Germlasm identification of bean 
(*Phaseolus vulgaris* L.) and lettuce 
(*Lactuca sativa* L.) resistant to avirulent 
populations of *Meloidogyne* spp. to Mi 
gene

Final project of grade 
Agricultural Engineering

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Resum

*Meloidogyne* spp. és un gènere de nematodes fitopàrasis causant de pèrdues importants en horticultura. En els darrers anys s’ha utilitzat la resistència vegetal per a controlar el nematode de forma sostenible.

En el present treball es determina la resistència vegetal en front *Meloidogyne* spp. de dues varietats d’enciam Grand Rapids i Salinas 88, i de tres varietats de mongeta Aporé, Macarrão Atibaia i Ouro Negro, de les quals s’han realitzat treballs previs a la Universitat Federal de Lavras (UFLA), Brasil. També s’avalua la possible resistència d’una varietat de mongeta, Cornell 49242, no caracteritzada prèviament. Com a control de tots els assajos portats a terme s’han utilitzat la varietat susceptible d’enciam Regina 71, i de mongeta Bolinha.

Per a la caracterització de la resistència es va realitzar un experiment en torretes per cada cultiu en el que es va enfrontar les diferents varietats a 11 poblacions de *Meloidogyne incognita* i *M. javanica*; i un experiment en condicions de camp on es va avaluar el comportament de les diferents varietats davant diferents densitats de *Meloidogyne incognita*.

Els resultats van confirmar la resistència per a poblacions de nematodes provinents d’Almería, Murcia i Catalunya de les varietats d’enciam Grand Rapids i Salinas 88, obtenint reproduccions inferiors del nematode en l’experiment en torretes i reduint la població en el sòl en l’experiment de camp. Les varietats de mongeta varen tenir resultats més variables. El cultivar Aporé es va mostrar resistent en tots els assajos. Macarrão Atibaia i Ouro Negro es van mostrar com a lleugerament resistentes i susceptibles (45%), en front la majoria de poblacions de nematodes utilitzades en l’assaig en torreta. En camp, Ouro Negro es va mostrar lleugerament resistant, però Macarrão Atibaia es va comportar com a susceptible amb un 81.9% de reproducció respecte Bolinha (varietat susceptible de control). El cultivar Cornell 49242 es comportar com a susceptible en front la majoria de poblacions de *Meloidogyne* spp. en l’assaig de torreta.
Resumen

*Meloidogyne* spp. Es un género de nematodos fitoparásitos causante de pérdidas importantes en horticultura. En los últimos años se ha utilizado la resistencia vegetal a fin de reducir los daños causados de forma sostenible.

En el presente trabajo se determina la resistencia frente *Meloidogyne* spp. de dos variedades de lechuga Grand Rapids y Salinas 88, y tres variedades de judía Aporé, Macarrão Atibaia y Ouro Negro, de las que se han realizado trabajos previos a la Universidad Federal de Lavras (UFLA), Brasil. También se evalúa la posible resistencia de una variedad de judía, Cornell 49242, no caracterizada previamente. Como control de todos los ensayos llevados a cabo se han utilizado las variedades susceptibles Regina 71, por lechuga y Bolinha, por judía.

Para la caracterización de la resistencia se realizó un experimento en macetas para cada cultivo en el que se enfrentó las diferentes variedades a 11 poblaciones de *Meloidogyne incognita* y *M. javanica*; y un experimento en condiciones de campo donde se evaluó el comportamiento de las diferentes variedades ante diferentes densidades de *Meloidogyne incognita*.

Los resultados confirmaron la resistencia para poblaciones de nematodos provenientes de Almería, Murcia y Cataluña de las variedades de lechuga Grand Rapids y Salinas 88, obteniendo reproducciones inferiores del nematodo en el experimento en macetas y reduciendo la población del suelo en el experimento de campo. Las variedades de judía tuvieron resultados más variables. El cultivar Aporé se mostró resistente en todos los ensayos. Macarrão Atibaia y Ouro Negro se mostraron como ligeramente resistentes y susceptibles (45%), frente a la mayoría de poblaciones de nematodos en el ensayo en maceta. En campo, Ouro Negro se mostró ligeramente resistente, pero Macarrão Atibaia se comportó como susceptible con un 81.9% de reproducción respecto Bolinha (variedad susceptible de control). El cultivar Cornell 49242 se comportó como susceptible frente la mayoría de poblaciones de *Meloidogyne* spp. en el ensayo de macetas.
Phytoparasitic nematodes of the genus *Meloidogyne* spp. are the cause of important losses in horticulture. In recent years, plant resistance has been used to reduce this damage in a sustainable way.

The present work determines the resistance against *Meloidogyne* spp. of two varieties of lettuce Grand Rapids and Salinas 88, and the resistance of three varieties of common bean Aporé, Macarrão Atibaia and Ouro Negro, which have previously been studied in the University Federal University of Lavras (UFLA), Brazil. Furthermore this study will assess the possibility of resistance of a common bean cultivar, Cornell 49242, which has not been previously tested. As a method of controlling all the tests carried out two cultivars were used – Regina 71 for lettuce and Bolinha for common bean.

In order to characterize the resistance, two experiments were carried out. Firstly, both crops were confronted with 11 populations of *Meloidogyne incognita* and *M. javanica* in a pot experiment. Secondly, a field conditions experiment was used to observe the behaviour of the different cultivars under different densities of *Meloidogyne incognita*.

The results confirmed the resistance of the Grand Rapids and Salinas 88 lettuce cv to nematode populations from Almeria, Murcia and Catalonia, as they obtained lower nematode reproductions in the pot experiment and reduced the soil population in the field experiment. The common bean cultivars showed more diverse results. Aporé cv. proved to be resistant in all experiments. The results for Macarrão Atibaia and Ouro Negro showed their slight resistance and susceptibility (of the 45%) to the majority of the nematode populations used in the pot experiment. In field conditions the results differed. Ouro Negro was slightly resistant, but Macarrão Atibaia behaved as susceptible with an 81.9% of reproduction compared to Bolinha (susceptible cultivar of control). The cultivar Cornell 49242 behaved as susceptible in front the majority of the *Meloidogyne* spp. populations of the pot experiment.
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Pel Matías i el Martí (m&m), la nova generació i amors de la meva vida, per un bon futur.
Germlasm identification of bean (*Phaseolus vulgaris* L.) and lettuce (*Lactuca sativa* L.) resistant to avirulent populations of *Meloidogyne* spp. to **Mi** gene.

“If what you are going to describe is true, leave the elegance to the tailor.”

Albert Einstein.

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UPC - BarcelonaTech
1. Introduction

In Spain, 53.7% of the land is dedicated to agricultural practices (The World Bank); and almost 50% of the vegetables produced is exported. Spain ranks first as a fruit and vegetable exporter in the European Union (Ministerio de Agricultura, Alimentación y Medio Ambiente, 2013). Horticulture production is mainly concentrated in the Mediterranean basin, where a high-input agricultural system is in place. This agricultural production system is characterized by cropping few high valuable crops, by monoculture in the majority of cases, and by high-cost inputs including pesticides and fertilizers. This leads to a loss of physicochemical and biological soil quality. The main crops belong to the Solanaceous (tomato, pepper, and aubergine) and the Cucurbitaceous (mainly cucumber, melon, zucchini squash and watermelon) families. However, in some growing areas, rotation sequences including members of the Compositae and Fabaceae families, mainly lettuce and green bean (Annex 1), are carried out. Lettuce crops reach 34.180 ha producing 853.000 tonnes per year. The production rate is 26.000 kg/Ha in irrigated land and 32.000 kg/ha in greenhouses. Lettuce is mainly grown in Murcia, Almería and Granada. Green bean is cultivated on 10.283 ha, which produce 188.210 tonnes per year. 36 % of the production is growing in under-protected conditions and represent 68% of the total production. Málaga, Almería and Granada are the main areas of production in Spain (MARM, 2013).

