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Original Article

Correlation of genome methylation of fig tree accessions with natural nematode and rust incidence

Correlação entre a metilação genômica de acessos de figueira e a incidência natural de nematoides e ferrugem

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Abstract

Commercial fig tree cultivation in Brazil involves a single cultivar, 'Roxo-de-Valinhos'. The use of a single cultivar results in serious diseases and related problems. The aim of this study was to characterize fig accessions by analyzing the natural root-knot nematode and leaf rust incidence in relation to the epigenomic profile of the plant, since epigenetic variations affect plant–pathogen interactions. All plants were attacked by nematodes, indicating susceptibility; *Meloidogyne incognita* was the root-knot nematode species involved. Joint analysis of data showed that methylation and leaf rust incidence were correlated when observed in the same phenological phase, presenting initial evidence of the same factorial pressure loads in genotypes, suggesting similar behavior within these genotypes.

Keywords: *Ficus carica* L., *Meloidogyne incognita*, *Cerotelium fici*, epigenetics, phenotypic plasticity.

Resumo

O Brasil é o maior produtor de figos da América do Sul, porém o cultivo comercial brasileiro da figueira baseia-se na plantação de uma única cultivar, o Roxo-de-Valinhos, resultando em sérios problemas relativos a pragas e doenças. Uma vez que há variações epigenéticas na interação planta-patógeno, principalmente por meio da regulação gênica, o presente trabalho objetiva realizar a caracterização in vivo de acessos de figo, por meio da análise de incidência natural de nematoides formadores de galha e de incidência natural de ferrugem, correlacionada ao seu perfil epigenômico, a fim de subsidiar trabalhos de conservação, melhoramento genético e produção da cultura. A análise dos componentes principais dos dados da caracterização dos acessos foi realizada por meio da matriz de correlação residual obtida pela análise de variância conjunta utilizando o programa GENES. Pôde-se constatar que todas as plantas foram atacadas por este patógeno, evidenciando que são suscetíveis ao mesmo. Já a análise conjunta dos dados demonstrou que a metilação e a incidência de ferrugem em folhas, quando observadas na mesma fase fenológica da planta, se correlacionam, apresentando evidências iniciais de mesmas cargas fatoriais de pressão nos genótipos, com a premissa de comportamento semelhante nos mesmos, indicando que, além do fator genético, fatores abióticos também são responsáveis pelas alterações no fenótipo das plantas, evidenciando a plasticidade fenotípica das mesmas.

Palavras-chave: *Ficus carica L.*, *Meloidogyne incognita*, *Cerotelium fici*, epigenética, plasticidade fenotípica.

1. Introduction

The common fig tree, *Ficus carica* L., is a deciduous fruit tree species belonging to the Moraceae family. It originated in the Mediterranean region (Patil, 2020); however, it is now widely cultivated in regions of the world with subtropical/temperate climates (Usai et al., 2020).

The fig is an economically important crop worldwide, particularly in the fresh and dried fruit markets (Gurung et al., 2021). It is among the twenty major fresh fruits exported by Brazil, the main producer in the southern hemisphere. The country produces approximately 23,000 tons of fruits per year from 2,208 hectares of planted area.

However, Brazilian commercial fig tree cultivation is based on a single cultivar, 'Roxo-de-Valinhos,' which results in serious problems related to pests and diseases. These infestations decrease production and profit, reduce

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fruit quality, impair plant development, and require costly inputs and culture treatments (Rodrigues et al., 2019a).

The main diseases affecting fig crops are fig tree rust, caused by the fungus *Cerotelium fici* (Solano-Baéz et al., 2017), and root-knot nematodes (*Meloidogyne incognita*) (Abdel-Sattar et al., 2020), which pose major challenges in cultivated areas worldwide.

Studies aimed at solving these problems would contribute significantly to the development of fig culture since genetic diversity is an important strategy for the survival and adaptation of the species to biotic and abiotic stressors (Van Dooren et al., 2020), the latter of which is characterized by the phenotypic plasticity of plants.

Strong selection pressures induced by extreme environments and specialized niches can lead to independent evolution of similar morphological and/or physiological characteristics in different lineages (Sackton and Clark, 2019). Thus, organisms adapted to extreme environments are especially important in studies concerning the fundamental questions of evolutionary biology.

Therefore, the aim of this study was to conduct an *in vivo* characterization of fig accessions through the analysis of natural root-knot nematode and leaf rust occurrences in relation to the epigenomic profile of the plant to support conservation work, genetic improvement, and crop productivity.

