Revista Brasileira de Ciências Agrárias
ISSN (on line) 1981-0997
v.15, n.2, e6412, 2020
Recife, PE, UFRPE. www.agraria.pro.br
DOI:10.5039/agraria.v15i2a6412
Protocol 6412 - 26/11/2018 • Approved in 04/03/2020

Biofortified mini lettuce advanced lines with resistance to *root-knot nematode*

Gabriel Mascarenhas Maciel[1], Bruno Sérgio Vieira[1], Adriany Pena de Souza[1], Rafael Resende Finzi[1], Ana Carolina Pires Jacinto[1], Igor Forigo Beloti[1]

[1] Universidade Federal de Uberlândia, Instituto de Ciências Agrárias, Monte Carmelo, MG, Brasil. E-mail: gabrielmaciel@ufu.br; brunovieira@iciag.ufu.br; adrianypena@gmail.com; rafaelfinzi@hotmail.com; carol.agro.ufu@gmail.com; agroifb@gmail.com

ABSTRACT: Biofortified mini lettuce resistant to root-knot nematode with high agronomic potential is not yet a reality in Brazil. Thus, the objective of this work was to evaluate the agronomic potential of lines of biofortified mini lettuces combined with resistance to *M. javanica*. Therefore, two experiments were performed: 1 - agronomic performance in the field; 2- resistance to *M. javanica* in the greenhouse. The experiments were arranged in a randomized block design (experiment 1) and in a completely randomized design (experiment 2), with ten treatments (six advanced lines and four commercial cultivars) and eight replications, conducted at the Federal University of Uberlândia (UFU). The following parameters were evaluated in experiment 1: total chlorophyll content; plant and stem diameter; and number of leaves plant^{-1}. In experiment 2: gall index; number of galls and eggs root^{-1}; reproduction factor. The evaluated genotypes which presented agronomic potential were generally not resistant to *M. javanica*, except UFU-66#6, an advanced lineage which is innovative in the segment of biofortified mini lettuce, combining agronomic potential and resistance to *M. javanica*.

Key words: Carotenoids; *Lactuca sativa*; *Meloidogyne javanica*

Linhagens avançadas de mini alface biofortificadas com resistência ao nematóide-das-galhas

RESUMO: Mini alface biofortificada e resistente ao nematóide das galhas Meloidogyne javanica, com elevado potencial agronômico, ainda não é uma realidade no Brasil. Assim, o objetivo deste trabalho foi avaliar o potencial agronômico de linhagens de mini-alfaces biofortificadas combinadas com a resistência ao *M. javanica*. Foram realizados dois experimentos:1- desempenho agronômico em campo; 2- resistência à *M. javanica* em casa de vegetação. Os experimentos foram dispostos no delineamento de blocos ao acaso (experimento 1) e no delineamento inteiramente casualizado (experimento 2); ambos constituídos de dez tratamentos (seis linhagens avançadas e quatro cultivares comerciais) e oito repetições, conduzidos nas dependências da Universidade Federal de Uberlândia (UFU). No experimento 1 foram avaliados: teor de clorofila total; diâmetro de copa e caule; e número de folhas planta^{-1}. No experimento 2: índice de galhas; número de galhas e ovos raiz^{-1}; e fator de reprodução. De maneira geral, os genótipos que apresentaram potencial agronômico não foram resistentes a *M. javanica*, exceto UFU-66#6. Concluiu-se que a linhagem avançada UFU-66 # 6 (segmento de mini-alface biofortificada) possui potencial agronômico aliado a resistência a *M. javanica*.

Palavras-chave: carotenoides; *Lactuca sativa*; *Meloidogyne javanica*

Introduction

Lettuce (*Lactuca sativa* L.*)* is considered the most important leafy vegetable in the world for fresh consumption in the form of salad, with mini lettuce standing out among the lettuce groups. The mini vegetables segment has presented a promising market with great potential for growth, as it presents commercial advantages such as ease of preparation and a differentiated visual aspect for gourmet dishes (Sala & Costa, 2012). The cultivated area of mini lettuce in protected cultivation in Brazil was over 200 hectares in 2015. The commercialization market value of this vegetable seed is more than 300 thousand dollars (Abcsem, 2015).