The intensification of agriculture has led to soil fatigue, because of the suboptimal physicochemical soil properties. Biotic factors also contributed to this by increasing plant pathogens, including plant parasitic nematodes. The genus Meloidogyne (Annex 2), the root-knot nematodes (RKN), is the main harmful plant parasitic nematode genus and limits horticulture production around 30 % worldwide (Netscher and Sikora, 1990). The most common RKN species in the Mediterranean basin are M. arenaria, M. incognita and M. javanica which are able to parasitize more than 2000 plant species (Ornat and Sorribas, 2008). In Spain, vegetable yield losses caused by RKN ranged from 30 to 88% (Giné et al., 2014, 2017a, 2017b; López-Gómez et al., 2014; Vela et al., 2014). RKN are mainly controlled by fumigant and non-fumigant nematicides (Talavera et al., 2012). However, the current legislation tries to reduce the use of pesticides by the Directive 2008/12/128/CE and promotes the use of non-chemical alternatives in order to reduce environmental and toxicological effects. Plant resistance is the main method of control used in integrated nematode management strategies due to its cost-effectiveness, its compatibility with other control methods, and its nil environmental impact (Starr et al., 2002). Resistant plants are able to suppress the development and reproduction of plant-parasitic nematodes (Roberts, 2002).
However, commercial vegetable resistant cultivars or rootstocks are only available for tomato, pepper, and aubergine, even though resistance has also been characterized on several *Cucumis metuliferus* and *Citrullus lanatus* var. citroides germplasm accessions which could be used as cucurbit rootstocks in the near future (Lee et al. 2010; Kokalis-Burelle and Rosskopf 2011; Thies et al. 2010, 2012, and 2015; Picó et al. 2013; Munera et al. 2014), and lettuce (Oliveira et al., 2015). In common bean resistance was first identified in 1928 (Isbell, 1931) and then other works came up and the gene that confers resistance to *Meloidogyne incognita* was identified as Me1 (Omwega et al., 1990; Ferreira et al., 2010, 2012). Therefore Omwega et al. (1990) and Mullin et al. (1991), determined the influence of high temperatures (below 28ºC to 30 ºC) on expression of resistance, increasing the nematode reproduction. Despite the benefits of using plant resistance, there are also some factors that can affect its expression. For example in tomato, the most studied one, resistance is conferred by the Mi-1.2 gene introgressed from *S. peruvianum* (Smith, 1944) which is active against *M. arenaria*, *M. incognita* and *M. javanica* (Roberts, 1992; Williamson, 1998). Nevertheless, plant resistance conferred against these nematode species by the Mi-1.2 gene is compromised when soil temperatures are sustained above 28 ºC (Dropkin, 1969), and/or against Mi-virulent populations or other RKN species such as *M. hapla*, *M. chitwoodi* race 3 (Brown et al., 1997), *M. enterolobii* (Kiewnick et al., 2009), or *M. exigua* (Silva et al., 2008). Moreover, the genetic background of the resistant tomato plant as well as of the nematode population has also shown to have an impact on the effectiveness of the Mi-1.2 mediated resistance to the targeted RKN species (Cortada et al., 2008; 2009; 2012). Despite resistant tomato cultivars and/or rootstocks are widely used, the repeated cultivation of resistant genotypes can select for Mi-virulent RKN-populations which could overcome the protective effect conferred by the Mi-1.2 gene (Williamson, 1998; Verdejo-Lucas et al., 2009). Thus, as the virulence is highly specific to a given resistant gene, the rotation with other single resistance genes and non-host crops could promote the durability of the resistance conferred by each resistance gene (Dijan-Caporalino et al., 2011).

The host status of a cultivar can be defined for a given set of conditions (Seinhorst, 1967) by the RKN population density at planting (*Pi*), which in absence of limiting factors, will increase proportionally and the multiplication rate (*Pf/Pi*, where *Pf* is the population density at the end of the crop) is at maximum with low *Pi* values. However, the multiplication rate decreases as *Pi* increases as a result of scarcity of food and competition (Seinhorst, 1970), tending to stabilize around an equilibrium density (*E*) at which the plant can supply enough food to maintain the population density at planting (*Pf=Pi*).
To estimate the dynamic of *Meloidogyne incognita* in field conditions the present work will determine the relationship between \( Pf/Pi \), the maximum rate of multiplication \((a)\), the maximum nematode density of the crop \((M)\) and the equilibrium density \((E)\).
2. OBJECTIVES

The aim of the experiments has been to test the lettuce and bean germplasm, previously studied for their partial or total resistance by Gomes et al. (2000), Alves and Campos (2001), Maluf et al. (2002), Wilcken (2005), Carvalho Filho et al. (2008), Ferreira (2010) and Oliveira et al. (2015), against several populations of Meloidogyne spp. found in different areas of the South of Spain and Catalonia.

The main objective can be divided into the following specific objectives:

- Screen of lettuce and bean cultivars against Meloidogyne spp. in pot experiments.
- Evaluation of the response of lettuce and bean cultivars against Meloidogyne incognita in field conditions.
3. MATERIALS AND METHODS

Experiments were carried out from May to July of 2016 inside the glasshouse and the plastic greenhouse of Agròpolis – UPC (Viladecans).

Figure 1. Agròpolis map. Situation of the glasshouse (A) and the plastic greenhouse (B).
3.1. Plant material

Five common bean cultivars were used in both pot and plastic greenhouse experiments. The origin and characteristics of the cultivars are shown in table 1.

<table>
<thead>
<tr>
<th>Bean cultivar</th>
<th>Origin</th>
<th>Resistance evaluated previously</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aporé</em></td>
<td>Brazil</td>
<td><em>Meloidogyne incognita, M. javanica, M. enterolobii</em></td>
<td>Ferreira et al., 2010; Melo et al., 2011; Ferreira et al., 2012; Oliveira et al., 2015</td>
</tr>
<tr>
<td><em>Ouro Negro</em></td>
<td>Brazil</td>
<td><em>Meloidogyne incognita, M. javanica, M. enterolobii</em></td>
<td>Ferreira et al., 2010; Melo et al., 2011; Oliveira et al., 2015</td>
</tr>
<tr>
<td><em>Macarrão</em></td>
<td>Brazil</td>
<td><em>Meloidogyne incognita, M. javanica</em></td>
<td>Ferreira et al., 2010</td>
</tr>
<tr>
<td><em>Atibaia</em></td>
<td>Brazil</td>
<td>No previous works with nematodes.</td>
<td>Trabanco et al., 2012</td>
</tr>
<tr>
<td><em>Cornell 49242</em></td>
<td>USDA – used in genetic improvement by SERIDA (Asturias)</td>
<td>Resistant to powdery mildew</td>
<td>Trabanco et al., 2012</td>
</tr>
<tr>
<td><em>Bolinha</em></td>
<td>Brazil</td>
<td>Susceptible cultivar (control)</td>
<td>Oliveira et al., 2015</td>
</tr>
</tbody>
</table>

The experiments did not make use of seedlings. Two seeds were sown in each pot or in soil to allow germination and plant growth. In the pot experiment, one plant per plot was removed one week after germination.
Three lettuce cultivars were used in both pot and plastic greenhouse experiments. The origin and characteristics of the cultivars are shown in Table 2.

