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SOURCES AND INHERITANCE OF LEAFMINER RESISTANCE IN YELLOW MELON ACCESSIONS[1]

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ABSTRACT - The use of resistant cultivars is an efficient and recommended method for the management of leafminers, which are the main phytosanitary problem in melons. The objectives of this study were to identify the sources of resistance to the leafminer in yellow melon accessions and to determine the resistance inheritance in acession AM-RT. Two field experiments were conducted in the municipalities of Baraúna, RN and Icapuí, CE, Brazil, to identify the sources of resistance. The design adopted was completely randomized blocks with 22 treatments and four replications. In this evaluation, the number of mines per leaf was quantified. The heterogeneity of the studied materials allowed for the identification of the accessions AM-RT and AM-TM as sources of resistance, considering that they revealed zero mines in the two evaluation environments. The accession AM-RT was selected and used to obtain the S_1 population (by self-fertilization), $S_{1:2}$ population derived from S_1, and crossing between AM-RT and 'Goldex', which were evaluated in a third laboratory trial to determine the genetic control of resistance in that material. By the segregation pattern of the populations S_1, $S_{1:2}$, and the crossing (AM-RT and 'Goldex') and the estimation of the chi-squared (χ^2) values, which were 1.33, 3.14, and 0.36, respectively, it was determined that the inheritance of resistance was controlled by only one gene with complete dominance. Therefore, in this study, two sources of resistance to the leafminer were identified, and resistance was conditioned by a gene with complete dominance in the accession 'AM-RT'.

Keywords: *Cucumis melo* L.. *Liriomyza* spp.. Genetic resistance. Dominant gene.

FONTES E HERANÇA DA RESISTÊNCIA À MOSCA MINADORA EM ACESSOS DE MELÃO AMARELO

RESUMO - O uso de cultivares resistentes é um método eficiente e recomendável para manejo da mosca minadora, principal problema fitossanitário na cultura do meloeiro. Nesse contexto, os objetivos deste trabalho foram identificar fontes de resistência à mosca minadora em acessos de melão amarelo e determinar sua herança genética. Para tanto, foram realizados dois experimentos em campo nos municípios de Baraúna - RN e Icapuí - CE para identificar fontes de resistência. O delineamento adotado foi em blocos completos casualizados com 22 tratamentos e quatro repetições. Na avaliação foi quantificado o número de minas por folha. A heterogeneidade dos materiais estudados possibilitou identificar os acessos AM-RT e AM-TM como fontes de resistência, considerando que apresentaram número de minas igual a zero nos dois ambientes de avaliação. O acesso AM-RT foi selecionado e utilizado para obter a população S_1 (autofecundação), população derivada de $S_{1:2}$ e o cruzamento entre AM-RT e 'Goldex', que foram avaliadas em um terceiro ensaio de laboratório visando determinar o controle genético da resistência em AM-RT. Portanto, por meio do padrão de segregação das populações S_1, $S_{1:2}$ e do cruzamento (AM-RT' and 'Goldex') avaliados, estimando-se os valores de qui quadrado (χ^2), que foram de 1,33, 3,14 e 0,36, respectivamente, foi determinado que a herança da resistência é controlada por apenas um gene com dominância completa. Então, nesse estudo foram identificadas duas fontes de resistência à mosca minadora e um gene com dominância completa condiciona à resistência no acesso AM-RT.

Palavras-chave: *Cucumis melo* L.. *Liriomyza* spp.. Resistência genética. Gene dominante.

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INTRODUCTION

Leafminer flies of the genus *Liriomyza* (Diptera: Agromyzidae) are one of the main phytosanitary problems in melon (*Cucumis melo* L.) in the semi-arid region of Northeast Brazil (NUNES et al., 2013), with *Liriomyza sativae* Blanchard reported as predominant in cultivated areas (FERREIRA et al., 2017; CELIN et al., 2017a).

The main damage caused by leafminers on melon is from larvae feeding on the leaf mesophyll tissue that create galleries or mines that reduce the photosynthetic area of the plant, causing the reduction of production and fruit quality (ARAUJO et al., 2013; COSTA et al., 2017).

