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REVIEW

Simple genetic inheritance conditions resistance to *Liriomyza sativae* in melon

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Abstract The leafminer *Liriomyza sativae* (Diptera: Agromyzidae) stands out as the main plant health problem in melon in the Northeast region of Brazil, which is the main region for production and export of the fruit. Genetic resistance of plants is an important strategy in management of this pest. The plant BAGMEL 56-R was selected as a new source of resistance to *L. sativae* through antibiosis; this resistance is characterized by the death of larvae soon after they begin feeding on the leaf mesophyll; the result is leaf mines that are small and insignificant in terms of yield reduction. Lines with contrasting levels of resistance were obtained from the progenies of this source of resistance through successive self-pollinations, conducted by the pedigree breeding method. Through the segregation pattern of the progenies and the test cross, the genetic nature of resistance was determined; one gene with complete dominance conditions resistance. The name *Liriomyza sativae* resistance and the symbol *Ls* are suggested to represent this new gene. In addition, through a non-preference test with lines in contrast for antibiosis and the susceptible hybrid 'Goldex', the presence of antixenosis was observed in this source of resistance. Probably, these different types of resistance in the source BAGMEL 56-R are associated with distinct defense mechanisms. Therefore, with this new source, introgression of resistance to *L. sativae* in elite lines or commercial hybrids of melon is possible.

Keywords Antibiosis · Antixenosis · *Cucumis melo* · Complete dominance · Insect resistance

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Introduction

Species of leafminer of the genus *Liriomyza* Mik (Diptera: Agromyzidae) are significant pests in several economically important crops in the world (Kang et al. 2009; Liu et al. 2011). In the Northeast region of Brazil, responsible for nearly all melon production and export from the country (IBGE 2016; MDIC 2016), *Liriomyza sativae* Blanchard 1938 (Diptera: Agromyzidae) has stood out as the main plant health problem in the crop, considerably limiting yield (Costa-Lima et al. 2009; Araújo et al. 2013; Oliveira et al. 2017).

Adult females of *L. sativae* perforate the adaxial surface of the melon leaf for feeding and oviposition;

however, the larvae cause the greatest damage. Upon emerging from the eggs, the larvae feed on the leaf mesophyll and form mines that reduce the photosynthetic ability of the plant and, depending on the level of infestation, reduce the yield and soluble solids content of the fruit, making sale of the fruit unviable (Dogimont et al. 1999; Araújo et al. 2007). High infestations cause premature leaf drop, exposing fruit to the sun and affecting external quality (Parrella 1987; Dogimont et al. 1999). In addition, the cost of controlling this pest makes the crop less profitable.

Leafminer is mainly controlled through chemical methods. However, some insecticides used are not selective and eliminate natural enemies, and few options of active ingredients have been registered (Nunes et al. 2013). This not only reduces the efficiency of biological control but also contributes to the development of resistant leafminer populations (Hernández et al. 2011; Liu et al. 2011; Guantai et al. 2015), favoring outbreaks of the pest in production areas. In addition, in the Northeast region, although production is concentrated from the months of July to January, melon is grown throughout the year. The lack of a fallow period, associated with the use of susceptible cultivars and climatic conditions favorable to the biotic potential of the insect ($\approx$31 °C, low rainfall), allows the pest to proliferate throughout the entire production period.

Melon breeding programs have sought to develop cultivars with genetic resistance to *Liriomyza* spp., aiming to avoid the aforementioned problems and make production more sustainable and economically viable. This control technique is considered effective and can easily be adopted by the producer and associated with other management methods, providing benefits to producers, consumers, and the environment (Basij et al. 2011; Dogimont and Boissot 2016). Direct resistance of the plant to insects can be through antixenosis (or non-preference), which changes insect behavior, resulting in the choice of an alternative host, or by antibiosis, negatively affecting the biology of the insect (Dogimont et al. 2010).

The melon genetic breeding program of Embrapa has prioritized identification of sources of resistance to leafminer (Oliveira et al. 2017). In a recent evaluation of melon germplasm at Embrapa Agroindústria Tropical regarding reaction to *L. sativae*, new sources of resistance were identified, some through reducing survival of larvae (antibiosis) and others through exhibiting lower insect infestation (antixenosis) (data not shown). Among these sources a plant of the accession BAGMEL 56 stood out through exhibiting mines of >1 cm, due to death of larvae soon after they begin feeding on the leaf mesophyll, characterizing antibiosis-type resistance. This plant was selected with a view toward introgression of this resistance in elite genotypes of melon, and came to be denominated BAGMEL 56-R.

Some studies deal with determination of inheritance of antibiosis-type resistance to *Liriomyza* spp. in melon (Kennedy et al. 1978; Dogimont et al. 1999); nevertheless, when a new source is identified, clarification of the genetic nature of the resistance is indispensable because this information assists the breeder in choosing the most adequate breeding method and selection strategy for introgression of resistance, leading to greater gains in selection.

Therefore, the aims of this study were to obtain resistant lines from the melon accession BAGMEL 56-R with antibiosis-type resistance to *L. sativae*, examine genetic inheritance of resistance, and investigate the occurrence of antixenosis in the resistant lines obtained.