Table 2. Cultivar name, origin and *Meloidogyne* resistance of the three lettuce cultivars used in both pot and plastic greenhouse experiments.

<table>
<thead>
<tr>
<th>Lettuce cultivar</th>
<th>Origin</th>
<th>Resistance evaluated previously</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regina 71</td>
<td>USDA</td>
<td>Susceptible variety (control)</td>
<td>Gomes <em>et al.</em>, 2000; Maluf <em>et al.</em>, 2002; Carvalho Filho <em>et al.</em>, 2007</td>
</tr>
</tbody>
</table>

Seeds of lettuce were sown in trays containing vermiculite, and then maintained at 25 °C with 16:8 light:darkness in a growth chamber until the second leaf appeared. After that, the plantlets were transplanted individually to seedling trays containing vermiculite. Plants were fertirrigated with Hoagland solution and maintained in the same conditions as before for three weeks prior to their transplantation to pots or soil.
3.2. Nematode populations

Twelve *Meloidogyne* populations coming from different vegetable growing areas from Spain were used in this study, eleven for pot experiments and one for the plastic greenhouse experiment (Table 3). Nematode populations were maintained on the susceptible tomato (*Solanum lycopersicum*) cv. Durinta growing in 3-L-pots containing sterilized sand. The *Meloidogyne* species were identified by perineal patterns and molecular SCAR – PCR markers (Zijlstra et al., 2000) (Annex 3).

<table>
<thead>
<tr>
<th>Experiment</th>
<th><em>Meloidogyne</em> population code</th>
<th>Origin</th>
<th><em>Meloidogyne</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amat</td>
<td>M. javanica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MJ 05</td>
<td>M. javanica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mi Al 30</td>
<td>M. incognita</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mi Al 09</td>
<td>M. incognita</td>
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<td>MJ Al 101</td>
<td>M. javanica</td>
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<td>Al 05</td>
<td>M. javanica</td>
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<tr>
<td>Viat or</td>
<td>M. javanica</td>
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</tr>
<tr>
<td>Adra</td>
<td>M. javanica</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P Almería</td>
<td>M. incognita</td>
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</tr>
<tr>
<td>P Murcia</td>
<td>M. incognita</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curas</td>
<td>M. javanica</td>
<td></td>
<td></td>
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<tr>
<td>Plastic greenhouse</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Agròpolis</td>
<td>M. incognita</td>
<td></td>
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</tbody>
</table>

The nematode inoculum used in the pot experiments consisted of second stage juveniles (J2) that emerged from eggs previously extracted from tomato roots through the Hussey and Barker’s method (1973) and left in Baermann trays during three weeks. Then J2 were collected, counted and maintained at 9°C until their use (Annex 4).
3.3. Greenhouse experiments

1- Screening lettuce and bean cultivars against *Meloidogyne* spp. in pot experiments.

Lettuce and common bean were cultivated in pots 0.5 L or 1 L, respectively. Plants were distributed on benches and maintained in the unheated greenhouse (Figure 2 and 3). The soil was infested at a rate of 1 J2 cm⁻³ of soil one week after transplanting the lettuce or two weeks after common bean germination. J2 nematodes were introduced in two opposite holes of 3 cm depth and two cm from the steam by using a pipette. Plants were watered by drip irrigation as needed and fertilised with a slow-release fertiliser (15% N + 10% P₂O₅ + 12% K₂O + 2% MgO₂ + microelements). Each combination cultivar-nematode population was replicated 10 times.

Figure 2A and 2B. Example of lettuce (up) and bean (down) pot assays distribution on bench inside the glasshouse. Ten replicates of each RKN population (nº population-nº repetition) alternated between cultivars.
The soil temperature and the water content of the soil at 8 cm depth from thereof the pots along the benches was registered in a datalogger by STS digital probes (Decagon devices) at 1 h intervals. The experiments were carried out from 11th of May to 27th of June with a final accumulation of 1257.3 ºC for lettuce; and from 16th of May to 7th of July with a final accumulation of 1560.8 ºC for common bean, calculated from an initial temperature base of 0 ºC.

At the end of the experiments, the aerial part of the plants was cut, and roots were gently washed with tap water, gently dried and weighted. The number of eggs per gram of root was determined by the Hussey and Barker method (1973), extracting the eggs from the entire root system in a 0.5%NaOCl solution for 10 min. The reproduction index (RI) was calculated as the number of eggs per plant produced on the resistant cultivar, and expressed as the percentage of those on the susceptible cultivar Regina 71 for lettuce or Bolinha for common bean. The level of resistance of each cultivar was classified as highly resistant (RI < 1%), resistant (1 ≤ RI ≤ 10%), moderately resistant (10 < RI ≤ 25%), slightly resistant (25 < RI ≤ 50%) or susceptible (RI > 50%) according to the categorization of Hadisoeganda and Sasser (1982).
2- Evaluate the response of lettuce and bean cultivars against *Meloidogyne incognita* under field conditions.

The experiment was carried out in a plastic greenhouse located at Agròpolis (Viladecans) infested with *M. incognita*. The soil was sandy loam with 83.8 % sand, 6.7 % silt and 9.5 % clay, pH 8.7; 1.8 % of organic matter and 0.5 dS/m of electric conductivity. The same lettuce and common bean cultivars assessed in pot conditions were used in plastic greenhouse, except common bean cv. Cornell 49242 that was not included. Plots of 0.5 m large and 2.5 m long were sampled to determine the initial nematode density previous sowing or transplanting. Composite samples consisted in 4 soil cores taken with an auger (2.5 cm diameter) from the first 30 cm of soil. The soil was homogenised and J2 were extracted from 500 cm³ of soil by Baermann trays (Whitehead and Hemming, 1965). After one week, J2 suspension was sieved thorough a 500 mesh and counted. In each plot all lettuce or common beans cultivars were growth but the resistant cultivars were arranged to growth between the standard susceptible ones (Figure 4). In total, ten plots of each lettuce or common bean were cultivated. Lettuce was transplanted the 13th May and cultivated until the 30th of June. Common bean was sowed the 13th of May and cultivated until the 5th of July. Plant were irrigated as needed by drip irrigation and fertilized with 130 NH₄NO₃, 81 KNO₃, MgSO₄ and NH₄H₂PO₄ grams per plant by fertirrigation 2 or 3 times per week depending on the needs.

![Figure 3. Distribution of plots and subplots in Agròpolis plastic greenhouse. Nomenclature and distribution of bean (yellow) and lettuce (green) subplots along the plot F.](image-url)
The soil temperatures and water content of the soil at 15 cm depth were recorded daily at 30 min intervals through temperature probes STM (Decagon devices Inc.).

The experiment finished after the accumulation of 1121 °C on lettuce and 1120.3 °C on bean, on the basis that the base temperature it was 0°C, plants were uprooted with a pitchfork, and the galling index was assessed in a 0 to 10 scale (Zeck, 1971). The roots were washed, gently dried, weighted and chopped in 2 cm long fragments. In addition to the eggs extraction from roots to calculate the RI and the assignation of host suitability as previously described; were determined the multiplication rate of the nematode in one generation \((Pf/Pi)\), the maximum rate of multiplication \((a)\) (Seinhorst, 1970), the maximum nematode density of the crop \((M)\) and the equilibrium density \((E)\), calculated according to the equation \(M = aE / (a - 1)\) (Schomaker and Been, 2006).
3.4. Statistical analysis

Statistical analysis was performed using SAS V9.0 (SAS institute Inc. Cary, NC) or JMP V 8. Data of root weight, gall index, and eggs per plant were transformed when needed using the best transformation from the Box-Cox transformation command from JMP to normalize them. All data from each crop were compared between cultivars per nematode population to determine the level of resistance, as well as between nematode populations per cultivar to know the putative variability due to the genetic background of the nematode population. When the analysis of variance was significant, means were separated by Tukey-Kramer HSD method.
4. RESULTS

Screening lettuce against *Meloidogyne* populations in pot experiments and *M. incognita* in plastic greenhouse.

