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F_4 families of crispleaf lettuce with tolerance to early bolting and homozygous for resistance to *Meloidogyne incognita* race 1

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ABSTRACT

The objective of this study was to evaluate yield, commercial characteristics, tolerance to early bolting and resistance to the root-knot nematode *Meloidogyne incognita* race 1 in 25 F_4 families of crispleaf lettuce, obtained out of crosses between cultivars Grand Rapids, Regina 71, and Verônica. In the first experiment, we evaluate leaf blade and borders characteristics, aboveground fresh weight, and number of days from sowing to the anthesis of first flower (tolerance to early bolting). In the second experiment, we evaluated the resistance to *Meloidogyne incognita* race 1 via gall index and number of galls and egg masses per root system. Five families had scores for leaf blade and borders similar to cultivars Verônica and Grand Rapids. Furthermore, 84% of the families were as tolerant to early bolting as cultivar Veronica, while 92% of the families were homozygous for resistance to *Meloidogyne incognita* race 1.

Keywords: *Lactuca sativa* L., breeding, root-knot nematode.

RESUMO

Famílias F_4 de alface de folhas crespas tolerantes ao florescimento precoce e homozigotas para resistência à *Meloidogyne incognita* Raça 1

O objetivo deste trabalho foi avaliar características comerciais, produtividade, tolerância ao florescimento precoce e resistência ao nematóide das galhas *Meloidogyne incognita* raça 1 em 25 famílias F_4 de alface, oriundas de cruzamentos envolvendo as cultivares Grand Rapids, Regina 71 e Verônica. No primeiro experimento, foram avaliadas as características de limbo e borda foliares, massa fresca da parte aérea e número de dias da semeadura até a antese da primeira flor (tolerância ao florescimento precoce). No segundo experimento, avaliou-se a resistência à *Meloidogyne incognita* raça 1 através do índice de galhas, número de galhas e massa de ovos por sistema radicular. Cinco famílias apresentaram notas para borda e limbo foliares semelhantes às cultivares Verônica e Grand Rapids. Além disso, 84% das famílias demonstraram tolerância ao florescimento precoce semelhante à cultivar Verônica e 92% delas foram consideradas homozigotas para resistência à *Meloidogyne incognita* raça 1.

Palavras-chave: *Lactuca sativa* L., melhoramento genético, nematóide das galhas.

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In regions with high temperatures, such as the summer in most regions of Brazil, lettuce blooms early, shortening the vegetative stage and, consequently, reducing leaf development. At the onset of the reproductive phase, with the elongation of the flowering stalk, the commercial value is lost due to latex production, which makes the leaf tastes bitter.

Air temperatures above 20°C promotes bolting (Silva *et al*., 1999), stimulus that is enhanced as temperature rises. Moreover, long days, when associated with high temperatures, accelerate the bolting process, which is also cultivar-specific (Wien, 2002). The solution has been to develop genotypes with high tolerance to heat. Cultivar Regina 71 has been used for this purpose in breeding programs, as a source of heat tolerance (Carvalho Filho *et al*., 2009, Silva *et al*., 2008, Fiorini *et al*., 2005). Therefore, it is of fundamental importance to search for alternatives to enable the production of high standard lettuce throughout the year.

In addition to early bolting, high temperature favors the development of nematodes, especially *Meloidogyne incognita* and *M. javanica*, which reduces plant fresh weight. Cultivar Babá, when evaluated in greenhouse, showed restriction in development when inoculated with races 1 and 2 of *M. incognita* (Krzyzanowski & Ferraz, 2000). In another experiment, cultivars Brisa and Lucy Brown had shoot and root fresh weight reduced due to the occurrence of *M. incognita* (Asuaje *et al*., 2004). Chemical control is occasionally used in the management of the root-knot nematode. However, chemicals, in addition to being toxic, have long lasting effects on leaves. Considering that lettuce has a relatively short cycle, the most safe and effective method for controlling nematodes would be the use of resistant cultivars.