Materials and methods

Germplasm

A plant of the accession BAGMEL 56 was used, a Charentais melon of the botanical variety *cantalupensis*, obtained from collections in melon agrobiodiversity areas from Maranhão State in northeastern Brazil, in 1994, and conserved in the Active Germplasm Bank of Cucurbitaceae for northeasthern Brazil (BAGMEL) of Embrapa Semiárido. In November 2014, this plant (BAGMEL 56-R) was selected through exhibiting antibiosis-type resistance to leafminer. As selection of this resistant plant occurred before flowering, it allowed both self-pollination (S_1 generation) and test cross with the hybrid 'Goldex', susceptible to leafminer.

Obtaining lines

Segregating population

The pedigree breeding method was used to generate the segregating population, obtained by self-pollination of the BAGMEL 56-R plant until obtaining lines

resistant and susceptible to leafminer, important for the study of inheritance. Selection trials were carried out in young plants under controlled infestation in cages. In addition, to validate selection, families of the second ($S_{1:2}$) and third ($S_{2:3}$) generations of self-pollination were evaluated in the field under natural infestation. In both environments, samples of leafminer were collected and sent for taxonomic identification through morphological and molecular evaluations in the Agricultural Entomology Laboratory of the Universidade Federal Rural de Pernambuco—UFRPE in Recife, Pernambuco State, Brazil.

Evaluation under controlled infestation

The S_1, $S_{1:2}$, and $S_{2:3}$ generations were evaluated in 02/2015, 07/2015, and 01/2016, respectively, and were conducted in the greenhouse and in the Plant Breeding and Genetic Resources Laboratory (LMRGV) of Embrapa Agroindústria Tropical (latitude 3°44′S, longitude 38°33′W, and altitude of 19.5 m) in Fortaleza, Ceará, Brazil. To obtain plants, progenies of each generation were sown in polyethylene trays (200 cells) filled with a substrate composed of coconut fiber powder and HS-florestais® at the proportion of 1:1. Ten days after planting, the seedlings were transplanted to polyethylene pots with 0.3 L of substrate composed of HS-florestais® and earthworm humus at the proportion of 3:1. The plants remained in the greenhouse where they had been sown up to the time of infestation, and they were irrigated twice a day.

Controlled infestations were carried out in plants with three true leaves (22 days after planting), which were taken from the greenhouse to the laboratory and distributed in cages ($60 \times 80 \times 50$ cm and/or $115 \times 380 \times 90$ cm; covered with voile cloth). In these cages, eight flies up to 48 h of age, were released per plant. The insects used in these trials were raised in a laboratory, coming from periodic collections made in melon production areas in the Jaguaribe-Açu agricultural region (Icapuí, CE) and multiplied in jack bean, *Canavalia ensiformis* L. (Fabaceae).

At 24 h after infestation, the plants were removed from the cages and returned to the greenhouse. From the fifth to the tenth day after infestation, larval development was observed, and the plants were classified as resistant (they did not allow development of larvae to pupae) or susceptible (they allowed development of at least one larva to the pupa stage). In the S_1 and $S_{1:2}$ generations, the number of mines per plant was also evaluated (intensity of infestation) on the fourth day after infestation. Data analysis was carried out in a descriptive manner.

Field evaluation

The experiment was conducted from 11/2015 to 01/2016 in the Pacajus Experimental Field (latitude 4°10′S, longitude 38°27′W, and altitude of 60 m), belonging to Embrapa Agroindústria Tropical in the municipality of Pacajus, Ceará, Brazil. Ten $S_{1:2(R)}$, five $S_{1:2(S)}$ and fourteen $S_{2:3(R)}$ families were evaluated. The symbols $_{(R)}$ and $_{(S)}$ indicate selection for resistant and susceptible genotypes, respectively. Plots composed of 10 plants were distributed in a randomized block design with two replications.

Seedlings were obtained as described in the previous item. Ten days after seeding, the plants were transplanted to the field with a spacing of 0.4 m between plants and 2.0 m between rows. Throughout the growing period, a drip irrigation system was adopted, and fertilization was carried out daily through fertigation. No insecticide was used for pest control. Evaluation was made at 46 days after transplanting, classifying the plants as resistant or susceptible, according to the previous item. Data analysis was performed in a descriptive manner.

Selection strategy

To obtain the resistant line, in the first trial, the resistant S_1 plants with a smaller number of mines per plant (NM/plant) were selected, self-pollinated, and harvested individually, generating the $S_{1:2(R)}$ families. In the $S_{1:2(R)}$ generation, families with the highest proportion of resistant plants were selected, and, within families, resistant plants with the smallest NM/plant were selected. This selection strategy was repeated until obtaining a homozygous resistant family, which containing only resistant plants with progenies with the same phenotypic pattern. Parallel to this, in the opposite direction, susceptible plants were selected with the highest NM/plant for the purpose of obtaining a susceptible line.