Due to the high index of nutritional deficiencies in the population, strategies aiming to enhance the biofortification of foods such as the increase in iodine (Lawson et al., 2016), selenium (Smoleń et al., 2014) and carotenoids (Cassetari et al., 2015) are of great interest. Carotenoids participate in various functions of the human organism, they are antioxidants and can help prevent diseases (Nascimento et al., 2013). There are reports of a smooth type lettuce genotype (Uberlândia 10000) that was biofortified, being rich in carotenoids (Sousa et al., 2007).

Despite the importance of food biofortification, there are still few commercial mini lettuces which are rich in carotenoids and have agronomic potential and resistance. The susceptibility of most of the cultivars to the root-knot nematode (*Meloidogyne incognita* and *M. javanica*) is one of the major problems faced by producers (Carvalho Filho et al., 2011). Moreover, this nematoid genus is a top ten parasitic plant nematode in molecular plant pathology (Jones et al., 2013). They cause losses in a wide range of agricultural crops, with direct and indirect damage resulting in delayed maturity, toppling, a reduction in the yields and quality of crop produce, thereby culminating in high production costs and loss of income (Onkendi et al., 2014).

In view of the above, it is necessary to obtain genotypes which are rich in carotenoids and have satisfactory levels of resistance. It is possible to select superior plants in segregated populations from the cross-breeding between divergent parents, and thereby develop new cultivars (Azevedo, 2013; Damerum et al., 2015). Thus, the objective of this work was to evaluate the agronomic potential of biofortified mini lettuce lines combined with a resistance to *M. javanica.*

Materials and Methods

The experiment was conducted in two stages: 1- agronomic evaluation of advanced lettuce lines; and 2-reaction of advanced lettuce lines to *Meloidogyne javanica*. Both stages were conducted at the Vegetable Experiment Station of Federal University of Uberlândia (*UFU*), Monte Carmelo - MG (18°42'43.19"S; 47°29'55.8" W; 873 meters of altitude).

Ten lettuce genotypes were evaluated, being four commercial cultivars: Robusta, Grand Rapids, Belíssima and UFU-BIOFORT1; and six advanced mini biofortified lettuce lines (F_4) belonging to the Germplasm Bank of Biofortified Lettuce of UFU: UFU-104#5, UFU-104#3D, UFU-104#1D, UFU-104#4D, UFU-66#6, Uberlândia10000 [rich in carotenoid (Sousa et al., 2007) and susceptible to *M. javanica*]. The advanced lines were obtained after hybridization between Belíssima cv. (Resistant to nematodes) x Uberlândia 10000 (susceptible to nematodes), followed by three successive self-fertilizations. Only plants with mini lettuce characteristics and high carotenoid levels were selected during the generations'advancements.

Experiment I: Agronomic evaluation. The experiment was conducted in the field between July and August, 2016, in a completely randomized block design (CRBD) with eight replications of each genotype. The analysis of variance (ANOVA) was performed according to the mathematical model: $Y_{ij} = \mu + g_i + b_j + \varepsilon_{ij}$, where, Y_{ij}: is the observation of the i^{th} genotype in the j^{th} block; μ: overall mean; g_i: is the effect of the i^{th} genotype; bj: is the effect of the j^{th} block; εij: is the random error associated with each observation Yij. The soil presented the following characteristics: pH (H_2O) = 5.9; P available = 30.1 mg.dm^{-3}; K = 0.22; Ca^{+2} = 2.8 cmolc.dm^{-3}; Mg = 1.0 cmolc.dm^{-3}; H^+ Al exchangeable = 3.40 cmolc.dm^{-3}; organic matter = 4.2 dag Kg^{-1}; SMP index = 3.4; Al = 0.0 cmolc.dm^{-3}; CTC pH 7.0 = 7.42 cmolc.dm^{-3}; Copper; 2.3 mg.dm^{-3}; Zinc = 6.6 mg.dm^{-3} and Manganese = 6.6 mg.dm^{-3}.