Genetic resistance to the root-knot nematode has been observed on lettuce. The head lettuce cultivars Salinas 88, Chalenge, Vanguard 75, Calgary, Classic, and La Jolla showed reproduction index below 1 for *M. incognita* race 2, i.e., these cultivars produced fewer eggs than the number of eggs initially inoculated. Thus, these cultivars are considered promising sources of resistance for breeding programs (Wilcken *et al*., 2005). The crisp lettuce cultivar Grand Rapids has also proven to be resistant to *M. incognita* races 1, 2, 3, and 4 (Gomes *et al*., 2000), and to *M. javanica* (Maluf *et al*., 2002).

This study aimed to select crisp lettuce F_4 families with tolerance to early bolting and resistance to the root-knot nematode *M. incognita* race 1.

MATERIAL AND METHODS

Two experiments were carried out. At the first, we evaluated commercial characteristics and tolerance to early bolting, and, at the second, resistance to *M. incognita* race 1. The experiments were performed in the field, at HortiAgro Seeds Ltda., in Ijaci (21°10'S, 44°55'W, 832 m of altitude, average annual temperature of 19.4°C, average minimum temperature of 14.8°C, average maximum temperature of 26.1°C), located in the south of Minas Gerais State. The experiments were carried out from October 2006 to March 2007, a period in which the average temperatures ranged between 25 and 32°C. Treatments consisted of the parental cultivars, namely Regina 71, Grand Rapids, and Veronica, in addition to the F_4 families. Cultivar Regina 71 has smooth leaves, high resistance to early bolting, and susceptibility to *M. incognita* race 1. Cultivar Grand Rapids belongs to the crisp type and is resistant to *M. incognita* races 1, 2, 3, and 4 (Gomes *et al*., 2000). Cultivar Verônica is tolerant to bolting, but susceptible to root-knot nematodes (Charchar *et al*., 1996; Silva *et al*., 2008).

The F_4 families were obtained out of the cross between cultivars Regina 71 and Grand Rapids. The F_1 was selfed, resulting in the F_2 population. According to the pedigree method (Borém & Miranda, 2005), we evaluated the F_2 population for resistance to *M. incognita* spp. and tolerance to early bolting. The selected plants were again selfed to produce the F_3 families that were selected for the crisp leaf type, resulting in the F_4 families. The selected F_4 families were then crossed to cultivar Verônica, starting the second phase of the program, also using the pedigree method. The F_1 population was selfed and the resulting F_2 population was evaluated for resistance to *M. incognita* spp. (Fiorini *et al*., 2007) and tolerance to early bolting. The selected families originated the F_3 families, which were evaluated for commercial characteristics. The selected plants led to the F_4 families whose performance is reported in this paper.

In experiment 1, families were evaluated for commercial characteristics and tolerance to early bolting. We evaluated 25 F_4 families and cultivars Regina 71, Grand Rapids, and Verônica, used as controls. Seeds were sown in polystyrene 128-cell trays, with commercial substrate (Plantmax®) and, 26 days after sowing, plants were transplanted to 1.20 wide seedbeds prepared beforehand and conducted in an organic farming system. We used blocks at random with three replications, and 16 plants per plot, spaced by 0.3 x 0.3 m.

For assessing leaf blade and borders, scores from 1 to 5 were assigned independently for each plant and trait, as follows: 1= crispy blade or border, 2= very wrinkled blade or border, 3= wrinkled blade or border, 4= slightly wrinkled blade or border 5= smooth blade or border (Carvalho Filho *et al*., 2009). Then, to assess plant fresh weight, eight plants per plot were cut at soil level and weighed on an electronic scale. These characteristics were subjected to analysis of variance. Means were separated by the Scott-Knott test, at 5% probability.

Tolerance to early bolting was evaluated in the remaining eight plants of each plot, conducted up to harvesting the seeds. Each plant was tutored with a stake to avoid bending. We evaluated the number of days from sowing to the anthesis of first flower for each plant, and then calculated the plot average. The values of each family, as well as those of the control cultivars were subjected to the analysis of variance, with means compared by the Dunnett test, at 5% probability.