In each generation, the plants selected were transferred to polyethylene pots filled with 5.0 L of substrate (HS-florestais® and earthworm humus;

3:1). At flowering, artificial self-pollination of the female flowers was performed, which were protected with gelatin capsules to avoid pollen contamination. The plants were grown until obtaining seeds, which were harvested individually per plant aiming to constitute the families of the next generation.

Genetic inheritance of resistance

The performance data of the antibiosis trait of the segregating population progenies of the BAGMEL 56-R accession and of the test cross (BAGMEL 56-R x 'Goldex') were used with the aim of clarifying genetic inheritance of resistance. The progeny of the test cross was evaluated as described in the item "Evaluation under controlled infestation" in an experiment conducted in 02/2015 in the LMRGV.

The data obtained in these populations were analyzed by the Chi square test ($P < 0.05$) for the purpose of identifying a genetic model suitable for inheritance of the trait.

Non-preference test

The A56-06-02 (resistant) and A56-16 (susceptible) contrasting lines for the antibiosis trait were evaluated together with the Goldex commercial hybrid in the experiment with and without an opporutunity for choice in the LMRGV. The trials were carried out in a completely randomized design, with nine replications, in which each plant constituted a plot.

The manner of obtaining and infesting the plants was carried out as described in the item "Evaluation under controlled infestation". In the test with choice, all the genotypes were placed in the same cages, such that the insects had the option of choosing the genotypes. In contrast, in the test without choice, the plants of each genotype were distributed separately in cages.

Four days after infestation, the number of mines (NM) per leaf of each plant was quantified. Under laboratory conditions, leaves with larvae showing normal development were kept in plastic cups for collection and determination of the number of pupae (NP) and, after that, the number of adults (NA). From the data collected, larval viability (LV = 100 NP/NM) and pupal viability (PV = 100 NA/NP) per plant were estimated.

Data on the number of mines, larval viability, and pupal viability were subjected to combined ANOVA and to the Tukey test at the level of 5% probability.

Results

Obtaining lines

Taxonomic identification of all samples of leafminer showed only the species *Liriomyza sativae* Blanchard 1938 (Diptera: Agromyzidae).

Of the 272 plants of the test with S_1 progenies, 77% exhibited antibiosis lethal to larvae of *L. sativae* (Table 1). The first individual selection of resistant S_1 plants allowed 10 $S_{1:2(R)}$ families to be obtained, of which four (A56.04, A56.06, A56.07, and A56.10) stood out by exhibiting totally resistant plants (Table 1). Among these four families, five plants of each with the smallest NM/plant were selected to form the next generation ($S_{2:3(R)}$ population). Nevertheless, only 14 $S_{2:3(R)}$ families were obtained because the plants selected from the A56.04 family were highly infested by powdery mildew (*Podosphaera xanthii*), and one plant of the A56.07 family did not reach fructification.

The $S_{2:3(R)}$ families were tested in the third trial in which 100% of the progeny (350 plants) showed resistance to leafminer (Table 1). This result initially led to speculation that the 14 $S_{2:3(R)}$ families, just as their respective parents, were homozygous for resistance to leafminer. However, field evaluation did not confirm this expectation.

Although the evaluations in the two environments were similar for most of the families, some that did not segregate under controlled infestation segregated in the field (Table 1). This happened to the families $S_{1:2(R)}$ A56.07 and $S_{2:3(R)}$ A56.07.01 and A56.07.04, the last two families being progenies of the first. This substantiated that the A56.07 family was not homozygous for resistance.

The $S_{1:2(R)}$ A56.06 and A56.10 family, just as the respective progeny families ($S_{2:3(R)}$), are noteworthy for excellent performance observed in all the trials, exhibiting only plants with antibiosis lethal to larvae of *L. sativae*. Therefore, considering the evaluations in both environments, the families within the progenies of A56.06 and A56.10 are homozygous for resistance and can be used as lines.

Table 1 Advance of generations of the segregating population of melon, obtained from the plant BAGMEL 56-R, aiming to obtain lines resistant to leafminer

Population[a]	Number of plants								
	Cage					Field			Total
	R		S		R (%)	R	S	R (%)	
S_1: A56	210	(7.54)	62	(10.95)	77.21	–	–	–	272
$S_{1:2(R)}$	173	(16.15)	25	(31.13)	87.37	145	42	77.54	385
A56.01	16	(13.8)	4	(33.8)	80.00	12	8	60.00	40
A56.02	16	(09.9)	4	(21.0)	80.00	6	10	37.50	36
A56.03	17	(16.8)	3	(22.5)	85.00	17	2	89.47	39
A56.04	20	(13.2)	0	–	100.00	19	0	100.00	39
A56.05	16	(15.1)	4	(24.8)	80.00	15	4	78.95	39
A56.06	20	(12.3)	0	–	100.00	28	0	100.00	48
A56.07	20	(25.1)	0	–	100.00	10	9	52.63	39
A56.08	15	(14.2)	3	(34.7)	83.33	8	4	66.67	30
A56.09	13	(22.1)	7	(40.0)	65.00	12	5	70.59	37
A56.10	20	(18.8)	0	–	100.00	18	0	100.00	38
$S_{2:3(R)}$	350	–	0	–	100.00	260	11	95.94	271
A56.06.01	25	–	0	–	100.00	30	0	100.00	55
A56.06.02	25	–	0	–	100.00	19	0	100.00	44
A56.06.03	25	–	0	–	100.00	20	0	100.00	45
A56.06.04	25	–	0	–	100.00	19	0	100.00	44
A56.06.05	25	–	0	–	100.00	19	0	100.00	44
A56.07.01	25	–	0	–	100.00	8	7	53.33	40
A56.07.02	25	–	0	–	100.00	20	0	100.00	45
A56.07.03	25	–	0	–	100.00	19	0	100.00	44
A56.07.04	25	–	0	–	100.00	16	4	80.00	45
A56.10.01	25	–	0	–	100.00	17	0	100.00	42
A56.10.02	25	–	0	–	100.00	17	0	100.00	42
A56.10.03	25	–	0	–	100.00	18	0	100.00	43
A56.10.04	25	–	0	–	100.00	20	0	100.00	45
A56.10.05	25	–	0	–	100.00	18	0	100.00	43