2. Material and Methods

The plant material consisted of 45 *Ficus* sp. accessions from the three-year-old Active Germplasm Bank of the Agricultural Sector, Faculty of Agrarian and Technological Sciences FCAT/UNESP, Campus of Dracena, São Paulo State, Brazil.

The experimental design consisted of randomized blocks of 45 treatments, with each accession considered as a treatment. There were two blocks and three plants per plot, totaling 270 plants with 1.5 m × 1.5 m spacing in an open cup format, each with 6 productive branches. The treatments, their respective accessions, and their places of origin are listed in Table 1.

Fertilization was performed according to the results of the soil analysis in the projection of fig tree canopies in the absence of mulching. Weeding was conducted when necessary, and irrigation was provided using drip tape three times a week, with an average precipitation of 2.0 mm/h from a 50 cm wide strip in the planting row. Phytosanitary treatment was conducted every 14th day by applying a 1% Bordeaux mixture.

Nematode incidence was evaluated in the root systems of plants in November and April by observing the occurrence or absence of *Meloidogyne incognita*, which were characterized by the formation of galls on the roots of infested plants. Thus, plants that presented galls in the root system were considered infested by nematodes.

The soil and root samples containing galls were sent to the Laboratory of Agricultural Nematology, Department of Plant Protection, Faculty of Agricultural Sciences at FCA/UNESP for identification and quantification of nematode

Table 1. Active Germplasm Bank of fig tree accessions of the Faculty of Agricultural and Technological Sciences (FCAT/UNESP). Dracena, SP, Brazil, 2021.

TREATMENT	ACCESSION	PLACE OF ORIGIN
1	Calimyrna	São José do Rio Preto
2	Nobile	IAC[1]
3	Genovese	IAC[1]
4	Roxo-de-Valinhos A	São Sebastião do Paraíso
5	Stanford	IAC[1]
6	White Adriatic	IAC[1]
7	Bonato	IAC[1]
8	White Genoa A	São José do Rio Preto
9	White Genoa B	IAC[1]
10	Smyrna A	IAC[1]
11	Smyrna B	São José do Rio Preto
12	Brunswick	IAC[1]
13	Caprifig A	IAC[1]
14	Pingo de Mel	Ilha Solteira
15	Roxo-de-Valinhos Gigante	Ilha Solteira
16	Palestine	Campinas
17	Trojan	IAC[1]
18	Red	Piracicaba
19	PI 440	Ilha Solteira
20	PI 301 (lost)	Ilha Solteira
21	PI 189	Ilha Solteira
22	PI 214	Ilha Solteira
23	PI 433	Ilha Solteira
24	Nazareth	Spain
25	Cuello Negro	Spain
26	Roxo-de-Valinhos B	Ilha Solteira
27	Accession 27	Monte Alto
28	Accession 28	Monte Alto
29	Accession 29	Monte Alto
30	Accession 30	Monte Alto
31	Accession 31	Monte Alto
32	Accession 32	Monte Alto
33	Accession 33	Monte Alto
34	Accession 34	Monte Alto
35	Accession 35	Monte Alto
36	Accession 36	Monte Alto
37	Accession 47	Bahia
38	Accession 44	Piracicaba
39	Accession 46	Piracicaba
40	Caprifig B	Ilha Solteira
41	Accession 41	Monte Alto
42	Accession 42	Monte Alto
43	Mini Fig	IAC[1]
44	Black	Piracicaba
45	Turkey	Campinas

[1] Instituto Agronômico de Campinas.

infection by sex. To identify the species of root-knot nematodes, the root samples were sent to the Laboratory of Nematology, Faculty of Agricultural and Veterinary Sciences, FCAV/UNESP.

The quantification of natural rust incidence in the field was conducted in November, coinciding with the beginning of fruit maturation. The experimental design was completely randomized; each material was considered a treatment and six replicates, and each plant was considered a replicate. Evaluation of the resistance of fig accessions was based on the infection efficiency, which considered the percentage of area with symptoms in a 1 cm^2 disk of a specific leaf region (upper right part of the leaf) in three leaves per replicate. Each leaf was collected from one part of the plant (new, medium, or basal leaf). The injured area was estimated through the area occupied with pustules inside a circle (2.5 cm in diameter) placed to the right on the underside of the leaves, based on the method described by Rodrigues et al. (2012).

To quantify the global genomic methylation content of fig trees, young leaves without spots or perforations were collected, washed under running water, and deveined. Total genomic DNA was extracted from the plant tissues using a modified cetyltrimethylammonium bromide technique.