In experiment 2, the 25 F_4 families and cultivars Regina 71 (susceptible) and Grand Rapids (resistant) were evaluated for resistance to *M. incognita* race 1. Seeds were sown in polystyrene 128-cell trays, containing commercial substrate (Plantmax®). Seedlings were infested with eggs of *M. incognita* race 1, 16 days after sowing, at a concentration of 1,200 eggs $cell^{-1}$, corresponding to 30 eggs cm^{-3} of substrate. Egg extraction and substrate infestation were carried out according to Hussey & Barker (1973), modified by Boneti & Ferraz (1981).

Plants remained in the trays in the greenhouse until 45 days after the inoculation with nematodes, when the evaluation was carried out. At this date, we observed a high incidence of both galls and egg masses in the roots of indicator plants (eight tomato plants per replication, cultivar Santa Clara,). Indicator plants were used to confirm the inoculation efficiency and infestation severity. We used a completely randomized design, with four replications and eight-plant plots.

The gall index was assessed using a scale and evaluating plant roots still with substrate, as follows: 1= few visible (<10 galls) and small (<1 mm) galls; 2= few visible galls, but with intermediate size (1 to 3 mm); 3= intermediate number of visible galls (10 to 30 galls), standard size, with some large galls (>3 mm); 4= many visible (>30 galls) predominantly large (>3 mm) galls, with few of intermediate size, some galls already coalescing; 5= clods with lots of large visible galls (>30 galls), many already coalescent. After washing the roots, the galls were counted. Then, roots were stained with an industrial dye containing 1% Bordeaux (Rocha *et al*., 2005) to count the number of egg masses. The figures for number of galls and egg masses were transformed into the square root and analyzed using the software SAS (SAS Institute, 1993). Means were contrasted via the Dunett test, at 5% probability. This test compares separately the mean of each control to the other treatments. Resistant families were considered as homozygous when means for all characteristics simultaneously differed significantly from cultivar Grand Rapids, but not from cultivar Regina 71. Susceptible families were considered as homozygous when means for all characteristics differed significantly from cultivar Regina 71, but not from cultivar Grand Rapids. In any condition other than those, families were considered as still segregating.

RESULTS AND DISCUSSION

Only six families had curly leaf borders significantly similar to cultivars Grand Rapids and Verônica (Table 1).

Cultivar Grand Rapids had curlier leaf blades than cultivar Verônica. Taking into consideration leaf blade, three families did not differ significantly from cultivar Grand Rapids and another four, from cultivar Verônica, indicating that these families produce curly leaf blades. Despite the potential increase in the frequency of alleles for curly leaves promoted by crossing the selected families to cultivar Verônica, two families, AFX 009B 51 18 and AFX 009B 78 02, had leaf blade and border similar to cultivar Regina 71. This may indicate that the character is governed by a large number of genes, which would

Table 1. Leaf blade and border, aboveground fresh mass, number of days to bolting, gall index and number, and number of egg masses in the roots in 25 F_4 families and cultivars of lettuce (limbo e bordos da folha, massa fresca da parte aérea, número de dias até o florescimento, índice e número de galhas e número de massas de ovos nas raízes em 25 famílias F_4 e cultivares de alface). Lavras, UFLA, 2007.