[a] *R* resistant; *S* susceptible; *R (%)* percentage of resistant plants; the mean number of mines per plant of each class in indicated between parentheses (R and S)

To obtain the susceptible line, initially, ten susceptible S_1 plants were selected as parents of the $S_{1:2(S)}$ families. In the trial in cages, these families did not segregate; all of them maintained the same susceptible response of the parents (Table 2). The same performance was observed in the field in the five families evaluated (Table 2). It was thus deduced that all the susceptible plants were homozygous for susceptibility to leafminer.

Inheritance of resistance

From the data observed in the test cross and segregating population trials (cages and field), the model of a gene with complete dominance was proposed, in which the allele that grants resistance (*Ls*) is dominant over that which grants susceptibility (*ls*) to explain genetic control of resistance by antibiosis to *L. sativae*, which was observed in the plant BAGMEL 56-R and in its progenies.

Through this model, *Lsls* can be attributed to the genotype of the BAGMEL 56-R plant because it was found to be heterozygous in regard to resistance. In the test cross, this heterozygous plant (*Lsls*) was crossed with the susceptible parent 'Goldex' (*lsls*), and it was expected that the progenies would exhibit a 1:1 proportion of resistant plants (*Lsls*) and susceptible plants (*lsls*); 85 resistant plants and 84 susceptible plants were observed (Table 3). In this respect, the S_1 progeny of the BAGMEL 56-R plant has resistant plants with *LsLs* or *Lsls* genotypes in an expected

Table 2 Advance of generations of the segregating population of melon, obtained from the plant BAGMEL 56-R, aiming to obtain lines susceptible to leafminer

Population[a]	Number of plants						
	Cage			Field			Total
	R^+	S	S (%)	R	S	S (%)	
S_1	210	62	22.79	–	–	–	272
$S_{1:2\ (S)}$	0	197	100.00	0	101	100.00	298
A56.11	0	20	100.00	–	–	–	20
A56.12	0	20	100.00	0	25	100.00	45
A56.13	0	20	100.00	–	–	–	20
A56.14	0	20	100.00	–	–	–	20
A56.15	0	20	100.00	0	23	100.00	43
A56.16	0	20	100.00	0	20	100.00	40
A56.17	0	20	100.00	–	–	–	20
A56.18	0	20	100.00	0	15	100.00	35
A56.19	0	20	100.00	0	18	100.00	38
A56.20	0	17	100.00	–	–	–	17

[a] *R* resistant; *S* susceptible; *R (%)* percentage of resistant plants

phenotypic proportion of $^3/_4$, and has susceptible plants with the genotype *lsls* at a proportion of $^1/_4$. The numbers observed were 210 resistant plants and 62 susceptible (Table 3). In both populations, the deviations between the frequencies expected and those observed were not significant by the Chi square test (Table 3). This suggests that the model proposed is suitable for inheritance of the trait in question.

The model was also tested in selection of resistant plants in the S_1 generation and in their progenies ($S_{1:2(R)}$ generation). The complete dominance of the trait did not allow differentiation of the genotypes *LsLs* and *Lsls*, hindering phenotypic selection of homozygous resistant plants. In the S_1 generation, with the exclusion of susceptible plants, only resistant plants remained, with the expectation that $^1/_3$ would have *LsLs* genotypes and $^2/_3$ would have *Lsls* genotypes; that is, a genotypic frequency of 1:2. It was observed that in ten resistant plants selected, three were homozygous (*LsLs*) and the others heterozygous (*Lsls*), confirmed by the segregation of the respective $S_{1:2(R)}$ progenies (Table 3). Therefore, the phenotypic frequency expected in the $S_{1:2(R)}$ generation is five resistant plants to one susceptible (5:1), considering that the homozygous parents have only resistant progenies (*LsLs*) and the heterozygous segregate in the proportion 1 *LsLs*:2 *Lsls*:1 *lsls*, that is, 3 resistant for every 1 susceptible. A total of 318 resistant plants and 67 susceptible ones were observed (Table 3). Likewise, the data on the seven $S_{1:2(R)}$ families which are segregating (i.e., excluding A56.04, A56.06 and A56.10), with the genotypic frequency expected of three resistant plants to one susceptible (3:1). In this case, a total of 193 resistant plants and 67 susceptible ones were observed. Therefore, in the three cases, the deviations between the expected frequencies and those observed were not significant by the Chi square test (Table 3).