The seedlings were produced in expanded polystyrene trays with 200 cells and filled with coconut fiber commercial substrate. After sowing, the trays remained in an arch greenhouse with dimensions of 5 x 6 m and headroom of 3.5 meters covered with transparent polyethylene film of 150 micra, protected against ultraviolet rays, and anti-aphid white screen side curtains. The seedlings were transplanted into definitive plant beds in the field at 28 days after sowing. The beds were formed with1.30 m wide. The plots were constituted by 20 plants distributed in four rows in the plant bed, with the six central plants of each plot being evaluated. The cultural treatments were carried out as recommended for lettuce cultivation (Filgueira, 2013).

The following characterizations were recorded 40 days after transplanting: Chlorophyll, indirectly representing the carotenoid content by the SPAD index (Cassetari et al., 2015); Plant diameter (cm) – measured with a graduated ruler; Stemdiameter (mm) – measured with a caliper; and Number of leaves - counting the number of commercial leaves in the plants, except necrotic leaves.

Experiment II: Resistance of mini lettuce genotypes to *M. javanica.* The experiment was conducted in a green house between August and September, 2016, in a completely randomized design (CRD) with eight replications of each genotype. The analysis of variance (ANOVA) was performed according to the mathematical model: Yij = μ + gi + εij, where, Yij: is the observation of the i^{th} genotype in the j^{th} repetition; μ: overall mean; gi: is the effect of the i^{th} genotype; εij: is the random error associated with each observation Yij.

The sowing occurred in expanded polystyrene trays with 128 cells containing commercial substrate based on coconut fiber. The substrate in the trays was then inoculated with *M. javanica* eggs 28 days after sowing .The inoculum contained 30 eggs.cm^{-3} on each substrate and was directly injected using a syringe into the substrate, next to each plant. The nematode eggs used as inoculum were extracted according to the technique proposed by Hussey & Barker (1973), modified by Bonetti& Ferraz (1981), obtained from a pure population of *M. javanica* that was maintained in greenhouse vessels with susceptible Santa Clara cv tomato plants.

The evaluations were started in the lettuce genotypes at 22 days after inoculation, when it was possible to observe their formation. The plants were removed from the tray and evaluated individually. The root system of each plant still with the clod was evaluated in order to determine the visual level of galls, and received a grade (1 to 5): 1: few visible galls (<10 galls), small (<1 mm); 2: few visible galls, with intermediate size (1 to 3 mm); 3: intermediate number of visible galls (10-30 galls), intermediate size and some large (> 3 mm) galls; 4: many visible galls (> 30 galls), predominantly large galls (> 3 mm), with a few intermediate size and some coalesced; 5: clod with many visible galls (> 30 galls), large and high number of coalescing root-knots, as proposed by Fioriniet al. (2007).

Next, each plant had its root system submerged in still water until the substrate was washed out of the roots in order to determine the number of root-knot nematode and eggs. Then, the number of root-knot and eggs on the root system was counted. In addition, the reproduction factor which corresponds to the ratio between the final (Pf) and initial (Pi) nematode population was calculated (Fr=Pf/Pi) (Ferreira et al., 2011).

The agronomic and nematode reaction data were submitted to analysis of variance and the F-test (p=0.05). The averages were compared by the Scott-Knott test (p=0.05). The mean of each control was separately compared for the visual level of galls, Uberlândia 10000 (Susceptible) and Grand Rapids (Resistant), with the average of each advanced line using Dunnett's test (p= 0.05). The significance of advanced lines and Grand Rapids cultivar (resistant) and the non-significance for Uberlândia 10000 (susceptible) for all genotypes identifies homozygous as resistant. On the other hand, the significance between the genotypes and the Uberlândia 10000 cultivar, and the non-significance for Grand Rapids for all the characteristics characterizes them as susceptible to homozygote. Any condition divergent from those mentioned above characterizes the genotypes as segregant. All the obtained data were analyzed using the Genes software program (Cruz, 2013). Relative superiority was also obtained for chlorophyll, number of galls, number of eggs and reproduction factor in relation to Uberlândia 10000cv. (rich in carotenoid and susceptible to *M. javanica*) by the equation: relative% = [(Advanced lines/Uberlândia 10000) – 1] 100.

