feces were oven-dried for 24 hours at 40 °C, and then weighed.

Dry weights were used to calculate nutritional indices, according to the method proposed by Waldbauer (1968): the relative consumption rate ($RCR = I/(B \times T)$), the relative growth rate ($RGR = B/(Bm \times T)$) and the efficiency of conversion of ingested food ($ECI = (B/I) \times 100;\%$), where T = duration of the feeding time (days); I = weight of the ingested food (g) during T; B = caterpillars weight gain (g) during T; F = weight of produced feces (g) during T; M = metabolized food during T ($M = [I - F] - B$), and Bm = caterpillars mean weight (g) during T.

The experiment to determine the nutritional indices was carried out in a completely randomized design with 27 treatments (genotypes) and 20 replications. Twenty caterpillars were weighed to obtain the aliquot for weight correction after drying in the oven. The experiment was carried out over a period of five days. Also, to evaluate de biological parameters, a completely randomized design with 27 treatments (genotypes) and 40 replications were used.

Genetic analysis by microsatellite markers

The mocó cotton genotypes were genotyped using four microsatellite markers linked to resistance genes: DC20027, linked to the Rghv1 gene that confers resistance to blue disease (*Cotton leafroll dwarf virus*, CLRDV) (Fang et al., 2010); CIR246, linked to the B12 gene, which confers resistance to angular leaf spot (*Xanthomonas axonopodis* pv. *malvacearum*) (Xiao et al., 2010); CIR316M, linked to the one of the genes of the QTL loci which control the resistance to the root-knot nematode (*Meloidogyne incognita*) (Shen et al., 2006; Guitiérrez et al., 2010) and BNL3279 linked to the

Table 6. Genomic and functional description of resistance genes linked to microsatellite loci obtained from homologous sequences of genes.

Primer	Resistance gene	Chromosome	NCBI gene locus ID	Protein	Biological Process		Molecular Function		UniProt Link
					GO	Description	GO	Description	
BNL3279	Ren	11	LOC107962236	glyceraldehyde-3-phosphate dehydrogenase GAPCP1, cloroplastic-like	GO:0006006	glucose metabolic process	GO:0016491	oxidoreductase activity	https://www.uniprot.org/uniprot/A0A1U8PS93
DC20027	Rghv1	10	LOC107931832	arginil-ARNt - proteína-transferase tipo 2	GO:0016598	protein arginylation	GO:0004057	arginyltransferase activity	https://www.uniprot.org/uniprot/A0A1U8LXM1
CIR316M	loco QTL que controla a resistência	11	LOC107942787	trafficking protein particle complex subunit 8-like	-	-	-	-	https://www.uniprot.org/uniprot/A0A1U8N2E0
CIR246	B12	14	LOC107902825	Uncharacterized	-	-	-	-	https://www.uniprot.org/uniprot/A0A1U8J273

resistance gene Ren^{lon}, conferring resistance to the reniform nematode (*Rotylenchulus reniformis*) (Dighe et al., 2009). Three *G. hirsutum* plants were included as allele size controls: Delta Opal, FMX966 and M315.

Genomic DNA was obtained from young leaves of cotton genotypes using the 2% CTAB method proposed by Doyle and Doyle (1990), with a modification related to the maceration method, in which the leaf tissue samples were manually crushed in 2.0 mL tubes, separately for each genotype, with a stainless steel sphere (6 mm) in liquid nitrogen (N_2). For this, the tubes were frozen in N_2 and transferred to a rack with a lid, where the leaf tissue sample was macerated through strong unidirectional movements (up and down) for 30 seconds. The extracted DNA was resuspended in 100 µl of TE-RNAase solution (1 M Tris HCL pH=8.0, 0.5 M EDTA pH=8.0 and 10 mg/mL RNAse). Aliquots of the extracted genomic DNA were quantified in 0.8% agarose gel electrophoresis and stained with ethidium bromide (0.5 µL/mL), by visually comparing the fluorescence intensity of the DNA bands in relation to the marker of known molecular mass of λ phage DNA (50, 100 and 200 ng/µL). Then, the genomic DNA was diluted to a final and use concentration of 10 ng/µL.