Leafminer control in melons is in accordance with integrated pest management programs, while the use of the chemical methods prevails (LIMA et al., 2012). However, the intensive use of insecticides can adversely affect natural enemies and pollinators and may also result in the emergence of insect resistance (LEIBEE, 1981; ASKARI-SARYAZDI et al., 2015; WEI et al., 2015). Therefore, it is essential that new control measures are developed to reduce the use of insecticides and improve the coexistence of leafminers in melon crops.

The use of host genetic resistance is recommended as an alternative strategy to the application of insecticide products, with resistant cultivars recognized as a more economical control method (BASIJ et al., 2011). Other advantages of this method include reduced damage to the environment, less technical knowledge on the part of the farmer to apply the method, and integration possibilities with other control methods (GIRÃO FILHO et al., 2012).

Evaluation of the level of infestation in plants belonging to the melon germplasm allowed the identification of sources of resistance to leafminers. Among these, the accessions 'PI282448' and 'PI 313970' have been characterized as having recessive resistance and incomplete dominance, respectively (KENNEDY et al., 1978). Subsequently, the 'Nantais Oblong' line was reported to exhibit dominant monogenic inheritance (DOGIMONT et al., 1995). In Brazil, the accession 'Bagmel' was the first source of resistance that had its inheritance characterized as a dominant monogenic type (CELIN et al., 2017a). These reports prove that although there is genetic variability, studies that characterize this resistance are scarce in the literature.

For the efficient use of resistance sources, it is necessary to understand the genetic variability of the germplasm available for selection. Once resistance sources have been identified, the inheritance of the resistance must be determined to adopt the most appropriate strategies for improvement programs to obtain resistant cultivars.

The objective of this study was to identify sources of resistance in yellow melon and determine the resistance inheritance in AM-RT for genetic improvement of melon in terms of its response to leafminer attacks.

MATERIALS AND METHODS

Selection of sources of resistance

Two experiments were carried out under field conditions to select sources of resistance at different locations. The first was realized in the municipality of Icapuí, CE (4º 42' S, 37º 20' W, 6 masl) and the second in the municipality of Baraúnas, RN (5º 05'S, 37º 38' W, 95 masl), from July to September 2017, and November 2017 to January 2018, respectively. The mean values of maximum and minimum temperature, relative humidity, and rainfall during the experimental periods were: Icapuí (T_{max} = 28.47 ºC, T_{min} = 27.05 ºC, RH = 59.12 %, and Rainfall = 0.0 mm); and Baraúna (T_{max} = 28.82 ºC, T_{min} = 27.59 ºC, RH = 65.71 %, and Rainfall = 0.06 mm).

The trials were conducted in a completely randomized block design with 22 treatments and four replications. The treatments were 21 accessions of yellow melon from the Melon Germplasm Collection of the Federal Rural University of the Semi-Arid (UFERSA), and a commercial hybrid ('Goldex') as a susceptibility standard (Table 1). The experimental unit consisted of 10 plants.

Table 1. Yellow melon genotypes evaluated for resistance to leafminer in field trials in the municipalities of Icapuí and Baraúnas in the years 2017 and 2018.

Treatments	
'Goldex'	HAC-11
HAC-01	HAC-12
HAC-02	HAC-13
HAC-03	HAC-14
HAC-04	HAC-15
HAC-05	HAC-16
HAC-06	HAC-17
HAC-07	HAC-18
HAC-08	HAC-19
HAC-09	AM-RT
HAC-10	AM-TM

Sowing was realized in polystyrene trays (200 cells) filled with commercial substrate (Tropstrato®), and transplanting occurred 12 days after sowing (DAS). The spacing was 0.3 m between plants and 2.0 m between rows. Field management was similar to commercial management, and no insecticide was applied for pest control. Resistance was evaluated in adult plants under natural infestation, 30 days after transplanting, by collecting three leaves per plant and quantifying the number of mines per leaf (NML).