Global DNA methylation was determined using the Imprint DNA Methylation Quantification kit (Sigma), following the manufacturer's instructions. This kit uses well strips that have been pre-treated with binding methylated DNA and applies DNA methylation-sensitive capture antibody and detection, allowing colorimetric detection of relative amounts of DNA methylation at an absorbance of 450 nm.

Statistical analyses were performed on the results of all the accessions in each evaluation dataset. The averages obtained were compared using Scott–Knott test at 5% probability significance threshold. The SISVAR software (Ferreira, 2019) was used for statistical analyses and experimental planning.

Subsequently, using data on the percentage of rust incidence and global genomic methylation content, principal component analysis was performed to define the clustering pattern between genotypes and the relative contribution of diversity per character by joint analysis of variance using GENES software (Cruz, 2013).

3. Results and Discussion

Evaluation of root-knot nematode incidence revealed that all plants had been attacked by this pathogen, indicating strong susceptibility. Analysis of nematode presence identified 120–150 adults per root sample, with 15 *Meloidogyne* sp. adults in the soil sample and 60 *Helicotylenchus* sp. adults in the root and soil samples.

The root-knot nematode species (*Meloidogyne incognita*) was identified under a photonic microscope by examining and documenting the perineal pattern of females and the morphology of the labial region of males (Figure 1).

Costa et al. (2015) evaluated six fig tree genotypes, including those in the present study, with regard to their response to *Meloidogyne javanica*, *M. incognita*, and *M. enterolobii* nematodes to identify potential rootstocks resistant to this pathogen. They concluded that all genotypes studied were susceptible to these species.

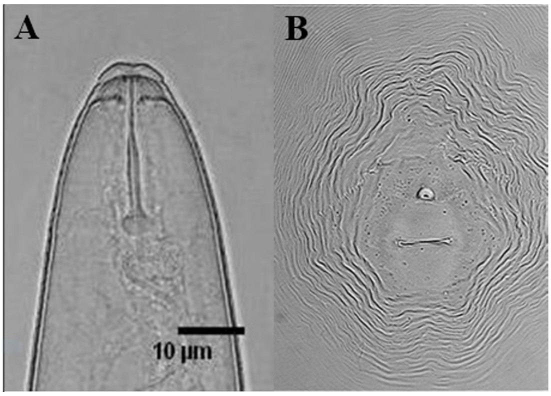

Figure 1. Morphological characteristics used to identify *Meloidogyne incognita*, showing the perineal configuration of females (A) and the labial region of males (B).

Rodrigues et al. (2012) selected fig plant mutants, formed by irradiating cuttings with gamma rays to increase their genetic variability in relation to vegetative and reproductive development, and found that all plants were carriers of *Meloidogyne incognita*, and that none of the plants were resistant to the disease. This validates data from the present study.

Various methods have been used to limit *Meloidogyne* damage, including the development of resistant cultivars (Park et al., 2020). To aid this effort, the identification and characterization of natural sources of resistance are important for the development of control strategies for root-knot nematodes. In woody crops, genetically resistant rootstocks that differ from cultivars of agronomic interest can be obtained (Saucet et al., 2016). However, this strategy is not yet viable in the case of fig trees.

Other *Ficus* species were selected for their resistance to these nematodes to compensate for the current lack of a resistant germplasm in cultivated fig trees. Despite the high resistance and graft compatibility in accessions of *Ficus racemosa* tropical species, no effective control strategy has yet been implemented even though *M. incognita* control is important for fig tree cultivation (Neugebauer et al., 2018).

Thus, the preparation of cultivation environments (soil improvement and sterilization by sunlight), treatment of plant diseases using chemical nematicides, and biological control of root-knot nematodes using microorganisms (Du et al., 2020; Sikandar et al., 2020) or plant extracts should be adopted, as prophylactic measures, for fig cultivation (Mukhtar et al., 2017).

Regarding rust incidence, Table 2 shows the leaf area percentages of *Cerotelium fici* pustules, with statistical differences between the evaluated accessions, with emphasis on Calimyrna, Genovese, Roxo-de-Valinhos A, Stanford, White Adriatic, White Genoa A, White Genoa B, Smyrna B, Brunswick, Pingo-de-Mel, PI 214, Accession 31, Accession 33, Accession 34, Accession 35, Accession 46, Caprifig B, Accession 41, Accession 42, Mini Fig, and

Table 2. Percentage of area covered with *Cerotelium fici* pustules in new (FFN), median (FFM), and basal (FFB) leaves of fig tree accessions. Dracena, 2020.