Results and Discussion

There was a significant effect regarding the agronomic potential among the chlorophyll content, plant diameter, stem diameter and leaf number variables (Table 1), indicating the existence of genetic variability among the genotypes. The chlorophyll content ranged from 15.8 to 30.6; plant diameter (cm) between 14.8 to 29.7; stem diameter (mm) from 11.9 to 28.9; and number of leaves from 13.9 to 46.8 (Table 1). The UFU-104#1D and UFU-104#4D, advanced lines stood out for their significant chlorophyll content (Scott-knott, 5% probability). It is worth noting that UFU-104#1D, UFU-104#4D, UFU-104#5 and UFU-66#6 advanced lines can be considered biofortified for presenting relative superiority in the chlorophyll content when compared to the control (Uberlândia 10,000), considered rich in carotenoids (Sousa et al., 2007) (92.45, 81.76, 69.81 and 67.92%, respectively). Cassetari et al. (2015) reported a high correlation between carotenoid and chlorophyll, suggesting a potential use of chlorophyll content as an indirect indicator of carotenoid content.

Regarding the plant diameter, the highest values (> 25 cm) were observed in commercial cultivars: Robusta, Grand

Table 1. Chlorophyll content, relative superiority (all genotypes versus Uberlândia 10,000), plant diameter, stem diameter and leaf number in six biofortified mini lettuce advanced lines (Uberlândia 10,000 x Belíssima) and four commercial cultivars.

Genotypes	Chlorophyll (SPAD index)[1]	Relative superiority (%)	Plant diameter (cm)	Stem diameter (mm)	Number of leaves (leaves plant^{-1})
UFU-104#5	27.0b	69.8	22.3c	24.1b	36.0c
Uberlândia 10000	15.9c	0.0	25.5b	24.0b	37.9b
UFU-104#1D	30.6a	92.4	21.7c	21.6b	32.9c
cv. Robusta[2]	18.8c	18.2	29.4a	22.8b	29.8c
cv. Grand Rapids[2]	25.6b	61.0	28.7a	26.1a	39.4b
UFU- BIOFORT1[2]	24.1b	51.6	29.7a	27.9a	31.7c
cv. Belíssima[2]	15.8c	-0.6	14.8d	11.9c	13.9d
UFU-104#3D	26.2b	64.8	23.5c	24.3b	46.8a
UFU-104#4D	28.9a	81.8	25.9b	28.9a	31.8c
UFU-66#6	26.7b	67.9	21.7c	20.8b	43.9a
Mean	23.9	-	24.3	23.2	34.4
CV (%)	14.9	-	9.5	15.8	15.6

Means followed by distinct capital letters, in the same column, differed from each other (p >0.05) by the Scott-Knott Test.[1]Represents the carotenoid content by the SPAD index (Cassetari et al., 2015). [2]Commercial cultivars.

Rapids and UFU-BIOFORT1. However, Belíssima cv. presented a smaller plant diameter (14.8 cm). There is a commercial preference for plants of intermediate size and lower diameter, thus avoiding food waste (Sala & Costa, 2012). When evaluating mini lettuce cultivars, Takahashi & Cardoso (2014) verified plant diameter of three commercial cultivars with values close to 20 cm. The UFU-104#1D, UFU-66#6, UFU-104#5 and UFU-104#3D genotypes also presented values close to 20 cm of plant diameter (21.7, 22.3 and 23.5 cm, respectively), and can be classified as mini lettuce.

Similar results were observed regarding the stem diameter between the evaluated advanced lines and the commercial cultivars, probably due to the high temperatures and long photoperiod. High temperatures combined with long photoperiod induce early weighing and consequently influence stem diameter by elongation (Souza et al., 2008). The observed visual differences in characteristics such as plant height and stem diameter can be attributed to variations in solar radiation and temperatures under experimental conditions.