The PCR reactions were performed for a final volume of 12 µL, containing 20 ng of DNA, 1x of buffer (50 mM KCl, 10 mM Tris HCl pH 8.3 and 1.5 mM MgCl2), 0.3 mM of each primer (forward and reverse), 0.25 mM of each dNTP and 1 U of Taq DNA polymerase. The reactions were carried out in a thermocycler with the following temperature conditions and steps: an initial denaturation step at 94 °C for 5 minutes; followed by 35 cycles with steps of denaturation (1 minute at 94°C), annealing (1 minute, gradient test temperature) and extension (1 minute at 72°C); and finally, the final extension step at 72 °C for another 7 minutes. The product of each PCR was separated by vertical electrophoresis in 4% polyacrylamide gel and stained with silver nitrate. After the revelation and drying of the gel, it was analyzed under white light, allowing the identification of the genotypes and their

quality with regard to the sharpness of the bands and the existence or not of nonspecific amplifications.

Statistical analysis

The data obtained were submitted to analysis of variance using the F test, and the means were compared using the Scott Knott test at the 5% probability level (R Core Team, 2017 – Scott Knott Package). The size of the amplified microsatellite fragments or alleles from each loci were estimated, separately, through a best-fit regression model based on the migration distance in centimeters and the size of DNA fragments obtained from the 50 bp marker standard.

Conclusion

Cotton genotypes PI0433, BA0502, BA05101, MA0407, MA0418, BRS Buriti, BRS Cedro and IAC 25 showed antibiosis to *Spodptera frugiperda*, as evidenced by high initial mortality and low larvae viability. Some genotypes are also resistant to blue disease and to the reniform nematode in cotton, as shown by SSR markers. Mechanisms of resistance may be common considering *Rotylenchulus reniformis* and *S. frugiperda*.

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Conflict of Interest

The authors declare no conflict of interest.

Table 7. Relationship and origin of local cotton cultivars for selection of resistance to *S. frugiperda* (Lepidoptera: Noctuidae).

Genotypes	States of Origin	Race or Variety
BRS BURITI[1]	Goiás	*G. hirsutum latifolium*
BRS CEDRO[1]	Piauí	*G. hirsutum latifolium*
IAC 25[1]	São Paulo	*G. hirsutum latifolium*
AC0602[2]	Acre	*G. hirsutum* r. *marie galante*
BA0477[2]	Bahia	*G. hirsutum* r. *marie galante*
BA0502[2]	Bahia	*G. hirsutum* r. *marie galante*
BA05101[2]	Bahia	*G. hirsutum* r. *marie galante*
BA0512[2]	Bahia	*G. hirsutum* r. *marie galante*
CE0461[2]	Ceará	*G. hirsutum* r. *marie galante*
CE0467[2]	Ceará	*G. hirsutum* r. *marie galante*
CE0474[2]	Ceará	*G. hirsutum* r. *marie galante*
CE0507[2]	Ceará	*G. hirsutum* r. *marie galante*
MA0407[2]	Maranhão	*G. hirsutum* r. *marie galante*
MA0409[2]	Maranhão	*G. hirsutum* r. *marie galante*
MA0418[2]	Maranhão	*G. hirsutum* r. *marie galante*
MA0423[2]	Maranhão	*G. hirsutum* r. *marie galante*
MA0425[2]	Maranhão	*G. hirsutum* r. *marie galante*
MA0430[2]	Maranhão	*G. hirsutum* r. *marie galante*
MA0438[2]	Maranhão	*G. hirsutum* r. *marie galante*
PI0416[2]	Piauí	*G. hirsutum* r. *marie galante*
PI0429[2]	Piauí	*G. hirsutum* r. *marie galante*
PI0432[2]	Piauí	*G. hirsutum* r. *marie galante*
PI0433[2]	Piauí	*G. hirsutum* r. *marie galante*
PI0438[2]	Piauí	*G. hirsutum* r. *marie galante*
PI0440[2]	Piauí	*G. hirsutum* r. *marie galante*
PI0467[2]	Piauí	*G. hirsutum* r. *marie galante*
RN0512[2]	Rio Grande do Norte	*G. hirsutum* r. *marie galante*

[1]Commercial cultivars. [2]Cotton mocó.

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