TREATMENT	ACCESSION	FFN (%)	FFM (%)	FFB (%)	Mean
1	Calimyrna	0 a	13 a	18 a	10.6 a
2	Nobile	1 a	27 b	25 b	17.4 a
3	Genovese	0 a	6 a	20 a	8.70 a
4	Roxo-de-Valinhos A	0 a	0 a	12 a	3.9 a
5	Stanford	0 a	1 a	5 a	1.90 a
6	White Adriatic	3 a	19 a	20 a	14.1 a
7	Bonato	6 a	23 a	87 c	38.7 b
8	White Genoa A	0 a	8 a	14 a	7.40 a
9	White Genoa B	0 a	1 a	23 a	8.20 a
10	Smyrna A	13 a	34 b	77 c	41.3 b
11	Smyrna B	2 a	5 a	14 a	6.80 a
12	Brunswick	1 a	0 a	7 a	2.60 a
13	Caprifig A	20 a	27 b	45 b	30.6 b
14	Pingo de Mel	27 b	0 a	3 a	10.0 a
15	Roxo-de-Valinhos Gigante	43 b	3 a	58 b	34.8 b
16	Palestine	2 a	14 a	44 b	20.0 a
17	Trojan	5 a	14 a	32 b	16.9 a
18	Red	2 a	39 b	70 c	36.8 b
19	PI 440	0 a	65 c	40 b	35.0 b
20	PI 301	-	-	-	-
21	PI 189	60 c	5 a	90 c	51.7 b
22	PI 214	20 a	5 a	15 a	13.3 a
23	PI 433	0 a	25 b	90 c	38.3 b
24	Nazareth	0 a	40 b	60 c	33.3 b
25	Cuello Negro	0 a	50 b	0 a	16.7 a
26	Roxo-de-Valinhos B	7 a	41 b	23 a	23.7 a
27	Accession 27	0 a	15 a	48 b	21.1 a
28	Accession 28	0 a	20 a	80 c	33.3 a
29	Accession 29	5 a	8 a	33 b	15.6 a
30	Accession 30	2 a	43 b	43 b	29.4 b
31	Accession 31	0 a	0 a	35 b	11.7 a
32	Accession 32	0 a	28 b	70 c	32.5 b
33	Accession 33	12 a	8 a	20 a	13.30 a
34	Accession 34	2 a	3 a	37 b	13.90 a
35	Accession 35	0 a	3 a	5 a	2.50 a
36	Accession 36	2 a	15 a	45 b	20.6 a
37	Accession 47	10 a	2 a	43 b	18.3 a
38	Accession 44	0 a	3 a	43 b	15.6 a
39	Accession 46	0 a	3 a	5 a	2.50 a
40	Caprifig B	0 a	7 a	8 a	5.00 a
41	Accession 41	2 a	0 a	0 a	0.60 a
42	Accession 42	0 a	5 a	5 a	3.30 a
43	Mini Fig	8 a	23 a	13 a	15.0 a
44	Black	3 a	37 b	35 b	25.0 b
45	Turkey	0 a	0 a	8 a	2.50 a

Different letters in the same column differ statistically from each other by the Scott-Knott test at 0.05% probability.

Turkey accessions, which had lower rust incidence averages (<15%). This indicates good adaptability to the climate conditions (subtropical Cwa climate type, mild and dry winters followed by very hot summers, according to the Köppen (1948) classification).

It was not possible to collect information on Accession 301 (represented by treatment 20). However, the treatment number was kept so that the other accessions did not have their treatment numbers changed and to avoid confusion.

'Roxo-de-Valinhos,' considered the base cultivar for the comparison of crop quality in Brazil, presented between 3.9 and 23.7% of rust lesions. These corroborated results found by Mezzalira et al. (2015), who compared the efficiency of alternative fungicides and insecticides to that of conventional products registered for fig tree culture in the state of Paraná and verified disease incidence in this cultivar with approximate values ranging from 4.39 to 30% for the control treatment.

In general, the mean values of the experiment ranged from 0.6 to 51.7%, corresponding to accession 41 and irradiated plant (PI) accession 189, respectively. However, the highest mean value was below the highest degree of disease severity, as observed in the literature. Sol-Rodríguez et al. (2021) evaluated the disease incidence and severity in fig trees in Mexico under moisture conditions similar to those in the present study and observed *C. fici* incidence values between 66.2% and 96.2%.

Pastore et al. (2015) analyzed the resistance of fig tree accessions to rust in different locations and observed significant differences between plants and locations. The authors concluded that less susceptible accessions may be associated with greater plant rusticity. However, they also determined that diversity could generate genotype-environment interactions, and environmental conditions may be more favorable to the development of fig trees naturalized in environments where genotypes had lower incidences.