In addition to presenting mini-lettuce size, UFU-104#3D (23.5 cm) and UFU-66#6 (21.7 cm) advanced lines were superior in relation to the number of leaves (Table 1). UFU-104#3D and UFU-66#6 hadmore leaves (46.8 and 43.9 leaves, respectively) than commercial cultivars (Robusta, Grand Rapids, Belíssima and UFU-BIOFORT1). In evaluating different progenies and lettuce cultivars, Souza et al. (2008) obtained the highest number of leaves with Regina cv.(42.9), while Santi et al. (2013) reported values close to 30 leaves per plant. The largest numbers are probably explained by the larger spacing used than is required for mini lettuce.

Reaction to root-knot nematode (*Meloidogyne javanica*)*:*The highest values for number of galls were verified in the UFU-104#1D, UFU-104#3D, UFU-104#4D advanced lines and in the Robusta and Uberlândia 100000 cultivars (124.2; 122.4; 118.6; 74.5, respectively) (Table 2). The Grand Rapids cv. presented a lower number of galls (33.0), in addition to having 55.7% higher susceptibility to *M. javanica* (Uberlândia 10000). Corroborating this fact, the resistance of the Grand Rapids cv. has already been confirmed by several authors (Ferreira et al., 2011; Carvalho Filho et al., 2011). The UFU-104#5 and UFU-66#6 advanced lines presented superiority related to the lowest number of galls in relation to the susceptible cultivar (Uberlândia 10000) of 38.3 and 20.3%. The UFU-BIOFORT1 and Belíssima cultivars were also superior to the susceptible cultivar (Uberlândia 10000) (29.53% and 20.27%) in relation to the lowest number of galls.

The highest value for number of eggs was verified in the UFU-104#1D advanced line (37.7) (Table 2). The Grand Rapids, UFU-BIOFORT1 and Belissima cultivars presented the lowest values for number of eggs (1.9, 3.0 and 3.9, respectively), in addition to presenting (88.1%, 81.3%, 75.6%, respectively) higher susceptible than the Uberlândia 10000 cv. The UFU-66#6 and UFU-104#3D advanced lines also presented superiority in relation to the susceptible cultivar of 65,6% and 56,9%, respectively, for number of eggs. UFU-104#4D showed the lowest superiority (13.1%) in relation to the number of eggs of the susceptible cultivar.

Regarding the reproductive factor, it was verified that all evaluated genotypes presented low values, which can be justified by high temperatures (> 35 °C) in the greenhouse. UFU-104#1D presented the highest reproduction factor (1.13). As expected, the Grand Rapids cv. had a lower reproductive factor (0.11) and 78% higher than the susceptible cultivar (Uberlândia 10000) to *M. javanica*. UFU-66#6 and UFU-104#5 presented similar behavior to Grand Rapids cv. (0.17 and 0.20, respectively), presenting superiority of 66% and 60%, respectively in relation to Uberlândia 10000.

A visual level of root-knot (VLRK) was verified when comparing the 10 advanced lines with the nematode resistant control (Grand Rapids) and the susceptible control (Uberlândia10000) (Table 3). UFU-66#6 and Belíssima cv. did not differ from the resistant Grand Rapids cultivar, however differing from the Uberlândia 10000 susceptible control, therefore being considered homozygous resistant for the

Table 2. *M. javanica* reaction in six biofortified mini lettuce advanced lines (Uberlândia 10000 x Belíssima) and four commercial cultivars.