Another observation that corroborates the suitability of the model suggested is that the ten susceptible plants selected in S_1 had only susceptible progenies, showing that they were recessive homozygous (*lsls*). Therefore, the frequency observed was identical to that expected, both in the selected plants and in the $S_{1:2(S)}$ population (Table 3).

Non-preference test

In the trials with the S_1 and $S_{1:2(R)}$ generations, the plants resistant through antibiosis showed amplitude

Table 3 Chi square test (χ^2) applied to the segregating population of BAGMEL 56-R and of the test cross (BAGMEL 56-R x 'Goldex')

Population	Absolute frequency			Ratio expected	χ^2	p
	Resistant		Susceptible			
Test cross	85		84	(1:1)	0.06	0.94
S_1	210		62	(3:1)	0.71	0.40
Selection $S_{1:2}$	(*LsLs*)	(*Lsls*)	(*lsls*)			
Resistant	3	7	–	(1:2)	0.50	0.82
Susceptible	–	–	10	–	–	–
$S_{1:2(R)}$—total	318		67	(5:1)	0.15	0.70
Heterozygotes	193		67	(3:1)	0.08	0.77
$S_{1:2(S)}$	0		298	(0:1)	–	–

of infestation (NM/plant) similar to the susceptible plants (data not shown). However, it was observed that the average NM/plant among the resistant progenies was always lower than the average observed in the susceptible ones (Table 1). This suggests that, in addition to antibiosis, there is also an antixenosis-type resistance to *L. sativae* in the progenies of the BAGMEL 56-R plant. This corroborated the results of the non-preference tests, with and without choice, carried out with contrasting lines obtained from the BAGMEL 56-R plant and the 'Goldex' hybrid (Table 4).

In the non-preference test, the resistant line A56-06-02 was less infested than the susceptible line A56-16, shown by the difference in NM/plant (Table 4). In the two trials, the response of the lines did not vary for this variable. Nevertheless, 'Goldex' exhibited unstable performance; its performance was similar to the resistant line in the test without choice since it was less attacked by leafminer in comparison with the test with choice. However, in the test with choice, it was similar to the susceptible line (Table 4).

The LV/plant data reinforce the finding of antibiosis-type resistance lethal to larvae of leafminer in the A56-06-02 line since they showed the NP/plant equal to zero, although oviposition (presence of mines) had occurred. The A56-16 line and 'Goldex' had similar high values for LV/plant (>91%), showing that they did not have any negative effect on larval development of the insect (Table 4). In addition, these two genotypes also showed similar high values for PV/plant (>81%). For the A56-06-02 line, this variable was not estimated because it did not allow development of the insects to the pupal and, consequently, adult phase.

Discussion

Taxonomic identification of exclusively *L. sativae* shows that it is the main species present in melon production areas in the Northeast region, which corroborates studies already carried out (Costa-Lima et al. 2009; Araújo et al. 2013; Ferreira 2014; Oliveira et al. 2017). The species *L. sativae*, *L. trifolii*, and *L. huidobrensis* are the main pests in the genus described as affecting various economically important crops, and they show wide geographic dispersion and polyphagous habits (Kang et al. 2009). Although the occurrence of *L. sativae* is prominent in the New World (Dogimont and Boissot 2016), it was cited as the most common species in the Sudan (Africa) in an evaluation of 100 melon accessions under field conditions (Gesmallah and Yousif 2004).

The hereditary nature of antibiosis-type resistance to *L. sativae*, shown by segregation of the characteristic in the progeny of the BAGMEL 56-R plant, led to the conclusion that the resistance detected was due to plant genetic defense mechanisms. A plant may be

Table 4 Mean number of mines and larval and pupal viability in three melon genotypes evaluated in regard to resistance to leafminer in testing with and without choice

Genotype[a]	Test without choice		Test with choice		Mean	
Number of mines/plant[b]						
A56-06-02 (R)	7.11	aA	4.90	aA	6.00	
Goldex	14.22	aA	27.56	bB	20.90	
A56-16 (S)	27.67	aB	28.00	aB	27.90	
Mean	16.33		20.15			
Larval viability/plant (%)						
A56-06-02 (R)	0.00		0.00		0.00	A
Goldex	92.23		91.10		91.66	B
A56-16 (S)	92.93		92.87		92.91	B
Mean	61.71	a	61.33	A		
Pupal viability/plant (%)[c]						
A56-06-02 (R)	–		–		–	
Goldex	85.68		85.42		85.43	A
A56-16 (S)	80.72		81.74		81.23	A
Mean	83.20	a	83.58	A		

[a] Resistant (R) and susceptible (S) line; [b] Mean values followed by the same letter, uppercase letter in the column and lowercase letter in the line, do not differ statistically among themselves by the Tukey test at 5% probability; [c] For the statistics of pupal/plant viability (%), the resistant line was not considered

wrongly characterized as resistant through exhibiting pseudo-resistance, which can occur through escape, induction, or other factors (Smith 2005).