The ability of a single genotype to generate alternative phenotypes based on changes in the environment (phenotypic plasticity) is a potential mechanism by which plants can respond quickly to external changes (Arnold et al., 2019); examples of this mechanism include growth responses to competition, and defense responses to attack by harmful organisms (Fernández-Milmanda et al., 2020).

DNA methylation, the most characterized epigenetic mechanism, affects various crucial aspects of the evolutionary biology of fig trees, such as varietal and behavioral differentiation of plants under environmental pressure (Rodrigues et al., 2019b).

DNA methylation is a conserved epigenetic marker that regulates several processes such as gene silencing, genome stability, and genomic imprinting (Zhang et al., 2018). It is also present in the gene-coding regions of many plant species, leading to overexpression of specific genes (Choi et al., 2020).

Figure 2 shows the quantification of global genomic methylation of the evaluated fig tree accessions, where dashed horizontal lines show values that were statistically different from the main commercial fig cultivar in Brazil, 'Roxo-de-Valinhos' (represented by treatment number 4 with an absolute value of 0.172).

Treatments, with their respective accessions whose absolute values were above the upper limit line, were 5 (Stanford), 6 (White Adriatic), 7 (Bonato), 10 (Smyrna), 12 (Brunswick), 13 (Caprifig IAC), 14 (Pingo de Mel), 15 (Roxo-de-Valinhos Gigante), 18 (Red fig), 19 (PI 440), 21 (PI 189), 35 (Accession 35 from Monte Alto), 40 (Caprifig ISA), and 41 (Accession 41 from Monte Alto). These accessions presented global genomic methylation content that was statistically higher than that of 'Roxo-de-Valinhos,' indicating that they were hypomethylated in relation to the selected parameter.

The absolute values observed below the lower limit line were those of treatments 22 and 27, 'PI 433,' and accession 27 from Monte Alto, respectively. They presented global genomic methylation contents statistically lower than that of 'Roxo-de-Valinhos,' indicating that they were

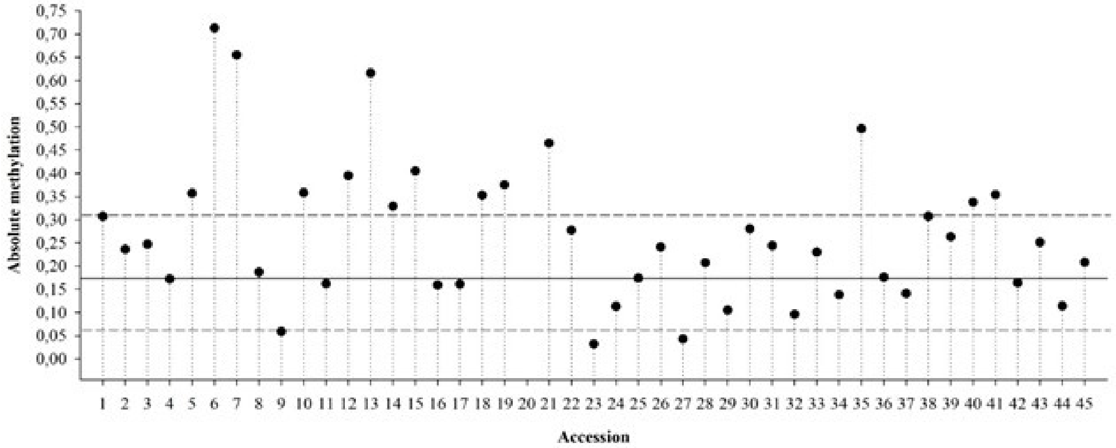

Figure 2. Quantification of global genomic methylation in fig tree accessions. Dashed horizontal lines indicate the limits of values that were statistically different from the 'Roxo-de-Valinhos' pattern, represented by the continuous horizontal line. Dracena, SP, 2021.

hypermethylated in relation to the selected parameter. Therefore, irradiation, including DNA demethylation, is an external factor capable of changing these patterns in plants.

Figure 3 presents principal component analysis results using the percentage of rust incidence in fig accessions and their global genomic methylation content. This figure shows that methylation and rust incidence in young leaves were correlated, with the premise of similar behavior in genotypes.

No correlation with the methylation content was observed for rust incidence in medium and basal leaves because the DNA of the accessions was extracted from new leaves. This indicates that there is a positive correlation between methylation and this disease before leaf development. However, global genomic methylation content may change as the leaf matures, making it impossible to correlate the variables observed at different phenological times.