Families	Leaf blade*	Leaf border*	Fresh mass (g plant^{-1})*	Days to bolting	Gall index	Gall number	Number of egg masses
AFX 009B 51 02	3.8 d	3.7 d	350.0 a	98**	1.80***	46.58***	17.01***
AFX 009B 51 03	3.7 d	3.0 c	397.0 a	91**	1.63***	35.40***	15.48***
AFX 009B 51 04	4.0 d	3.4 d	350.0 a	96**	1.69***	30.36***	10.00***
AFX 009B 51 06	2.9 c	2.7 b	316.7 b	95**	1.78***	44.78***	16.87***
AFX 009B 51 08	3.7 d	3.4 d	295.8 b	85***	1.63***	39.59***	12.39***
AFX 009B 51 18	4.9 e	4.0 e	329.2 b	85***	1.80***	42.29***	16.28***
AFX 009B 51 25	3.8 d	3.3 d	375.0 a	93**	2.03***	41.43***	16.94***
AFX 009B 51 34	2.1 b	3.0 c	312.5 b	95**	1.81***	31.29***	16.26***
AFX 009B 78 02	4.5 e	5.0 f	395.8 a	92**	1.46***	41.19***	11.78***
AFX 009B 78 03[1]	1.0 a	1.0 a	291.7 b	97**	2.09***	45.59***	25.33***
AFX 009B 78 11[1]	1.7 b	1.0 a	338.1 b	94**	2.02***	36.20***	16.80***
AFX 009B 78 26	3.7 d	3.6 d	300.0 b	94**	1.77***	32.70***	13.88***
AFX 009B 78 27[1]	1.6 b	1.3 a	300.0 b	101**	2.13***	42.88***	15.50***
AFX 009B 78 29[1]	2.2 b	1.3 a	358.3 a	91**	1.70***	30.55***	12.65***
AFX 009B 78 36[1]	1.0 a	1.0 a	333.3 b	87***	1.91***	48.25***	18.97***
AFX 012B 53 12	1.0 a	3.9 e	295.8 b	93**	2.16**	40.51***	16.48***
AFX 012B 53 13	4.0 d	3.5 d	312.5 b	91**	1.75***	38.64***	12.84***
AFX 012B 53 33	3.9 d	3.3 c	291.7 b	88***	1.79***	30.45***	16.61***
AFX 012B 53 39	4.0 d	3.0 c	283.3 b	94**	1.79***	36.61***	8.56***
AFX 012B 53 61	3.9 d	2.8 c	308.3 b	92**	1.54***	39.53***	14.69***
AFX 013B 68 11	3.2 c	2.4 b	314.6 b	95**	1.59***	46.49***	20.12***
AFX 013B 68 13	2.9 c	2.5 b	408.3 a	99**	1.50***	31.67***	13.52***
AFX 013B 68 37	3.6 d	1.3 a	333.3 b	101**	1.62***	31.55***	12.67***
AFX 013B 68 46	4.0 d	3.0 c	312.5 b	98**	1.85***	35.20***	14.61***
AFX 013B 68 66	4.5 e	2.5 b	316.7 b	98**	1.78***	39.15***	12.43***
Grand Rapids	1.0 a	1.0 a	304.2 b	86	1.53	23.28	12.90
Regina 71	5.0 e	5.0 f	304.7 b	110**	4.26**	94.23**	48.29**
Verônica	2.0 b	1.0 a	304.2 b	95**			

*Means followed by the same letter in the column did not differ significantly from each other, Scott-Knott test, $p<0.05$ (médias seguidas da mesma letra na coluna não diferem significativamente entre si, teste de Scott- Knott, p<0,05); **Means significantly higher than cultivar Grand Rapids, Dunett test, $p<0.05$ (médias significativamente superiores à cultivar Grand Rapids, teste de Dunnett, p<0,05); ***Means did not significantly differ from cultivar Grand Rapids, Dunett test, $p<0.05$ (médias não diferiram significativamente da cultivar Grand Rapids, teste de Dunnett, p<0,05); [1]Families considered similar to cultivars Grand Rapids and/or Verônica for leaf blade and border (famílias consideradas semelhantes às cultivares Grand Rapids e/ou Verônica para limbo e borda foliares); [2]Leaf blade and borders assessed by a visual scale from 1= crispy blade or border to 5= smooth blade or border (limbo e bordo das folhas avaliados através de escala visual de 1= limbo e bordo crespos a 5= limbo e bordo lisos).

hamper selection gains.

There were no significant differences among cultivars for aboveground fresh weight and all families had figures significantly greater than or equal to the cultivars (Table 1). However, as this is a character of additive genetic nature (Lédo *et al*., 2001) and high narrow sense heritability (79%) (Souza *et al*., 2008), selection for increasing the aboveground fresh weight will favor the accumulation of favorable alleles in the next generations.

In the screening for commercial characteristics, the families AFX 009B 78 36, AFX 009B 78 11, AFX 009B 78 03, AFX 009B 78 27, and AFX 009B 78 29 (Table 1) were selected due to their high similarity to the crispleaf lettuce cultivar used as the commercial standard for the three characteristics. It should be emphasized that new selections for these traits should be carried out in subsequent generations, to increase the frequency of favorable alleles to crisp leaves. Similar results were found by Carvalho Filho *et al*. (2009).