Genotypes	NG[1] (galls root^{-1})	SRNG (%)	NO (eggs root^{-1})	SRNO (%)	FR	SRFR (%)
UFU-104#5	46.0c	-38.3	19.0c	18.7	0.20d	-60.0
Uberlândia 10000 (S)	74.5b	0.0	16.0d	0.0	0.50b	0.0
UFU-104#1D	124.2a	66.7	37.7a	135.6	1.13a	126.0
cv. Robusta[2]	108.0a	44.9	27.2b	70.0	0.54b	8.0
cv. Grand Rapids[2] (R)	33.0d	-55.7	1.9g	-88.1	0.11d	-78.0
cv. UFU- BIOFORT1[2]	52.5c	-29.5	3.0g	-81.2	0.32c	-36.0
cv.Belíssima[2]	54.0c	-27.5	3.9g	-75.6	0.32c	-36.0
UFU-104#3D	122.4a	64.3	6.9f	-56.9	0.28c	-44.0
UFU-104#4D	118.6a	59.2	13.9e	-13.1	0.59b	18.0
UFU-66#6	59.4b	-20.3	5.5f	-65.6	0.17d	-66.0
Mean	76.7	-	14.1	-	0.4	-
CV%	21.4	-	22.2	-	29.7	-

Means followed by distinct capital letters, in the same column, differed from each other (p >0.05) by the Scott-Knott Test. [1]NG= number of galls; SRNG= relative superiority of NG [Genotypes versus Uberlândia 10000 (S)]; NO= number of eggs; SRNO= relative superiority of NO [Genotypes versus Uberlândia 10000 (S)]; FR= reproduction factor; and SRFR= relative superiority of FR [Genotypes versus Uberlândia 10000 (S)]. [2]Commercial cultivars. S = susceptible and R= resistant.

Table 3. Comparison of the mean scores for the visual level of root-knot (VLRK) of six biofortified mini lettuce advanced lines (Uberlândia 10000 x Belissima) and four commercial cultivars inoculated with *M. javanica* eggs.

Genotypes	VLRK[3]	Probability[1]		Reaction
		Uberlândia 10000 (S)	Grand Rapids (R)	
UFU-104#5	1.75	*	*	Segregating
UFU-104#1D	4.00	ns	*	Homozygous/Susceptible
cv. Robusta[2]	2.98	ns	*	Homozygous/ Susceptible
cv. UFU-BIOFORT1[2]	1.44	*	*	Segregating
cv. Belíssima[2]	1.25	*	ns	Homozygous/ Resistant
UFU-104#3D	2.00	*	*	Segregating
UFU-104#4D	3.50	*	*	Segregating
UFU-66#6	1.25	*	ns	Homozygous/ Resistant
Uberlândia 10000(S)		4,75		
cv. Grand Rapids[2] (R)		0,50		

[1]Probability of the means of the treatments were different from the means of the controls by Dunnett's Test, were * and ns: significant and not significant at 5% probability, respectively.[2]Commercial cultivars. [3]VLRK= 1: few visible galls (<10 galls), small (<1 mm); 2: few visible galls, with intermediate size (1 to 3 mm); 3: intermediate number of visible galls (10-30 galls), intermediate size and some large (> 3 mm) galls; 4: many visible galls (> 30 galls), predominantly large galls (> 3 mm), with few intermediate size and some coalesced; 5: clod with many visible galls (> 30 galls), large and high number of coalescing root-knots. S= susceptible and R= resistant.

characteristic to *M. javanica.* UFU-104#1D and cv. Robusta did not differ from Uberlândia 10000, therefore being considered homozygous susceptible to *M. javanica*. The UFU-104#5, UFU-104#3D, and UFU-104#4D advanced lines, as well as UFU-BIOFORT1 cv. differed from Grand Rapids and Uberlândia 10000 cv., therefore being segregated for such characteristic. The results of Ferreira et al. (2011) corroborate with the present work, showing that it is possible to find resistant advanced lines. Fiorini et al. (2007) also observed similar results, noting the existence of 10 homozygous families for resistance to the *Meloidogyne* spp. nematode in 39 $F_{2:3}$ lettuce families.