The lettuce cultivars Salinas 88 and Grand Rapids responded as resistant against 11 RKN populations after one nematode generation in pot test. The number of eggs per plant on those cultivars ranged from 2.9 to 6.8% of those eggs produced on the susceptible cv. Regina 71. In addition, the number of eggs produced on cv. Salinas 88 was a 42% of those produced on cv. Grand Rapids (Table 4).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fresh root weight (g)</th>
<th>Number of eggs plant</th>
<th>RI (%)</th>
<th>RL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand Rapids</td>
<td>5.32 ± 0.21</td>
<td>311 ± 50 B</td>
<td>6.8%</td>
<td>R</td>
</tr>
<tr>
<td>Salinas 88</td>
<td>5.67 ± 0.38</td>
<td>132 ± 20 C</td>
<td>2.9%</td>
<td>R</td>
</tr>
<tr>
<td>Regina 71</td>
<td>7.64 ± 0.56</td>
<td>4552 ± 1061 A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± standard error of 110 replicates. Data in the same column followed by different letter are significantly different (*P* < 0.05) according to the Tukey-Kramer HSD test. 

*Resistance index:* number of eggs per plant on the resistant cultivar as percentage of those on the resistant. 

*Resistance level:* HR= highly resistant (RI < 1%), R=resistant (1 ≤ RI ≤ 10%), MR=moderately resistant (10 < RI ≤ 25%), SL=slightly resistant (25 < RI ≤ 50%) or S=susceptible (RI > 50%) according to the categorization of Hadisoeganda and Sasser (1982).

The response of the lettuce cultivars varied according the nematode population (Table 5). Both Grand Rapids and Salinas 88 cultivars responded as resistant against 10 out 11 RKN populations assessed. They were only susceptible to the RKN population Curas who reproduced poorly on the susceptible cv. Regina 71. In general, the Grand Rapids cultivar was less resistant than the Salinas 88 according to the frequency of resistance levels. That is, Grand Rapids responded as resistant,
moderately resistant, or slightly resistant against a 36.4, 36.4 or 18.2 % of the RKN populations, respectively, whilst Salinas 88 responded as highly resistant, resistant or moderately resistant against the 27.3, 36.4 and 27.3 % of the RKN populations, respectively.
Germplasm identification of bean (*Phaseolus vulgaris* L.) and lettuce (*Lactuca sativa* L.) resistant to avirulent populations of *Meloidogyne* spp. to *M. isca*.

Data are mean ± standard error of 10 replicates of each combination cultivar – RKN population. Data in the same row for eggs per plant followed by different uppercase letter are significantly different (*P* < 0.05) according to the Tukey-Kramer HSD test indicating differences between lettuce cultivars per a given nematode population. Data in the same column followed by different lowercase letter are significantly different (*P* < 0.05) according to the Tukey-Kramer HSD test indicating differences between nematode populations for a given lettuce cultivar. *a* Resistance index: number of eggs per plant on the resistant cultivar as percentage of those on the resistant; Resistance level: HR= highly resistant (RI < 1%), R=resistant (1 ≤ RI ≤ 10%), MR=moderately resistant (10 < RI ≤ 25%), SL=slightly resistant (25 < RI ≤ 50%) or S=susceptible (RI > 50%) according to the categorization of Hadisoeganda and Sasser (1982).

**Table 5. Number of egg per plant, resistance index (RI) and resistance level (RL) of the resistant lettuce cultivars Grand Rapids and Salinas 88, and the susceptible Regina 71 against 11 *Meloidogyne* populations after 52 days to be inoculated with 500 J2 per plant**

<table>
<thead>
<tr>
<th><em>Meloidogyne</em> population</th>
<th>Eggs plant&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Regina 71</th>
<th>Grand rapids</th>
<th>Salinas 88</th>
<th>Grand rapids</th>
<th>Salinas 88</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adra</td>
<td>2571 ± 903 A&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>93 ± 23 B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>311 ± 121 B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3,6 R</td>
<td>12,1 MR</td>
<td></td>
</tr>
<tr>
<td>Al05</td>
<td>2046 ± 404 A&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>785 ± 259 A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107 ± 29 B&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>38,4 SR</td>
<td>5,2 R</td>
<td></td>
</tr>
<tr>
<td>Amat</td>
<td>30862 ± 8613 A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>576 ± 273 B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81 ± 33 B&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1,9 R</td>
<td>0,3 HR</td>
<td></td>
</tr>
<tr>
<td>Curas</td>
<td>128 ± 84 A&lt;sup&gt;d&lt;/sup&gt;</td>
<td>217 ± 47 A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>178 ± 36 A&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>168,8 S</td>
<td>138,4 S</td>
<td></td>
</tr>
<tr>
<td>MJ05</td>
<td>3310 ± 1058 A&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>533 ± 233 B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>148 ± 117 B&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16,1 MR</td>
<td>4,5 R</td>
<td></td>
</tr>
<tr>
<td>MJAl101</td>
<td>5554 ± 2682 A&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>154 ± 85 B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>45 ± 14 B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2,8 R</td>
<td>0,8 HR</td>
<td></td>
</tr>
<tr>
<td>MJAl09</td>
<td>461 ± 253 A&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>141 ± 65 B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55 ± 15 B&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>30,5 SR</td>
<td>12,0 MR</td>
<td></td>
</tr>
<tr>
<td>MJAl10</td>
<td>700 ± 372 A&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>102 ± 43 B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>52 ± 30 B&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14,6 MR</td>
<td>7,5 R</td>
<td></td>
</tr>
<tr>
<td>P Almeria</td>
<td>1010 ± 821 A&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>154 ± 67 A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>185 ± 84 A&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>15,3 MR</td>
<td>18,3 MR</td>
<td></td>
</tr>
<tr>
<td>P Murcia</td>
<td>4040 ± 2040 A&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>564 ± 453 A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>193 ± 40 A&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14,0 MR</td>
<td>4,8 R</td>
<td></td>
</tr>
<tr>
<td>Viator</td>
<td>8069 ± 3820A&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>321 ± 125 B&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72 ± 20 C&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4,0 R</td>
<td>0,9 HR</td>
<td></td>
</tr>
</tbody>
</table>

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UPC - BarcelonaTech
In the plastic greenhouse experiment, $Pi$ ranged from 218 to 7.613 J2 per 500 cm$^3$ of soil in plots cropped with lettuce. At high $Pi$, most of the plants of the susceptible cv. Regina 71 died, and then a galling index of 10 was considered for statistical analysis. The lettuce cultivars Grand Rapids and Salinas 88 responded as slight resistant and moderately resistant, respectively (Table 6).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Galling Index</th>
<th>Root weight</th>
<th>Eggs plant$^{-1}$ (x10$^3$)</th>
<th>RI (%)</th>
<th>RL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grand Rapids</td>
<td>3.2 ± 0.4 B</td>
<td>9.8 ± 0.7 B</td>
<td>81.4 ± 12.9 B</td>
<td>33.1</td>
<td>SR</td>
</tr>
<tr>
<td>Salinas 88</td>
<td>2.8 ± 0.2 B</td>
<td>9.2 ± 0.9 B</td>
<td>25.9 ± 10.7 C</td>
<td>10.5</td>
<td>MR</td>
</tr>
<tr>
<td>Regina 71</td>
<td>6.1 ± 0.3 A</td>
<td>23.7 ± 1.8 A</td>
<td>245.7 ± 27.6 A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± standard error of 40 replicates for Regina 71, 20 replicates for Grand Rapids and 20 for Salinas 88. Data in the same column followed by different letter are significantly different ($P < 0.05$) according to the Tukey-Kramer HSD test. $^a$ Resistance index: number of eggs per plant on the resistant cultivar as percentage of those on the resistant. $^b$ Resistance level: HR= highly resistant (RI < 1%), R= resistant (1 ≤ RI ≤ 10%), MR=moderately resistant (10 < RI ≤ 25%), SL=slightly resistant (25 < RI ≤ 50%) or S=susceptible (RI > 50%) according to the categorization of Hadisoeganda and Sasser (1982).