Data analysis was performed considering the mean obtained for the plot and applying the non-parametric Kruskal-Wallis test at the level of 5% of probability, using the statistical software R (R CORE TEAM, 2018).

Study of the genetic inheritance of leafminer resistance in melon

Germplasm

The resistant accession AM-RT was used in the inheritance study. This accession belongs to the *inodorus* group of the yellow type. In the inheritance study, the S_1 population obtained by self-pollination of AM-RT, $S_{1:2}$ populations derived from S_1, and crossing between AM-RT and 'Goldex' hybrid, which is susceptible to leafminers, were evaluated.

Obtaining segregating populations

The AM-RT seeds were placed to germinate in polystyrene trays containing commercial substrate (Tropstrato®). Twelve days after germination, 10 seedlings were transplanted into the field. At the time of flowering, self-pollination was conducted in five plants to obtain S_1 population and the other five were crossed with the hybrid 'Goldex' (susceptible) to obtain crossing, respectively. In the second self-pollination cycle, the $S_{1:2}$ generation was obtained from S_1.

The self-pollination method involved manual and controlled pollination. Flowers were isolated before anthesis and at 48–72 h after pollination. At the time of pollination, the male flower was detached and pollen was gently deposited on the stigma of the female flower. Information on parents, date, and type of pollination was specified using tags attached to the peduncle of the female flower.

After obtaining the S_1 and $S_{1:2}$ populations, the single seed descent (SSD) breeding method was used to obtain melon lines with potential resistance to the leafminer.

Inheritance study trial

The trials were conducted at the Laboratory of Applied Entomology of the Federal Rural University of the Semi-Arid (UFERSA), Mossoró, RN, where the leafminer *L. sativae* brood was maintained. Three trials were performed to study inheritance, in which 100 plants of the S_1 population, 150 plants of the $S_{1:2}$ population, and 100 plants of the crossing (AM-RT x 'Goldex') were evaluated. Seeds of all three treatments were sown in 200-cell polystyrene trays containing a commercial substrate (Tropstrato®). The trays were maintained in a greenhouse protected by an anti-aphid screen. Twelve DAS, the plants were transplanted into 0.3 L pots containing the same substrate used for sowing. The plants remained in the greenhouse and were irrigated twice a day.

When the plants reached the stage of three well-expanded definitive leaves (25 d after sowing), they were transferred to the laboratory, placed in cages with a wooden frame, coated with an anti-aphid screen, and exposed to infestation. Each cage contained approximately 300 pairs of leafminers. After 60 min of infestation, the plants were removed from the cages and taken to the greenhouse, where they remained until evaluation. Four days after infestation, the number of mines per leaf (NML) was determined. The leaves were collected and taken to the laboratory, where they were kept with their petioles in 30 mL plastic containers and isolated in trays to obtain pupae. After five days, pupae were collected and quantified.

Plants were defined as resistant when larvae did not develop to pupation, and considered susceptible when larvae developed prior to pupation.

The data obtained by distinguishing resistant and susceptible plants were analyzed using the chi-squared test ($P < 0.05$) to identify a genetic model suitable for the inheritance of the character.

Obtaining lines

The lines were obtained using the single seed descent (SSD) breeding method with modifications. For this, 20 plants evaluated as resistant in the first generation of self-pollination (S_1) were selected, transplanted to the field, and self-pollinated to obtain the $S_{1:2}$ population. From each $S_{1:2}$ progeny, 10 plants were selected to be subjected to infestation by the leafminer.

The S_1 and $S_{1:2}$ populations were evaluated, respectively, in 09/2018 and 03/2019 at the Laboratory of Applied Entomology at UFERSA. Each generation of self-pollination obtained was subjected to infestation by the leafminer in tests to identify resistant plants for self-pollination to obtain the next generation. The infestation method was similar to that described for segregating populations, and the selection criterion was the infestation index (the NML). This process was repeated until the sixth self-fertilation generation was achieved.