No family was as tolerant as cultivar Regina 71 (average of 110 days for flowering) to early bolting. Conversely, when compared to cultivar Grand Rapids (86 days), 21 families were more tolerant to early bolting. Cultivar Veronica, considered as tolerant to early bolting among the crispleaf types, flowered, on average, after 95 days, differing significantly from Grand Rapids, but not from cultivar Regina 71. Among the 25 families studied, only families AFX 009B 51 08, AFX 51 18 009B, AFX 009B 78 36, and AFX 009B 53 33 flowered significantly earlier than the cultivar Veronica, respectively after 85, 85, 87 and 88 days. The lack of families as tolerant to early bolting as cultivar Regina 71 can be due to the reduction in the proportion of cultivar Regina 71 alleles within the F_4 families as consequence of the crosses with cultivar Veronica. Nevertheless, there was variation left within families, as there were plants with flowering dates similar to those observed for cultivar Regina 71.

When we consider the distribution frequency of individual plants for number of days to bolting (upper and lower limits of 85 and 115 days, respectively, with five classes and 9-day intervals in between classes), we noted that 27.8% of the plants flowered after 96 days, which corresponds to the lower limit observed in the tolerant cultivar Regina 71. Thus, further selection within each family may lead to an increase in the number of days to bolting in the following generations due to the increase in the additive variance level (between and within families). Thus, we expect a rise in the likelihood of obtaining genotypes with tolerance to early bolting (Borém & Miranda, 2005). This possibility is supported also by the high heritability in the narrow sense, about 67%, observed for the characteristic (Souza *et al*., 2008).

The averages for gall index in cultivars Regina and Grand Rapids 71 (Table 1) were respectively 1.53 and 4.26, both corresponding to extreme figures, hence confirming respectively the resistance and susceptibility of the two cultivars to *M. incognita* race 1. Family AFX 012B 53 33 was the only to differ significantly from cultivars Grand Rapids and Regina 71. All other families differed significantly from cultivar Regina 71, but not from cultivar Grand Rapids. When the average for number of galls in the roots was considered, only the family AFX 012 53 33 differed significantly from both cultivars, while the other families differed significantly from cultivar Regina 71, but not from cultivar Grand Rapids (Table 1). Finally, for number of egg masses only family AFX 009B 78 11 did not differ significantly from cultivar Regina 71, if we consider a probability slightly higher than 5%, which was the significance threshold we adopted. When we took cultivar Grand Rapids as control, only cultivar Regina 71 was significantly different (Table 1), the latter with a higher number of egg masses than the former.

When we take into account the three characteristics related to the reaction to the root-knot nematode, all families were resistant to *M. incognita* race 1, except families AFX 012B 53 33 and AFX 009B 78 11. Therefore, we concluded that 22 families were homozygous for resistance. Carvalho Filho *et al*. (2007) also succeeded in obtaining F_4 families of lettuce homozygous for resistant to the root-knot nematode using cultivar Salinas 88 as one of the parents, due to the inheritance of the other parent, cultivar Grand Rapids. Given the monogenic inheritance of the resistance (Gomes *et al*., 2000), we already expected these results, as the plants used to breed these families were previously selected for resistance. Gomes *et al*. (2000) in their work observed that the resistance against the root-knot nematode was stable, even when plants were challenged with a mixture of races. Thus, those authors suggested that the same gene would control the resistance to all races. If this is true, there is a chance that the families report here are resistant also to other races of *M. incognita* and *M. javanica*. However, further evaluations are needed with races 2, 3, and 4 of *M. incognita*, as well as with *M. javanica*, to confirm this premise. This universal resistance would be very convenient, since *M. incognita* and *M. javanica* populations are endemic in similar regions and can develop in the same area (Tihohod, 2000).

Families AFX 009B 78 27 and AFX 009B 78 29 are crispleaf lettuce, tolerant to early bolting, and homozygous for resistance to *M. incognita* race 1. Consequently, these two families will be used to continue our breeding program.

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