Epigenetic variations influence plant–microbe interactions, mainly through gene regulation (Kumar and Mohapatra, 2021). Epigenetic mechanisms associated with interactions between plants and pathogens can play positive roles in promoting plant defense, particularly bacterial and fungal pathogens. However, the role of DNA methylation alteration in plant preparation for pest and disease attacks is not yet known (Wang et al., 2019).

Stress alters the epigenetic profile of plants and can improve their stress-tolerance capacity (Varotto et al., 2020). Depending on the species, this can be either through DNA hypermethylation or hypomethylation.

DNA hypomethylation is a general feature of the promoters of many genes associated with fruit ripening, as these promoters contain binding sites for transcription factors associated with this growth stage (Lang et al., 2017; Zhaobo et al., 2017). Huang et al. (2019) analyzed the influence of global DNA methylation on orange fruits and observed that DNA hypermethylation is critical for proper fruit ripening.

Differences were observed in the rust incidence and global methylation content of the DNA of different fig tree accessions from the active germplasm bank evaluated in this study. In addition, it was possible to correlate the disease with methylation when observed in the same phenological phase of the plant, showing initial evidence of the same factorial pressure loads in genotypes. This indicates that, in addition to genetic factors, abiotic factors are also responsible for changes in the phenotype of plants.

Future studies on comparing gene expression between treatments would be important for understanding the complex regulatory systems. Such studies may lead to the identification of genes of agronomic interest for fig tree crops and facilitate the subsequent manipulation and propagation of improved cultivars for commercial cultivation.

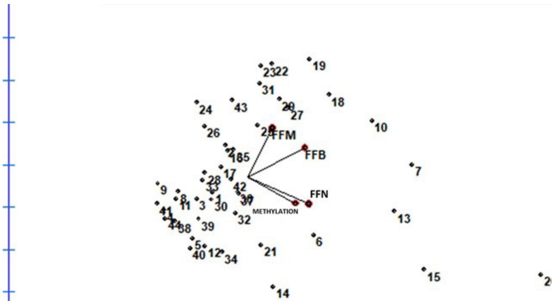

Figure 3. Principal component analysis using percentage of rust incidence and global genomic methylation content of fig tree accessions.

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References

ARNOLD, P.A., KRUUK, L.E.B. and NICOTRA, A.B., 2019. How to analyse plant phenotypic plasticity in response to a changing climate. *The New Phytologist*, vol. 222, no. 3, pp. 1235-1241. http://dx.doi.org/10.1111/nph.15656. PMid:30632169.

CHOI, J., LYONS, D.B., KIM, M.Y., MOORE, J.D. and ZILBERMAN, D., 2020. DNA methylation and histone H1 jointly repress transposable elements and aberrant intragenic transcripts. *Molecular Cell*, vol. 77, no. 2, pp. 310-323. http://dx.doi.org/10.1016/j.molcel.2019.10.011. PMid:31732458.

COSTA, M.G.S., CORREIA, E.C.S.S., REIS, L.L. and WILCKEN, S.R.S., 2015. Reação de figueiras a três espécies de nematoides-das-galhas. *Revista Brasileira de Fruticultura*, vol. 37, no. 3, pp. 617-622. http://dx.doi.org/10.1590/0100-2945-168/14.

CRUZ, C.D., 2013. GENES - a software package for analysis in experimental statistics and quantitative genetics. *Acta Scientiarum*, vol. 35, pp. 271-276.

DU, B., XU, Y., DONG, H., LI, Y. and WANG, J., 2020. *Phanerochaete chrysos*porium strain B-22, a nematophagous fungus parasitizing *Meloidogyne incognita. PLoS One*, vol. 15, no. 1, p. e0216688. http://dx.doi.org/10.1371/journal.pone.0216688. PMid:31931510.

FERNÁNDEZ-MILMANDA, G.L., CROCCO, C.D., REICHELT, M., MAZZA, C.A., KOLLNER, T.G., ZHANG, T., CARGNEL, M.D., LICHY, M.Z., FIORUCCI, A., FANKHAUSER, C., KOO, A.J., AUSTIN, A.T., GERSHENZON, J. and BALLARÉ, C.L., 2020. A light-dependent molecular link between competition cues and defence responses in plants. *Nature Plants*, vol. 6, no. 3, pp. 223-230. http://dx.doi.org/10.1038/s41477-020-0604-8. PMid:32170284.

FERREIRA, D.F., 2019. SISVAR: a computer analysis system to fixed effects split plot type designs. *Revista Brasileira de Biometria*, vol. 37, no. 4, pp. 529-535. http://dx.doi.org/10.28951/rbb.v37i4.450.