In addition, segregation of the progeny indicates that the parent was not homozygous for resistance. The segregation observed in the accession and in the progeny of the selected plant shows that the BAGMEL 56 accession is composed of more than one genotype. This may be a result of the accession collection process itself since the accession comes from the crop areas of small producers in the Northeast of Brazil. It should be noted that melon has a mixed reproductive system, which favors expansion of genetic variability in production fields. In addition, the presence of more than one genotype in the accession can occur through mixture of seeds, natural crosses, or mutation (Fehr 1987).

In this context, self-pollination of the BAGMEL 56-R plant allowed the resistance characteristic to be maintained through the progenies, as observed in the results obtained in the segregating population. Thus, segregation of the antibiosis characteristic in two distinct classes allowed contrasting lines to be obtained, that is, resistant and susceptible lines.

Furthermore, it is important to note that the selection made in young plants under controlled infestation was effective in obtaining these lines. This allows selection and advances of generations at any time of the year, as long as the pest is available. Nevertheless, the evaluation performed in the field showed segregation of some families considered to be homozygous in the cage trials; field evaluation proved to be fundamental in selection of resistant lines. Discrepant results in the field and the greenhouse may occur due to different conditions inherent to each condition (Nunes et al. 2013).

Some suppositions may explain these differences, as for example, time of exposure of the plant to the pest. In the field, the plant is exposed throughout all phenological phases, increasing the possibility of expressing the phenotype of resistance or susceptibility. In contrast, controlled infestation exposes the plant to the insect for a day, and only in the initial phase of phenological development. Thus, evaluations of resistance in the field must be carried out to complement the results obtained under controlled conditions.

In addition, the number of individuals evaluated per family can be considered a limiting factor in selection of homozygous families. Nevertheless, 16 plants that are descendants of self-pollination of a resistant parent would be sufficient to conclude, with 99% certainty, that the parent is homozygous or heterozygous, considering a model with a gene with complete dominance (Cruz 2016). The number of plants evaluated in the two environments was greater than the minimum necessary for observation of segregation of the trait.

The BAGMEL 56-R plant is the first source with antibiosis-type resistance lethal to larvae of *L. sativae* registered in a melon accession collected in Brazil, though this resistance was previously described in the accessions PI 282448 (African) and PI 313970 (Indian), also resistant to *L. sativae* (Kennedy et al. 1978), and in the Nantais Oblong line (French), resistant to *L. trifolii* (Dogimont et al. 1995). It is noteworthy that resistance is characterized by death of the larvae soon after they begin to feed on the leaf mesophyll, resulting in almost imperceptible galleries (>1 cm) in the leaves; when compared to galleries created in susceptible plants, they are insignificant. It was apparent that the presence of mines in the resistant progenies did not reduce the photosynthetic capacity of the plant and, consequently, these mines did not affect fruit yield and quality. In addition, as these progenies do not allow larval development, they contribute to reducing the population of the insect in the field, and they provide benefits to humans and the environment through reducing the use of insecticides in pest management.

Simple inheritance of the antibiosis trait made it easier to obtain contrasting lines rapidly, requiring only three self-pollination generations, considering the last generation, which was used to confirm supposition of homozygosity of the trait in the parents. Inheritance of the antibiosis-type resistance of the BAGMEL 56-R plant was explained by a model of complete dominance in a gene composed of two alleles (*Ls* and *ls*). Similar inheritance was obtained in the analysis of generations carried out with the resistant source Nantais Oblong (France) (Dogimont et al. 1999; Dogimont 2011). That line has antibiosis-type resistance to the larvae of *L. trifolii*, with resistance controlled by a gene with complete dominance, which was denominated *Lt*. In contrast, accessions PI 282448 (Africa) and PI 313970 (India), which exhibited a smaller number of mines and greater larval mortality to *L. sativae*, have apparent recessive resistance and incomplete dominance, respectively (Kennedy et al. 1978).

Although resistance is similar in BAGMEL 56-R and the French line, there is evidence that they are controlled by distinct genes or distinct alleles of a same locus. Under field conditions, the Nantais Oblong line was evaluated in a trial juxtaposed to the experiment for evaluation of the progenies of the BAGMEL 56-R plant; however, unlike the latter source, Nantais Oblong did not manifest resistance to *L. sativae*, that is, the larvae exhibited normal development in the Nantais Oblong line (data not shown). Resistance in the French line was also not manifested when infested with *L. huidobrensis* (Dogimont 2011). Therefore, a new dominant gene for resistance with the name *Liriomyza sativae* resistance and the symbol *Ls* is suggested, present in resistant lines obtained from the BAGMEL 56-R plant.

Thus, the specificity of resistance to the determined species of *Liriomyza* is demonstrated in resistant melon genotypes. However, new studies dealing with the specificity of the proposed *Ls* gene against the different species of leafminer that attack melon need to be carried out so that resistance is used in a reliable manner. This concern exists due to the occurrence of more than one species of *Liriomyza* in melon (Musundire et al. 2012; Dogimont and Boissot 2016), emphasizing the importance of identification of the species in studies of resistance, given the specificity of resistance.