To identify homozygous resistant plants, S_1 individuals were subjected to infestation by the leafminer, and the plants selected as resistant were

self-pollinated to obtain the $S_{1:2}$ progenies. The $S_{1:2}$ progenies, consisting of 10 plants each, were again evaluated for resistance. From these, seven homozygous resistant families were selected, corresponding to plants homozygous for resistance. Three plants from each homozygous family were selected for self-pollination until the sixth cycle.

RESULTS AND DISCUSSION

Selection of leafminer resistance sources

In this study, significant differences in infestation (number of mines per leaf) were found among the evaluated melon accessions in the municipalities of Icapuí and Baraúna when subjected to the Kruskal-Wallis test ($P<0.05$) (Table 2). According to the analysis, the accessions resistant were AM-RT and AM-TM, with a mean number of mines of zero. The other accessions presented mean values that ranged from 10.75 to 17.50 in the Baraúna environment, and from 13.25 to 22.75 in the Icapuí environment, but did not differ statistically from the susceptible control (Table 2).

Despite the differences identified, the results showed low heterogeneity between the accessions evaluated, since the Nemenyi test showed the formation of only two groups: one composed of 19 accessions that were considered susceptible and the other consisting of two accessions classified as resistant (Table 2).

Table 2. Kruskal-Wallis test, mean, group, and reaction of yellow melon hybrids to leafminer infestation.

Baraúna				Icapuí			
Treatment	Mean	Group	Reaction	Treatment	Mean	Group	Reaction
Goldex	23.75	a	S	Goldex	26.25	a	S
HAC-03	17.50	a	S	HAC-07	22.75	a	S
HAC-19	17.50	a	S	HAC-03	19.75	a	S
HAC-10	17.25	a	S	HAC-11	19.00	a	S
HAC-05	16.25	a	S	HAC-08	18.50	a	S
HAC-04	16.00	a	S	HAC-13	18.25	a	S
HAC-09	16.00	a	S	HAC-14	18.25	a	S
HAC-13	16.00	a	S	HAC-17	18.25	a	S
HAC-07	15.75	a	S	HAC-19	18.25	a	S
HAC-14	15.75	a	S	HAC-18	17.00	a	S
HAC-08	15.50	a	S	HAC-16	16.50	a	S
HAC-06	15.25	a	S	HAC-04	16.00	a	S
HAC-18	15.25	a	S	HAC-06	15.50	a	S
HAC-01	15.00	a	S	HAC-10	15.50	a	S
HAC-17	15.00	a	S	HAC-05	14.75	a	S
HAC-16	14.75	a	S	HAC-12	14.50	a	S
HAC-12	14.50	a	S	HAC-09	14.00	a	S
HAC-11	14.00	a	S	HAC-15	14.00	a	S
HAC-15	12.75	a	S	HAC-02	13.50	a	S
HAC-02	10.75	a	S	HAC-01	13.25	a	S
AM-RT	0.00	b	R	AM-RT	0.00	b	R
AM-TM	0.00	b	R	AM-TM	0.00	b	R
χ^2 value	37.509*			χ^2 value	42.882*		
df	21			df	21		
P-value of χ^2	0.014700			P-value of χ^2	0.003255		

*Significant ($p < 0.05$) by the Kruskal-Wallis test. R: Resistant; S: Susceptible.

Kennedy et al. (1978) reported that the accessions 'PI 282448' (Africa) and PI '313970' (India) were resistant to *L. sativae*. Another reported source was the French line 'Nantais Oblong' with resistance to *Liriomyza trifolii* (DOGIMONT et al., 1995).

In studies in Brazil, differences in resistance levels between yellow melon hybrids were observed by Guimarães et al. (2009). In this study, the genotypes'PR-13-3-2-1-1 × 9278-2-1-2-1-1-1-1' and

'G 1-1 × PR 62-1-4- 1-1-1' showed a high level of resistance to *Liriomyza huidobrensis*. In another study evaluating 22 melon accessions collected from small properties in the Northeast region, 'AC-22 and AC-10' were the most promising (NUNES et al., 2013). More satisfactory results were observed in field and laboratory evaluations with 48 melon accessions from Embrapa, with the identification of four new sources of resistance to *L. sativae*: 'CNPH 11-1072', 'CNPH 11-1077', 'CNPH 00-915' and 'BAGMEL' (CELIN et al., 2017a).