The maximum multiplication rate of $M. incognita$ occurred at the lowest $Pi$ (218 J2 per 250 cm$^3$ of soil) in all lettuce cultivars. However, on the cultivars Salinas 88 and Grand Rapids $a$ was 2 and 27.6 % respectively, on the susceptible cv. Regina 71. The equilibrium density on the resistant cultivars Salinas 88 and Grand Rapids was 21.3 and 32.4 % on the susceptible cv. Regina 71, respectively (Table 7; Figure 6).
Germplasm identification of bean (*Phaseolus vulgaris* L.) and lettuce (*Lactuca sativa* L.) resistant to avirulent populations of *Meloidogyne* spp. to *Mi* gene

Table 7. Maximum multiplication rate (*a*), maximum population density (*M*) and equilibrium density (*E*) of *Meloidogyne incognita* on the resistant lettuce cv. Grand Rapids and Salinas 88 and on the susceptible cv. Regina 71, 50 days after transplanting on plastic greenhouse.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>a</th>
<th>E</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regina 71</td>
<td>305812</td>
<td>511033</td>
<td>511035</td>
</tr>
<tr>
<td>Gran rapids</td>
<td>84430</td>
<td>165578</td>
<td>165580</td>
</tr>
<tr>
<td>Salinas 88</td>
<td>5988</td>
<td>108802</td>
<td>108820</td>
</tr>
</tbody>
</table>

Figure 6. Relationship between initial (*Pi*) and final (*Pf*) population densities of *M. incognita* on the resistant lettuce cultivars Grand Rapids (GRP) and Salinas 88 (SAL) and on the susceptible cv. Regina 71 (REG) 50 days after transplanting on plastic greenhouse.
Screening common bean against *Meloidogyne* populations in pot experiments and against *M. incognita* in plastic greenhouse.

The common bean cv. Aporé responded as resistant considering the mean reproduction of 11 RKN populations after one nematode generation in pot test, whilst Macarrão Atibaia and Ouro Negro responded as slightly resistant, in which RKN produced 29.4 and 39.7% more eggs per plant than on cv. Aporé. The common bean cv. Cornell 49242 was susceptible because the mean of RKN reproduction was 64.5% on the susceptible cv. Bolinha. (Table 8).

Table 8. Fresh root weight, number of egg per plant, resistance index (RI) and resistance level (RL) of the resistant common bean cultivars Aporé, Macarrão Atibaia and Ouro Negro, the susceptible Bolinha, and the unknown resistance Cornell 49242, against all 11 *Meloidogyne* populations after 53 days to be inoculated with 1000 J2 per plant

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Fresh root weight (g)</th>
<th>Number of eggs plant (^{-1})</th>
<th>RI (%)(^a)</th>
<th>RL(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aporé</td>
<td>8,43 ± 0,49</td>
<td>8548 ± 5211,36 D</td>
<td>7,50%</td>
<td>R</td>
</tr>
<tr>
<td>Bolinha</td>
<td>9,74 ± 0,44</td>
<td>15236 ± 3225,7A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cornell 49242</td>
<td>10,23 ± 0,73</td>
<td>1953 ± 850,1 AB</td>
<td>64,50%</td>
<td>S</td>
</tr>
<tr>
<td>Macarrão Atibaia</td>
<td>7,58 ± 0,82</td>
<td>229 ± 98,57 C</td>
<td>32,40%</td>
<td>SR</td>
</tr>
<tr>
<td>Ouro Negro</td>
<td>7,75 ± 0,6</td>
<td>119 ± 90,17 BC</td>
<td>47,20%</td>
<td>SR</td>
</tr>
</tbody>
</table>

Data are mean ± standard error of 550 replicates. Data in the same column followed by different letter are significantly different (\(P < 0,05\)) according to the Tukey-Kramer HSD test. \(^a\) Resistance index: number of eggs per plant on the resistant cultivar as percentage of those on the resistant. \(^b\) Resistance level: HR= highly resistant (RI < 1%), R=resistant (1 ≤ RI ≤ 10%), MR=moderately resistant (10 < RI ≤ 25%), SL=slightly resistant (25 < RI ≤ 50%) or S=susceptible (RI > 50%) according to the categorization of Hadisoeganda and Sasser (1982).

The response of the common bean cultivars against nematode populations differed. The cv. Aporé responded as highly resistant and resistant to 8 out 11 RKN populations, but cv. Macarrão Atibaia responded as highly resistant and resistant to 3 RKN populations whilst Ouro Negro as resistant to 3 RKN populations. All three cultivars responded as susceptible to the population *P Murcia*, mainly due to the low reproduction of the RKN population on the susceptible cv. Bolinha. In addition, the RKN population *Adra* was able to reproduce on the resistant cultivars that responded as susceptible or slight resistant (Table 9).
Table 9. Number of egg per plant, resistance index (RI) and resistance level (RL) of the resistant cultivars common bean cultivars Aporé, Macarrão Atibaia and Ouro Negro, the susceptible Bolinha, and the unknown resistance Cornell 49242, against 11 Meloidogyne populations after 53 days to be inoculated with 1000 J2 per plant.