Most of the mini lettuce advanced lines generally presented higher carotenoid content (indirectly) compared to the Uberlândia 10000 (rich in carotenoids) cultivar, which leads them to be considered biofortified lines. Among the evaluated genotypes, UFU-104#5 and UFU-66#6 are highlighted for nematode resistance, with the lowest number of galls in relation to the susceptible cultivar (Uberlândia 10000) and similar behavior to the resistant cultivar (Grand Rapids) for nematode reproductive factor. However, only UFU-66#6 did not differ from the resistant Grand Rapids cultivar in relation to galls, being homozygous resistant to the *M. javanica*. Furthermore, UFU-66#6 was superior in relation to the number of leaves in comparison with the other mini lettuce advanced lines. Therefore, UFU-66#6 is a biofortified mini-lettuce advanced line which can combine great agronomic potential and resistance to *M. javanica*.

Conclusion

It is concluded that only the UFU-66#6 advanced line (segment of biofortified mini lettuce) showed agronomic potential combined with resistance to *M. javanica*.

Acknowledgements

We are grateful for research support from the Lettuce-breeding program at the Federal University of Uberlandia, and for financial support from the Brazilian *CAPES*, *CNPq* and *FAPEMIG* institutions.

Literature Cited

Associação Brasileira do Comércio de Sementes e Mudas - Abcsem. Dados do setor. 2015. http://www.abcsem.com.br/dados-do-setor. 02 Nov. 2018.

Azevedo, A. M.; Andrade Júnior, V. C.; Oliveira, C. M.; Fernandes, J. S. C.; Pedrosa, C. E.; Dornas, M. F. S.; Castro, B. M. C. Seleção de genótipos de alface para cultivo protegido: divergência genética e importância de caracteres. Horticultura Brasileira, v. 31, n.2, p. 260-265, 2013. https://doi.org/10.1590/S0102-05362013000200014.

Bonetti, J. I. S.; Ferraz, S. Modificação do método de Hussey e Barker para extração de ovos de *Meloidogyne exigua* do cafeeiro. Fitopatologia Brasileira, v.6, n.3, p.553, 1981.

Carvalho Filho, J. L. S; Gomes, L. A. A.; Silva, R. R.; Ferreira, S.; Carvalho, R. R. C.; Maluf, W. R. Parâmetros populacionais e correlação entre características da resistência a nematoides de galhas em alface. Revista Brasileira de Ciências Agrárias, v. 6, n. 1, p. 46-51, 2011. https://doi.org/10.5039/agraria.v6i1a819.

Cassetari, L. S.; Gomes, M. S.; Santos, D. C.; Santiago, W. D.; Andrade, J.; Guimarães, A. C.; Souza, J. A.; Cardoso, M. G.; Maluf, W. R.; Gomes, L. A. B-carotene and chlorophyll levels in cultivars and breeding lines of lettuce. Acta Horticulturae, v. 1083, p. 469-473, 2015. https://doi.org/10.17660/ActaHortic.2015.1083.60.

Cruz, C. D. GENES: a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum Agronomy, v. 35, n.3, p. 271-276, 2013. https://doi.org/10.4025/actasciagron.v35i3.21251.

Damerum, A.; Selmes, S. L.; Biggi, G. F.; Clarkson, G. J.; Rothwell, S. D.; Truco, M. J.; Michelmore, R. W.; Hancock, R. D.; Shellcock, C.; Chapman, M. A.; Taylor, G. Elucidating the genetic basis of antioxidant status in lettuce (*Lactuca sativa*). Horticulture Research, v. 2, p. 15055, 2015. http:s//doi.org/10.1038/hortres.2015.55.

Ferreira, S.; Vieira, V. L. F.; Gomes, L. A. A.; Maluf, W. R.; Carvalho Filho, J. L. S. Identificação de linhagens avançadas de alface quanto à resistência a *Meloidogyne javanica*. Ciência e Agrotecnologia, v. 35, n. 2, p. 270-277, 2011. https://doi.org/10.1590/S1413-70542011000200006.

Filgueira, F. A. R. *Novo manual de olericultura:* agrotecnologia moderna na produção e comercialização de hortaliças. 3.ed. Viçosa: UFV, 2013. 412 p.