However, it is important to emphasize that even with the effort to identify new sources of resistance, only a few have been determined at present, which limits the advances in breeding programs aimed at obtaining resistant melon cultivars in Brazil.

The identification of sources of resistance to the main phytosanitary problems of any crop is of paramount importance, considering that the genotype of the crop is the basis for management strategies to be adopted in the field. Regarding management to reduce the impact of insect pests on crop yield or quality, host resistance can be intentionally employed alone or in combination with other strategies (TRAPERO et al., 2016).

Genetic inheritance of leafminer resistance in melon crops

Based on the phenotypic proportions of resistant (R) and susceptible (S) plants in the segregating populations (S_1 and $S_{1:2}$), and crossing AM-RT x 'Goldex', a model was proposed to explain the genetic control of resistance to *L. sativae* in AM-RT. The model proposed was the one with a gene with complete dominance, as the allele that confers resistance (*Lm*) is dominant over the allele that confers susceptibility (*lm*) when comparing the observed values for each phenotypic class with the expected values, the corresponding chi-squared values were calculated ($\chi^2 = 0.36$, $\chi^2 = 1.33$, and $\chi^2 = 0.30$). These values were not significant in relation to the theoretical value ($\chi^2_{0.05} = 3.84$), suggesting the adequacy of the model to explain the observed segregation (Table 3).

Table 3. Chi-squared test (χ^2) applied to segregating populations of AM-RT (R) and the crossing between AM-RT (R) and 'Goldex'.

Population	Absolute frequency		Expected ratio	χ^2
	Resistant	Susceptible		
AM-RT x 'Goldex'	53	47	1:1	0.36^{ns}
AM-RT S_1	80	20	3:1	1.33^{ns}
AM-RT S_1	(*LmLm*) (*Lmlm*)	(*lmlm*)		
AM-RT S_1 (R)	4 11		1:2	0.30^{ns}
AM-RT $S_{1:2}$	111	39	5:1	3.14^{ns}

^{ns} not significant by chi-squared test at 5% probability ($\chi^2 = 3.84$).

Considering the dominant monogenic genetic control, the genotype of the resistant plant AM-RT (R) that originated in the S_1 population was heterozygous for resistance. Thus, the S_1 progenies of the AM-RT (R) plant were resistant plants with *LmLm* or *Lmlm* genotypes, with an expected phenotypic proportion of ¾, and susceptible plants with the *lmlm* genotype, with an expected proportion of ¼. The observed values were 80 resistant and 20 susceptible for the AM-RT S_1 accession (Table 3).

In the crossing AM-RT x 'Goldex', the heterozygous plant AM-RT (R) was crossed with a susceptible parent 'Goldex', with a 1:1 ratio of resistant to susceptible plants expected for the progeny, respectively. The results showed 53 resistant and 47 susceptible plants (Table 3).

Of the 80 S_1 plants identified as resistant, 20 were selected and self-pollinated for laboratory evaluation of progeny resistance to the leafminer. Of the total number of self-pollinated plants, five did not produce viable seeds, while of the 15 resistant plants evaluated, four were homozygous (*LmLm*), as they presented only resistant plants in the progenies, and 11 were heterozygous (*Lmlm*), presenting segregating progenies, with resistant and susceptible plants (Table 4). Considering that susceptible plants were eliminated, the expected phenotypic frequency for $S_{1:2}$ (R) progenies was five resistant plants for one susceptible plant (5:1). The results revealed 111 resistant and 39 susceptible plants (Table 3).

The inheritance of resistance to leafminers (*L. sativae*) in melon is of the dominant monogenic type according to the above observations. Other studies have reported similar inheritance in the genotypes 'Nantais Oblong' (DOGIMONT et al., 1999) and 'BAGMEL 56-R' (CELIN et al., 2017b). There are no known reports of polygenic genetic control of leafminers in melons. Genetic control of resistance to *L. sativae* was also determined for the accessions 'PI 282448' and 'PI 313970', which were monogenic recessive and incompletely dominant, respectively (KENNEDY et al., 1978). More recently, the

A915.34.01.08 lineage has been characterized as oligogenic (LEITÃO, 2018).