<table>
<thead>
<tr>
<th>Meloidogyne population</th>
<th>Bolinha</th>
<th>Aporé</th>
<th>Cornell 49242</th>
<th>Macarrão Atibaia</th>
<th>Ouro Negro</th>
<th>Aporé</th>
<th>Cornell 49242</th>
<th>Macarrão Atibaia</th>
<th>Ouro Negro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adra</td>
<td>$5119 \pm 1105 \text{ A ab}$</td>
<td>$8548 \pm 5211 \text{ A a}$</td>
<td>$11482 \pm 7138 \text{ A abc}$</td>
<td>$2956 \pm 2522 \text{ A a}$</td>
<td>$3418 \pm 1960 \text{ A abc}$</td>
<td>$166,97 \text{ S}$</td>
<td>$224,28 \text{ S}$</td>
<td>$57,74 \text{ S}$</td>
<td>$66,77 \text{ S}$</td>
</tr>
<tr>
<td>AI05</td>
<td>$10686 \pm 4560 \text{ A ab}$</td>
<td>$97 \pm 31 \text{ B c}$</td>
<td>$5944 \pm 3596 \text{ A abc}$</td>
<td>$2420 \pm 953 \text{ A a}$</td>
<td>$3426 \pm 2533 \text{ A abcd}$</td>
<td>$0,91 \text{ HR}$</td>
<td>$55,62 \text{ S}$</td>
<td>$22,65 \text{ MR}$</td>
<td>$32,06 \text{ SR}$</td>
</tr>
<tr>
<td>Amat</td>
<td>$9842 \pm 4304 \text{ A ab}$</td>
<td>$1953 \pm 850 \text{ B ab}$</td>
<td>$26606 \pm 10567 \text{ A ab}$</td>
<td>$1532 \pm 949 \text{ B ab}$</td>
<td>$350 \pm 289 \text{ B d}$</td>
<td>$19,84 \text{ MR}$</td>
<td>$270,33 \text{ S}$</td>
<td>$15,56 \text{ MR}$</td>
<td>$3,55 \text{ R}$</td>
</tr>
<tr>
<td>Curas</td>
<td>$29989 \pm 19053 \text{ A ab}$</td>
<td>$229 \pm 98 \text{ C c}$</td>
<td>$3025 \pm 1287 \text{ AB bc}$</td>
<td>$858 \pm 549 \text{ BC ab}$</td>
<td>$1984 \pm 401 \text{ AB abc}$</td>
<td>$0,76 \text{ HR}$</td>
<td>$10,09 \text{ MR}$</td>
<td>$2,86 \text{ R}$</td>
<td>$6,62 \text{ R}$</td>
</tr>
<tr>
<td>MJ05</td>
<td>$3904 \pm 2188 \text{ A ab}$</td>
<td>$119 \pm 90 \text{ B c}$</td>
<td>$15212 \pm 6960 \text{ A ab}$</td>
<td>$2238 \pm 2038 \text{ A a}$</td>
<td>$10044 \pm 7943 \text{ A a}$</td>
<td>$3,06 \text{ R}$</td>
<td>$389,63 \text{ S}$</td>
<td>$57,33 \text{ S}$</td>
<td>$257,27 \text{ S}$</td>
</tr>
<tr>
<td>MJAl01</td>
<td>$5466 \pm 1842 \text{ A ab}$</td>
<td>$139 \pm 74 \text{ B c}$</td>
<td>$1956 \pm 1725 \text{ AB ab}$</td>
<td>$8240 \pm 7022 \text{ A a}$</td>
<td>$12672 \pm 9991 \text{ A a}$</td>
<td>$2,54 \text{ R}$</td>
<td>$35,79 \text{ SR}$</td>
<td>$150,74 \text{ S}$</td>
<td>$231,82 \text{ S}$</td>
</tr>
<tr>
<td>MiAl09</td>
<td>$2743 \pm 664 \text{ A b}$</td>
<td>$115 \pm 39 \text{ B c}$</td>
<td>$1120 \pm 436 \text{ A abc}$</td>
<td>$7391 \pm 3938 \text{ A a}$</td>
<td>$597 \pm 549 \text{ B cd}$</td>
<td>$4,18 \text{ R}$</td>
<td>$40,83 \text{ S}$</td>
<td>$269,42 \text{ MR}$</td>
<td>$21,76 \text{ MR}$</td>
</tr>
<tr>
<td>MiAl30</td>
<td>$16380 \pm 4555 \text{ A ab}$</td>
<td>$72 \pm 26 \text{ C c}$</td>
<td>$13901 \pm 6803 \text{ A abc}$</td>
<td>$2177 \pm 1027 \text{ B a}$</td>
<td>$8142 \pm 3860 \text{ AB a}$</td>
<td>$0,44 \text{ HR}$</td>
<td>$84,86 \text{ S}$</td>
<td>$13,29 \text{ MR}$</td>
<td>$49,71 \text{ SR}$</td>
</tr>
<tr>
<td>P Almeria</td>
<td>$55356 \pm 10691 \text{ A a}$</td>
<td>$2249 \pm 1017 \text{ B a}$</td>
<td>$20792 \pm 9880 \text{ AB a}$</td>
<td>$7809 \pm 2168 \text{ AB a}$</td>
<td>$11366 \pm 4131 \text{ AB a}$</td>
<td>$4,05 \text{ R}$</td>
<td>$37,44 \text{ SR}$</td>
<td>$14,06 \text{ MR}$</td>
<td>$20,47 \text{ MR}$</td>
</tr>
<tr>
<td>P Murcia</td>
<td>$50 \pm 11 \text{ AB c}$</td>
<td>$282 \pm 131 \text{ AB bc}$</td>
<td>$182 \pm 26 \text{ A c}$</td>
<td>$74 \pm 39 \text{ B b}$</td>
<td>$350 \pm 101 \text{ A bcd}$</td>
<td>$565 \text{ S}$</td>
<td>$363,6 \text{ S}$</td>
<td>$147,6 \text{ S}$</td>
<td>$700 \text{ S}$</td>
</tr>
<tr>
<td>Viator</td>
<td>$13637 \pm 5620 \text{ Aab}$</td>
<td>$53 \pm 3 \text{ B c}$</td>
<td>$6626 \pm 1510 \text{ Aab}$</td>
<td>$24556 \pm 10758 \text{ A a}$</td>
<td>$34597 \pm 25449 \text{ Aab}$</td>
<td>$0,39 \text{ HR}$</td>
<td>$48,59 \text{ SR}$</td>
<td>$180,06 \text{ S}$</td>
<td>$253,69 \text{ S}$</td>
</tr>
</tbody>
</table>

Data are mean ± standard error of 10 replicates of each combination cultivar – RKN population. Data in the same row for eggs per plant followed by different uppercase letter are significantly different ($P < 0.05$) according to the Tukey-Kramer HSD test indicating differences between lettuce cultivars per a given nematode population. Data in the same column followed by different lowercase letter are significantly different ($P < 0.05$) according to the Tukey-Kramer HSD test indicating differences between nematode populations for a given lettuce cultivar. \(^a\) Resistance index: number of eggs per plant on the resistant cultivar as percentage of those on the resistant; Resistance level: HR= highly resistant (RI < 1%), R=resistant (1 ≤ RI ≤ 10%), MR=moderately resistant (10 < RI ≤ 25%), SL=slightly resistant (25 < RI ≤ 50%) or S=susceptible (RI > 50%) according to the categorization of Hadisoeganda and Sasser (1982).
In the plastic greenhouse experiment, the $Pi$ ranged from 121 to 5749 $J^2 250 \text{ cm}^3$ of soil. The common bean cv. Aporé responded as resistant to *M. incognita*. The galling index and number of eggs per plant on cv. Aporé were 23 and 7.5% on the susceptible cv. Bolinha. However, cv. Ouro Negro and Macarrão Atibaia responded as slightly resistant and susceptible (Table 10).

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Galling index</th>
<th>Root weight (g)</th>
<th>Number of eggs plant$^{-1}$</th>
<th>Resistance index (%)</th>
<th>Resistance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aporé</td>
<td>1,1 ± 0,31 C</td>
<td>7,2 ± 0,75 B</td>
<td>57084 16921 C</td>
<td>7,5</td>
<td>R</td>
</tr>
<tr>
<td>Macarrão Atibaia</td>
<td>3,36 ±0,62 AB</td>
<td>17,63 ± 1,3 A</td>
<td>623073 ± 132968 A</td>
<td>81,9</td>
<td>S</td>
</tr>
<tr>
<td>Ouro Negro</td>
<td>2,78 ± 0,81 B</td>
<td>9,46 ± 0,91 B</td>
<td>269449 ± 82684 A</td>
<td>35,4</td>
<td>SR</td>
</tr>
<tr>
<td>Bolinha</td>
<td>4,68 ± 0,41 A</td>
<td>16,73 ± 1,05 A</td>
<td>760886 ± 115092 A</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± standard error of 30 replicates of Bolinha and 10 replicates of Aporé, Macarrão Atibaia and Ouro Negro. Data in the same column followed by different letter are significantly different ($P < 0,05$) according to the Tukey-Kramer HSD test. *Resistance index: number of eggs per plant on the resistant cultivar as percentage of those on the resistant.* †Resistance level: HR= highly resistant ($RI < 1\%$), R=resistant ($1 \leq RI \leq 10\%$), MR=moderately resistant ($10 < RI \leq 25\%$), SL=slightly resistant ($25 < RI \leq 50\%$) or S=susceptible ($RI > 50\%$) according to the categorization of Hadisoeganda and Sasser (1982).