GURUNG, A.B., ALI, M.A., LEE, J., FARAH, M.A. and ALANAZI, K.M., 2021. Molecular docking and dynamics simulation study of bioactive compounds from *Ficus carica* L. with important anticancer drug targets. *PLoS One*, vol. 16, no. 7, p. e0254035. http://dx.doi.org/10.1371/journal.pone.0254035. PMid:34260631.

HUANG, H., LIU, R., NIU, Q., TANG, K., ZHANG, B., ZHANG, H., CHEN, K., ZHU, J.K. and LANG, Z., 2019. Global increase in DNA methylation during orange fruit development and ripening. *Proceedings of the National Academy of Sciences of the United*

States of America, vol. 116, no. 4, pp. 1430-1436. http://dx.doi.org/10.1073/pnas.1815441116. PMid:30635417.

KÖPPEN, W., 1948. *Climatologia: con un estudio de los climas de la tierra*. Ciudad de México: Fondo de Cultura Económica, 479 p.

KUMAR, S. and MOHAPATRA, T., 2021. Dynamics of DNA methylation and its functions in plant growth and development. *Frontiers in Plant Science*, vol. 12, p. 596236. http://dx.doi.org/10.3389/fpls.2021.596236. PMid:34093600.

LANG, Z., WANG, Y., TANG, K., TANG, D., DATSENKA, T., CHENG, J., ZHANG, Y., HANDA, A.K. and ZHU, J.K., 2017. Critical roles of DNA demethylation in the activation of ripening-induced genes and inhibition of ripening-repressed genes in tomato fruit. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, no. 22, pp. E4511-E4519. http://dx.doi.org/10.1073/pnas.1705233114. PMid:28507144.

MEZZALIRA, E.J., PIVA, A.L., NAVA, G.A., PAULUS, D. and SANTIN, A., 2015. Rust and fig tree borer control by different fungicides and insecticides. *Revista Ceres*, vol. 62, pp. 44-51. http://dx.doi.org/10.1590/0034-737X201562010006.

MUKHTAR, T., AROOJ, M., ASHFAQ, M. and GULZAR, A., 2017. Resistance evaluation and host status of selected green gram germplasm against *Meloidogyne incognita. Crop Protection*, vol. 92, pp. 198-202. http://dx.doi.org/10.1016/j.cropro.2016.10.004.

NEUGEBAUER, W.C., GOMES, C.B., MOURA, A.B., CAMPOS, A.D., SCHAFER, J.T. and BRUM, D., 2018. Potencial de rizobactérias no controle de *Meloidogyne incognita* em figueira. *Revista de la Facultad de Agronomía*, vol. 117, pp. 23-30.

PARK, E.J., JANG, H.J., PARK, C.S., LEE, S.J., LEE, S., KIM, K.H., YUN, B.S., LEE, S.W. and RHO, M.C., 2020. Evaluation of nematicidal activity of Streptomyces yatensis KRA-28 against *Meloidogyne incógnita. Journal of Microbiology and Biotechnology*, vol. 30, no. 5, pp. 700-707. http://dx.doi.org/10.4014/jmb.1908.08038. PMid:32482935.

PASTORE, R.L., BOFF, P., GOLINSKI, N.G. and BOFF, M.I.C., 2015. Resistência de figueiras "crioulas" à ferrugem sob sistema orgânico de cultivo. *Cadernos de Agroecologia*, vol. 10, pp. 1-6.

PATIL, S.P., 2020. *Ficus carica* assisted green synthesis of metal nanoparticles: a mini review. *Biotechnology Reports*, vol. 28, p. e00569. http://dx.doi.org/10.1016/j.btre.2020.e00569. PMid:34094890.

RODRIGUES, M.G.F., CORRÊA, L.S., SANTOS, P.C. and TULMANN NETO, A., 2012. Seleção de clones de figueira cv. Roxo de Valinhos formados por gemas irradiadas. *Revista Brasileira de Fruticultura*, vol. 34, no. 2, pp. 604-611. http://dx.doi.org/10.1590/S0100-29452012000200036.

RODRIGUES, M.G.F., SANTOS, T.P., FERREIRA, A.F.A., MONTEIRO, L.N.H., NAKANISHI, E.S. and BOLIANI, A.C., 2019a. Morphological characterization of active germoplasm bank fig tree accessions. *Revista Brasileira de Fruticultura*, vol. 41, no. 5, p. e074. http://dx.doi.org/10.1590/0100-29452019074.

RODRIGUES, M.G.F., MONTEIRO, L.N.H., FERREIRA, A.F.A., SANTOS, T.P., PAVAN, B.E., NEVES, V.A.B. and BOLIANI, A.C., 2019b. Biometric characteristics among fig tree genotypes in Brazil. *Genetics and Molecular Research*, vol. 18, no. 2, p. gmr18191. http://dx.doi.org/10.4238/gmr18191.