Although antibiosis is the most notable type of resistance to *L. sativae* in resistant progenies of the BAGMEL 56-R plant, antixenosis may also perform resistance in the adult phase of the insect. This was also a point of speculation in the Nantais Oblong line (Dogimont et al. 1995).

In this study, inheritance was proposed only for antibiosis because antixenosis was only observed from the results of the trials of the segregating population and subsequently confirmed in the non-preference tests. However, it is believed that the two types of resistance are not associated with the same gene because in the first generations, there was similar amplitude of infestation in plants with contrasting levels of antibiosis. However, in proceeding with the segregating population, in addition to selection of resistant and susceptible plants through the antibiosis trait, plants with a smaller and a larger number of mines were selected; in other words, plants were selected that contrast due to antixenosis. Therefore, this may explain the results obtained in the non-preference test, in which plants with antibiosis were less preferred than the susceptible line.

Identification of some sources with antixenosis in regard to *Liriomyza* spp. is reported; however, the genetic nature of the trait is not known for any of them (Kennedy et al. 1978; Dogimont et al. 1995; Gesmallah and Yousif 2004; Guimarães et al. 2009; Nunes et al. 2013). To clarify inheritance of antixenosis, a study with a classic genetic design is recommended, which simultaneously evaluates the contrasting parents, P_1 and P_2, the F_1 ($P_1 \times P_2$) and F_2 ($F_1 \times F_1$) generations, and backcrosses, BC_1 ($F_1 \times P_1$) and BC_2 ($F_1 \times P_2$) (Cruz et al. 2012). This may be possible with the lines obtained in this study.

In addition, upon identifying new sources of resistance, it is important not only to clarify the genetic nature of the resistance but also to investigate the defense mechanisms responsible for the reaction since the same phenotype may be due to distinct mechanisms. In general, resistance to *Liriomyza* spp. in different crops was associated both with structural defense mechanisms (trichomes, wall thickness, etc.) (Wei et al. 2000) and chemical defense mechanisms (secondary metabolites, antidigestive proteins, etc.) (Kang et al. 2009). The defense mechanism(s) to *L. sativae* of the BAGMEL 56-R source need to be clarified. Since the genetic nature of the resistance has now been discovered, our current research projects are focused on finding the gene's location on the melon genome. This may pave the way towards developing marker assisted selection.

It should be emphasized that genetic resistance to insects should be used together with other control methods, such as biological control and crop practices (HansPetersen et al. 2010; Simmons et al. 2010), in addition to correct use of selective chemical control. The combination of control methods has additive effects to the plant resistance method since it reduces the possibility of the insect breaking this resistance and impedes rapid evolution of pest populations. In addition, it should be emphasized that resistant lines obtained from the BAGMEL 56-R plant have other favorable characteristics, such as good leaf cover, high production, resistance to powdery mildew, and good fruit quality. Thus, genetic breeding methods such as SSD (Single Seed Descent) and backcrossing are recommended for introgression of this resistance to *L. sativae*, above all in melons of the Charentais type.

The expectation is that this source of resistance be made available to melon producers in the future through

cultivars with good agronomic characteristics and resistance to *L. sativae*, promoting a sustainable production system with high yield and competitiveness.

Conclusion

Melon lines were obtained with antibiosis-type resistance to *Liriomyza sativae*. A complete dominance gene conditions antibiosis-type resistance to *L. sativae* in the source BAGMEL 56-R. In addition to antibiosis, this source of resistance also exhibits antixenosis.

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References

Araújo EL, Fernandes DRR, Geremias LD et al (2007) Occurrence of leafminer *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), losses and its parasitoid, in *Cucumis melo* L., in the semiarid of the Rio Grande do Norte. Rev Caat 20:210–212

Araújo EL, Nogueira CHF, Menezes Netto AC et al (2013) Biological aspects of the leafminer Liriomyza sativae (Diptera: Agromyzidae) on melon (Cucumis melo L.). Cienc Rural 43:579–582. doi:10.1590/S0103-84782013000400003

Basij M, Askarianzaeh A, Asgari S et al (2011) Evaluation of resistance of cucumber cultivars to the vegetable leafminer (*Liriomyza sativae* Blanchard) (Diptera: Agromyzidae) in greenhouse. Chil J Agric Res 71:395–400. doi:10.4067/S0718-58392011000300008

Costa-Lima TC, Geremias LD, Parra JRP (2009) Effect of temperature and relative-humidity on the development of *Liriomyza sativae* Blanchard (Diptera: Agromyzidae) in *Vigna unguiculata*. Neotrop Entomol 38:727–733. doi:10.1590/S1519-566X2009000600004

Cruz CD (2016) Genes software—extended and integrated with the R, matlab and selegen. Acta Sci Agron. doi:10.4025/actasciagron.v38i4.32629

Cruz CD, Regazzi AJ, Carneiro PCS (2012) Modelos biométricos aplicados ao melhoramento genético. UFV, Viçosa