Fiorini, C. V. A.; Gomes, L. A. A.; Libânio, R. A.; Maluf, W. R.; Campos, V. P.; Licursi, V.; Moretto, P.; Souza, L. A. Identificação de famílias F2:3 de alface homozigotas resistentes aos nematoides das galhas. Horticultura Brasileira, v. 25, n. 4, p. 509-513, 2007. https://doi.org/10.1590/S0102-05362007000400004.

Hussey, R. S.; Barker, K. R. A comparison of methods collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter**,** v.57, n.12, p.1025-1028, 1973. https://babel.hathitrust.org/cgi/pt?id=mdp.39015001262776;view=1up;seq=459. 29 Abr. 2018.

Jones, J. T.; Haegeman, A.; Danchin, E. G. J.; Gaur, H. S.; Helder, J.; Jones, M. G. K.; Kikuchi, T.; Manzanilla-López, R.; Palomares-Rius, J. E.; Wesemael, W. M. L.; Perry, R. N. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology*,* v. 14, n. 9, p. 946–961, 2013. https://doi.org/10.1111/mpp.12057.

Lawson, P. G.; Daum, D.; Czauderna, R.; Meuser, H.; Härtling, J. W. Soil versus foliar iodine fertilization as a biofortification strategy for field-grown vegetables. Frontiers in Plant Science, v. 6, p. 450, 2015. https://doi.org/10.3389/fpls.2015.00450.

Nascimento, K. O.; Rocha, D. G. C. M.; Da Silva, E. B.; Barbosa-Junior, J. L.; Barbosa, M. I. M. J.; Nascimento, C. O. Caracterização química e informação nutricional de fécula de batata-doce (*Ipomoea batatas* L.) orgânica e biofortificada. Revista Verde, v. 8, n. 2, p. 132-138, 2013. https://www.gvaa.com.br/revista/index.php/RVADS/article/view/1774/1520. 22 Abr. 2018.

Onkendi, E. M.; Kariuki, G. M.; Marais, M.; Moleleki, L. N. The threat of root-knot nematodes (*Meloidogyne* spp.) in Africa: a review. Plant. Pathology, v. 63, n.4, p. 727-737, 2014. https://doi.org/10.1111/ppa.12202.

Sala, F. C.; Costa, C. P. Retrospectiva e tendência da alfacicultura brasileira. Horticultura Brasileira, v. 30, n. 2, p. 187-194, 2012. https://doi.org/10.1590/S0102-05362012000200002.

Santi, A.; Scaramuzza, W. L. M. P.; Neuhaus, A.; Dallacort, R.; Krause, W; Tieppo, R. C. Desempenho agronômico de alface americana fertilizada com torta de filtro em ambiente protegido. Horticultura Brasileira*,* v. 31, n. 2, p. 338-343, 2013. https://doi.org/10.1590/S0102-05362013000200027.

Smolén, S.; Kowalska, I.; Sady, W. Assessment of biofortification with iodine and selenium of lettuce cultivated in the NFT hydroponic system. Scientia Horticulturae, v. 166, p. 9-6, 2014. https://doi.org/10.1016/j.scienta.2013.11.011.

Sousa, C. S.; Bonetti, A. M.; Goulart Filho, L. R.; Machado, J. R. A.; Londe, L. N.; Baffi, M. A.; Ramos, R. G.; Vieira, C. U.; Kerr, W. E. Divergência genética entre genótipos de alface por meio de marcadores AFLP. Bragantia, v. 66, n. 1, p. 11-16, 2007. https://doi.org/10.1590/S0006-87052007000100002.

Souza, M. C. M.; Resende, L. V.; Menezes, D.; Loges, V.; Soute, T. A.; Santos, V. F. Variabilidade genética para características agronômicas em progênies de alface tolerantes ao calor. Horticultura Brasileira, v. 26, n. 3, p. 354-358, 2008. https://doi.org/10.1590/S0102-05362008000300012.

Takahashi, K.; Cardoso, A. I. I. Plant density in production of mini lettuce cultivars in organic system management. Horticultura Brasileira, v. 32, n. 3, p. 342-347, 2014. https://doi.org/10.1590/S0102-05362014000300017.