SACKTON, T.B. and CLARK, N., 2019. Convergent evolution in the genomics era: new insights and directions. *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 374, no. 1777, p. 20190102. http://dx.doi.org/10.1098/rstb.2019.0102. PMid:31154976.

SAUCET, S.B., VAN GHELDER, C., ABAD, P., DUVAL, H. and ESMENJAUD, D., 2016. Resistance to root-knot nematodes *Meloidogyne* spp. in woody plants. *The New Phytologist*, vol. 211, no. 1, pp. 41-56. http://dx.doi.org/10.1111/nph.13933. PMid:27128375.

SIKANDAR, A., ZHANG, M., WANG, Y., ZHU, X., LIU, X., FAN, H., XUAN, Y., CHEN, L. and DUAN, Y., 2020. *In vitro* evaluation of *Penicillium chrysogenum* Snef1216 against *Meloidogyne incognita* (root-knot nematode). *Scientific Reports*, vol. 10, no. 1, p. 8342. http://dx.doi.org/10.1038/s41598-020-65262-z. PMid:32433576.

SOLANO-BÁEZ, A.R., JIMÉNEZ-JIMÉNEZ, B. and CAMACHO-TAPIA, M., 2017. First confirmed report of *Cerotelium fici* causing leaf rust on *Ficus carica* in Mexico. *Plant Pathology and Quarantine*, vol. 7, no. 2, pp. 160-163. http://dx.doi.org/10.5943/ppq/7/2/9.

SOL-RODRÍGUEZ, N., GUILLÉN-SÁNCHEZ, D., ALIA-TEJACAL, I., JUÁREZ-LÓPEZ, P. and LÓPEZ-MARTÍNEZ, V., 2021. Incidencia y severidad de roya causada por *Cerotelium* fici en higo (*Ficus carica*) en Morelos, México. *Centro Agrícola*, vol. 48, pp. 43-51.

USAI, G., MASCAGNI, F., GIORDANI, T., VANGELISTI, A., BOSI, E., ZUCCOLO, A., CECCARELLI, M., KING, R., HASSANI-PAK, K., ZAMBRANO, L.S., CAVALLINI, A. and NATALI, L., 2020. Epigenetic patterns within the haplotype phased fig (*Ficus carica* L.) genome. *The Plant Journal*, vol. 102, no. 3, pp. 600-614. http://dx.doi.org/10.1111/tpj.14635. PMid:31808196.

VAN DOOREN, T.J.M., SILVEIRA, A.B., GILBAULT, E., JIMÉNEZ-GÓMEZ, J.M., MARTIN, A., BACH, L., TISNÉ, S., QUADRANA, L., LOUDET, O. and COLOT, V., 2020. Mild drought in the vegetative stage induces phenotypic, gene expression and DNA methylation plasticity in Arabidopsis but no transgenerational effects. *Journal of Experimental Botany*, vol. 71, no. 12, pp. 3588-3602. http://dx.doi.org/10.1093/jxb/eraa132. PMid:32166321.

VAROTTO, S., TANI, E., ABRAHAM, E., KRUGMAN, T., KAPAZOGLOU, A., MELZER, R., RADANOVIĆ , A. and MILADINOVIĆ , D., 2020. Epigenetics: possible applications in climate-smart crop breeding. *Journal of Experimental Botany*, vol. 71, no. 17, pp. 5223-5236. http://dx.doi.org/10.1093/jxb/eraa188. PMid:32279074.

WANG, C., WANG, C., ZOU, J., YANG, Y., LI, Z. and ZHU, S., 2019. Epigenetics in the plant–virus interaction. *Plant Cell Reports*, vol. 38, no. 9, pp. 1031-1038. http://dx.doi.org/10.1007/s00299-019-02414-0. PMid:31065780.

ZHANG, H., LANG, Z. and ZHU, J.-K., 2018. Dynamics and function of DNA methylation in plants. *Nature Reviews. Molecular Cell Biology*, vol. 19, no. 8, pp. 489-506. http://dx.doi.org/10.1038/s41580-018-0016-z. PMid:29784956.

ZHAOBO, L., YIHAI, W., KAI, T., DENGGUO, T., TATSIANA, D., JINGFEI, C., YIJING, Z., AVTAR, K.H. and JIAN-KANG, Z., 2017. Active DNA demethylation in fruit ripening. *Proceedings of the National Academy of Sciences of the United States of America*, vol. 114, pp. e4511-e4519.