Dogimont C (2011) Gene list for melon. Cucurbit Genet Coop Rep 33:104–133

Dogimont C, Boissot N (2016) Insect resistance in melon and its modification by molecular breeding. In: Ezura H, Ariizumi T, Garcia-Mas J, Rose J (eds) Functional genomics and biotechnology in solanaceae and cucurbitaceae crops. Springer, Heidelberg, pp 199–219. doi:10.1007/978-3-662-48535-4_12

Dogimont C, Bordat D, Pages C et al (1995) Characterization of resistance to *Liriomyza trifolii* (Burgess) in melon (*Cucumis melo*). Fruits 50:449–452

Dogimont C, Bordat D, Pages C et al (1999) One dominant gene conferring the resistance to the leafminer, *Liriomyza trifolii* (Burgess) Diptera: Agromyzidae in melon (*Cucumis melo* L.). Euphytica 105:63–67. doi:10.1023/A:1003436428847

Dogimont C, Bendahmane A, Chovelon V, Boissot N (2010) Host plant resistance to aphids in cultivated crops: genetic and molecular bases, and interactions with aphid populations. Comptes Rendus Biol 333:566–573. doi:10.1016/j.crvi.2010.04.003

Fehr WR (1987) Principles of cultivar development: theory and technique, vol 1. McGraw-Hill Inc, New York, p 536

Ferreira ECB (2014) Genetic structure of natural populations *Liriomyza sativae* Blanchard (Diptera: Agromyzidae). Dissertation, Universidade Federal Rural de Pernambuco, Pernambuco

Gesmallah AEE, Yousif MT (2004) Resistance in melons (*Cucumis melo* L.) to leafminers (*Liriomyza* spp.; Diptera: Agromyzidae). J Agric Sci 2:125–130

Guantai MM, Ogol CPKO, Salifu D et al (2015) Differential effects of pesticide applications on *Liriomyza huidobrensis* (Diptera: Agromyzidae) and its parasitoids on pea in Central Kenya. J Econ Entomol 108:662–671. doi:10.1093/jee/tov006

Guimarães JA, Oliveira VR, Michereff Filho M et al (2009) Evaluation of resistance of yellow melon hybrids to leafminers *Liriomyza* spp. Embrapa, Embrapa Hortaliças, p 16

HansPetersen HN, McSorley R, Liburd OE (2010) The impact of intercropping squash with non-crop vegetation borders on the above-ground arthropod community. Fla Entomol 93:590–608. doi:10.1653/024.093.0418

Hernández R, Harris M, Liu T-X (2011) Impact of insecticides on parasitoids of the leafminer, *Liriomyza trifolii*, in Pepper in South Texas. J Insect Sci 11:61. doi:10.1673/031.011.6101

IBGE (2016) Instituto Brasileiro de Geografia e Estatística. Banco de dados agregados: produção agrícola municipal. http://www2.sidra.ibge.gov.br/bda/tabela/listabl.asp?c=1612&z=t&o=11. Accessed 15 November 2016

Kang L, Chen B, Wei JN, Liu TX (2009) Roles of thermal adaptation and chemical ecology in *Liriomyza* distribution and control. Annu Rev Entomol 54:127–145. doi:10.1146/annurev.ento.54.110807.090507

Kennedy GG, Bohn GW, Stoner AK, Webb RE (1978) Leafminer resistance in muskmelon. J Am Soc Hortic Sci 103:571–574

Liu T, Kang L, Lei Z, Hernandez R (2011) Hymenopteran parasitoids and their role in biological control of vegetable *Liriomyza* leafminers. In: Liu T, Kang L (eds) Recent advances in entomological research. Springer, Heidelberg, pp 376–403

MDIC (2016) Ministry of Industry, foreign trade and services. The system of analysis of foreign trade information (AliceWeb). http://aliceweb.mdic.gov.br/. Accessed 15 Nov 2016

Musundire R, Chabi-Olaye A, Krüger K (2012) Host plant effects on morphometric characteristics of *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* (Diptera: Agromyzidae). J Appl Entomol 136(1–2):97–108. Available at http://doi.wiley.com/10.1111/j.1439-0418.2010.01597.x

Nunes GHS, Medeiros AC, Araujo EL, Nogueira CHF, Sombra KDS (2013) Resistance of melon accessions to leafminer *Liriomyza* spp. (Diptera: Agromyzidae). Rev Bras Frutic 35:746–754

Oliveira FIC, Fiege LBC, Celin EF, et al (2017) Screening of melon genotypes for resistance to vegetable leafminer and your phenotypic correlations with colorimetry. An Acad Bras Ciênc 89 (in press)

Parrella MP (1987) Biology of *Liriomyza*. Ann Rev Entomol 32:201–204

Simmons AM, Kousik CS, Levi A (2010) Combining reflective mulch and host plant resistance for sweet potato whitefly (Hemiptera: Aleyrodidae) management in watermelon. Crop Prot 29:898–902

Smith CM (2005) Plant resistance to arthropods: molecular and conventional approaches. Kluwer Academic Publishers, Dordrecht

Wei J, Zou L, Kuang R, He L (2000) Influence of leaf tissue structure on host feeding selection by pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae). Zool Stud 39:295–300