The published list of melon genes includes resistance genes to diseases and pests of leaves, flowers, fruits, seeds, and in relation to productivity (DOGIMONT; SARI, 2022). Regarding insect resistance, the largest number of genes reported show resistance to *Aphis gossypii* (Hemiptera: Aphididae), while only the genes *Lt* (DOGIMONT et al. 1999) and *Ls* (CELIN et al., 2017b) (DOGIMONT; SARI, 2022) have been reported for leafminers, highlighting the need for research into new sources of resistance and their characterization, thereby contributing to advances in the use of host resistance.

Research on improvements for resistance to insects is limited owing to the difficulty in ensuring adequate conditions for insect infestation of the materials tested, and the slow transfer of characteristics because of the complex and polygenic nature of the heritage (DHILLON; SHARMA, 2012). Regarding the last factor, the source of resistance to the leafminer identified and characterized in terms of inheritance in this work offers the advantage of being monogenic dominant, which facilitates the process of introgression of this gene in other cultivated genotypes.

Considering the identification of the accession AM-RT as a source of resistance and that its genetic control is dominant monogenic, the strategy adopted to contribute to genetic improvement was to obtain resistant lines from the S_1 and $S_{1:2}$ progenies for the study of inheritance. The advantage of using lines is that at the end of the process, their main characteristics are fixed. A resistant line that has good agronomic characteristics can be crossed with another elite line to obtain resistant simple hybrids.

Obtaining lines

Plants of the accession AM-RT were self-pollinated to obtain S_1 populations. Subsequently, 100 individuals from one of these populations were evaluated for resistance to *L. sativae* in the laboratory. Of the 80 plants identified as resistant, 20 were selected, transferred to the field, and self-pollinated to obtain $S_{1:2}$ generations. This evaluation enabled the identification of four progenies (AM-RT.L1, AM-RT.L5, AM-RT.L8, and AM-RT.L12), which presented completely resistant plants (Table 4).

Table 4. $S_{1:2}$ progenies evaluated for leafminer resistance.

Progeny ($S_{1:2}$)	Number of plants		
	Reaction		Total
	Resistant	Susceptible	
AM-RT. L1	10	0	10
AM-RT. L2	-	-	-
AM-RT. L3	3	7	10
AM-RT. L4	1	9	10
AM-RT. L5	10	0	10
AM-RT. L6	6	4	10
AM-RT. L7	7	3	10
AM-RT. L8	10	0	10
AM-RT. L9	-	-	-
AM-RT. L10	-	-	-
AM-RT. L11	-	-	-
AM-RT. L12	10	0	10
AM-RT. L13	7	3	10
AM-RT. L14	8	2	10
AM-RT. L15	8	2	10
AM-RT. L16	6	4	10
AM-RT. L17	8	2	10
AM-RT. L18	9	1	10
AM-RT. L19	8	2	10
AM-RT. L20	-	-	-
Total	111	39	150

To continue the process of obtaining lineages from each of the four $S_{1:2}$ progenies, three plants without leaf mines were taken to the field and self-pollinated to obtain the next generation ($S_{2:3}$). Subsequently, 12 plants were self-pollinated and two plants from each were selected, totaling 24 plants. In the following stages, the plants were cultivated only by self-pollination, without testing in the laboratory for infestation until the $S_{5:6}$ generation. Twenty-four lineages were obtained at the end of the process.

After the cultivation of segregating plants and the obtained lines, it is necessary to determine the main characteristics of each lineage, particularly those related to production, fruit quality, and resistance expression.

CONCLUSIONS

Among the melon accessions evaluated, there was genetic variability in resistance to the leafminer, and the accessions AM-RT and AM-TM were identified as sources of resistance.Resistance to leafminer is conditioned by a gene with complete dominance in the accession AM-RT.Twenty-four lines were obtained from successive self-pollinations of the resistant accession AM-RT.

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