The maximum multiplication rate of *M. incognita* occurred at the lowest $Pi$ (181 $J^2$ per 250 cm$^3$ of soil) in all common bean cultivars. However, on cultivars Aporé, Macarrão Atibaia, and Ouro Negro $a$ was 11, 70 and 11 %on the susceptible cv. Bolinha, respectively. The equilibrium density on the resistant cultivars Aporé, Macarrão Atibaia, and Ouro Negro was 10, 75 and 32 % on the susceptible cv Bolinha, respectively (Table 11; Figure 7).
Table 11. Maximum multiplication rate (a), Maximum population density (M) and equilibrium density (E) of *Meloidogyne incognita* on the resistant common bean cultivars Aporé, Macarrão Atibaia and Ouro Negro, and the susceptible Bolinha after 58 days of sowing in plastic greenhouse.

Eggs + J2 per 250 cm$^3$ of soil

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>a</th>
<th>E</th>
<th>M</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aporé</em></td>
<td>33500</td>
<td>160995</td>
<td>161000</td>
</tr>
<tr>
<td><em>Macarrão Atibaia</em></td>
<td>206500</td>
<td>1164594</td>
<td>1164600</td>
</tr>
<tr>
<td><em>Ouro Negro</em></td>
<td>32000</td>
<td>496424</td>
<td>496440</td>
</tr>
<tr>
<td><em>Bolinha</em></td>
<td>293650</td>
<td>1560973</td>
<td>1560978</td>
</tr>
</tbody>
</table>

Figure 7. Relationship between initial ($P_i$) and final ($P_f$) population densities of *M. incognita* on the resistant common bean cultivars Aporé (APO), Macarrão Atibaia (MAT) and Ouro Negro (ONO), and the susceptible Bolinha (BOL) after 58 days of sowing in plastic greenhouses.
Evaluation *Meloidogyne incognita* density on plastic greenhouse soil

The \( P_i \) values for the cropping cycle ranged from 218 to 7.613 \( J_2 \) per 250 cm\(^3\) of soil. The results obtained from the greenhouse experiment were analysed and it significant differences were not found in relation to the cross interaction between the response of the cultivars and the nematode \( P_i \). This fact does not exclude the significance of the results in relation to the resistance – susceptibility of the cultivars in front different densities of \( J_2 \) RKN.

Table 12. Density of RKN (\( P_i \)) found in each plot of the assay from the extraction of \( J_2 \) from 250 cm\(^3\) of soil by Baermann trays (Whitehead and Hemming, 1965).

<table>
<thead>
<tr>
<th>Left subplots</th>
<th>RKN ( P_i )</th>
<th>Right Subplots</th>
<th>RKN ( P_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>F10E</td>
<td>1.420</td>
<td>F10D</td>
<td>3.210</td>
</tr>
<tr>
<td>F9E</td>
<td>952</td>
<td>F9D</td>
<td>4.730</td>
</tr>
<tr>
<td>F8E</td>
<td>7.613</td>
<td>F8D</td>
<td>445</td>
</tr>
<tr>
<td>F7E</td>
<td>2.797</td>
<td>F7D</td>
<td>1.351</td>
</tr>
<tr>
<td>F6E</td>
<td>4.652</td>
<td>F6D</td>
<td>766</td>
</tr>
<tr>
<td>F5E</td>
<td>3.887</td>
<td>F5D</td>
<td>5.749</td>
</tr>
<tr>
<td>F4E</td>
<td>604</td>
<td>F4D</td>
<td>1.653</td>
</tr>
<tr>
<td>F3E</td>
<td>547</td>
<td>F3D</td>
<td>181</td>
</tr>
<tr>
<td>F2E</td>
<td>218</td>
<td>F2D</td>
<td>630</td>
</tr>
<tr>
<td>F1E</td>
<td>372</td>
<td>F1D</td>
<td>1.649</td>
</tr>
</tbody>
</table>

Data expressed in \( J_2 \) per cm\(^3\) of soil. Green cells (F8E, F7E, F5E, F4E, F2E, F9D, F8D, F7D, F6D and F1D) correspond to plots where lettuce experiment was placed. White cells (F10E, F9E, F6E, F3E, F1E, F10D, F5D, F4D, F3D and F2D) correspond to the plots where common beans where seeded for the experiment.
5. DISCUSSION

The results obtained in this study have confirmed the resistance of the lettuce cv. Grand Rapids and Salinas 88 to RKN populations from Spain as it was previously reported against several populations of *Meloidogyne incognita* and *Meloidogyne javanica* from Brazil (Gomes et al., 2000; Wilcken et al., 2005; Silva et al., 2008; Carvalho Filho et al., 2011). The values of RI categorize both cultivars as highly resistant (RI ≤ 10%). Melo (2011) reported resistance of both cultivars against *Meloidogyne enterolobii*. The genetic nature of the resistance in both cultivars is under study. It seems that resistance of cv. Salinas 88 may be controlled by a single locus and its action may be influenced by additional modifier genes (Carvalho Filho et al., 2008).

Both lettuce cultivars responded as susceptible against the *Curas* population, probably due to a low reproduction on the susceptible cv. Regina 71. The reproductive capability of this RKN population on cv. Regina 71 was around 2.8% than the rest of RKN populations used in this study. Several studies have been reported variability on the reproductive ability between RKN populations on a given plant species (Castagnone-Sereno, 2002; Robers, 1995, Sasser et al., 1984). Thus, this variability justify this kind of studies because allows to know the frequency at which a given germplasm will respond as resistant or susceptible in field conditions.

In plastic greenhouse conditions in which lettuce cultivars were submitted to a gradient of *Pi*, Salinas 88 maintained its resistance, but cv. Grand Rapids responded as slightly resistant, according to the RI values, as well as the relative maximum multiplication rate (*a*). Consequently, the optimal use of these lettuce cultivars in field conditions has to consider the nematode densities at planting to suppress nematode reproduction at levels that do not cause yield losses to the following crop.

About cv. Grand Rapids, Maluf et al. (2002) reported that the resistance is due predominantly to additive genes, and has incomplete penetrance and variable expressivity. In comparison to Grand Rapids, Salinas 88 resistance gene has partial dominance and seems to have an n-allelism of the major genes controlling resistance (Carvalho Filho et al., 2008).
Germplasm identification of bean (*Phaseolus vulgaris* L.) and lettuce (*Lactuca sativa* L.) resistant to avirulent populations of *Meloidogyne* spp. to *M. incognita*

Regarding the common bean cultivars, the results of this study has confirmed the resistance of the cv. Aporé reported previously against several populations of *M. incognita* races 1 and 3, and *M. javanica* from Brazil (Ferreira *et al.*, 2010; Ferreira *et al.*, 2012). In addition, common bean cv. Aporé has also been reported as resistant to *M. enterolobii* (Melo *et al.*, 2011) and *M. chitwoodi* (Oliveira *et al.*, 2015).

Common bean cv. Ouro Negro and Macarrão Atibaia responded as slightly resistant considering the mean reproduction of all the assessed RKN populations (47.2 and 32.4 % respectively). Against *Meloidogyne enterolobii*; cv. Macarrão Atibaia responded as susceptible and cv. Ouro Negro as slightly resistant. For *M. incognita* races 1 and 3, and *M. javanica*, results by Melo *et al.* (2011) described the cultivars as moderate and slightly resistant. The common bean cv. Cornell 49242 was susceptible to the majority of the RKN populations. Despite this bean cultivar is used as source of resistance to powdery mildew and other air-borne fungal disease (Trabanco *et al.*, 2012; Pérez-Vega *et al.*, 2012), it is not resistant to RKN. The specific response of these cultivars to specific RKN populations differed, but in most cases they responded from slight resistant to susceptible. Thus, the specificity of the resistance to a given RKN population reported by Ferreira *et al.* (2010), Melo *et al.* (2011) and Oliveira *et al.* (2015) has been confirmed. The low reproduction of the RKN *P. Murcia* on the susceptible cv. Bolinha was the cause of the susceptible response of all the common bean cultivars. It needs some more studies to confirm the poor reproduction of the RKN on common bean.

In the plastic greenhouse experiment, the cv. Aporé and Ouro Negro responded as resistant and slightly resistant according to the relative maximum multiplication rate (*a*) values, 11 % for both cultivars. However, equilibrium rates were 10 and 32% respectively. This means that Aporé has limiting factors to the nematode reproduction what is interesting for future research for classic genetic improvements. Macarrão Atibaia cv. responded as susceptible in front the different RKN densities, with a maximum multiplication rate and equilibrium density of 70 and 75% on the susceptible cv. Bolinha.
Conclusions

- The resistance of lettuce cv. Grand Rapids and Salinas 88 in front of *Meloidogyne incognita* and *Meloidogyne javanica* populations has been confirmed in pot and plastic greenhouse conditions. However, the resistance of cv. Grand Rapids is affected by the nematode density at transplanting.

- The green bean cultivar Aporé is resistant in front the most *M. incognita* and *M. javanica* populations assessed. The resistant response of cv. Macarrão Atibaia and Ouro Negro are less frequent and less intense than on cv. Aporé, in fact, the resistance is more dependent of the RKN population. The cv. Cornell 49242 is susceptible to the majority of RKN populations. In plastic greenhouse conditions, Aporé is the only cultivar able to effectively suppress the nematode reproduction irrespective of the *Pi*, whilst cv. Ouro Negro reduce its effectiveness at increasing *Pi*.
Germplasm identification of bean (Phaseolus vulgaris L.) and lettuce (Lactuca sativa L.) resistant to avirulent populations of Meloidogyne spp. to Mi gene

Bibliography


Germplasm identification of bean (Phaseolus vulgaris L.) and lettuce (Lactuca sativa L.) resistant to avirulent populations of Meloidogyne spp. to Mi gene


6. Annex 1 – Lettuce and bean

Lettuce (*Lactuca sativa* L.)

The origin of lettuce remains uncertain, some experts accept that it comes from India, but many botanists *Lactuca serriola* L. could be the wild ancestor from North America (Mallar, 1978). Lettuce has been cultivated since 2.500 years ago, being the Romans and Greeks the first to use it (Sasias *et al.*, 2011).

Lettuce is an autogamy and annual plant belonging to the *Compositae* family. The roots are not longer than 25 cm, pivoting and with secondary roots. The leaves are distributed in rose, the first are separated in some varieties remaining in this position for all the development (as Romana) and others shape a heart.

The improvement of lettuce has been focused mainly on commercial characteristics and productivity. However, in recent years, due to the occurrence of several diseases, the incorporation of genes for resistance to different pathogens has been sought, especially resistance to Lettuce Mosaic Virus (LMV), *Bremia lactucae* and root-knot nematodes *Meloidogyne incognita* and *Meloidogyne javanica* (Krause-sakate *et al.*, 2001) among others.

Bean (*Phaseolus vulgaris* L.)

Bean comes from America ensured by archaeology discoveries and botanical evidences. This species arrived to Europe and Spain at the beginning of 16th century.

They belong to Fabaceae family and is an annual plant. The root system is shallow with a wide branching of secondary roots. The stalk can grow from 30 cm to 2 or 3 meters. The flowers can present a wide variety of colours, but is usually to find white flowers in the most common cultivars. The fruit is a legume, which shape, colour and size can change depending on the variety (Sasias *et al.*, 2011).

Regarding the occurrence of diseases, the incorporation of genes for resistance has been one of the main objectives of the improvement. In regions where temperature is high, as in the case of
lettuce, to root-knot nematode resistance (*M. incognita* and *M. javanica*) becomes an important objective due to the higher incidence of this pathogen.

Lettuce and beans are common crops in areas of the Mediterranean in which the presence of *Meloidogyne* spp. can cause losses in productivity and product quality.

Plant-parasitic nematodes (PPN) are constrained parasites. A large group are living in soil feeding plant roots but others can be found feeding aerial parts.

The majority of plant-parasitic nematodes have a worm shape with unsegmented bodies. All of them are characterized by stylets that basically, allows them to feed themselves and in some cases the penetration inside of the plant. Symptomatology is not defined because are similar to others that can be produced by other pathogens. For these reason is necessary to use specific diagnosis techniques. Their life will occur into the first 15 to 30 cm of the soil. Their development would be influenced for many different factors as humidity, temperature and aeration of the soil. The distribution of these microorganisms in cultivated soils is irregular depending on the susceptibility of the crops and the management of the soil.

PPN are classified into two orders: *Tylenchida* and *Dorylaimida*. The first one compromises the majority of them, being *Heteroderidae* the family that belong the three genus that cause the most important damage in agriculture: *Globodera*, *Heterodera* (cyst nematodes) and *Meloidogyne* (root-knot nematode) (Agrios, 2005).

The genus *Meloidogyne*, the root-knot nematodes (RKN) is the main restrain in horticulture production, producing around a 30% of crop yield losses at global level (Netscher and Sikora, 1990). In Catalonia, Spain, yield losses registered are up to 88% losses in cucumber (Giné *et al.*, 2014). The most common species in the Mediterranean areas are *M. arenaria*, *M. incognita* and *M. javanica* (Ornat and Sorribas, 2008). More than 2.000 plant species can be affected by *Meloidogyne* spp. and they cause damage to the most important crops belong to *Cucurbitaceae* (cucumber, pumpkin, melon), *Solanaceae* (tomato, potato, aubergine) and *Fabaceae* (beans, peas) families and others as lettuce or carrot.

The RKN morphology’s changes during their life cycle and it is characterized because of the presence of marked sexual dimorphism, males are longer than juveniles but they maintain the warm shape and females turn into round shape.
The life cycle begins with second stage juvenile (J2), the only infective stage of the nematode in soil. When the J2 reaches the roots, penetrate using the stylet and chemical segregations. Once the J2 are inside the root they migrate toward the vascular cylinder where it induces the feeding site. In this point, the nematode will become sedentary and will fix the feeding place transforming between 5 to 7 vegetal cells to feeding cells. The transformation occurs through the increase of metabolic activity, the number of cell organelles, and become multinucleated cells. Then the knot development will start, which will be more or less remarkable depending on the combination of the specie of *Meloidogyne* and the host plant. Once the infection is done the nematode will pass through the third (J3) and fourth (J4) juvenile stage until arrive to adult stage. In the last moult occurs the adult differentiation to males or females. In favourable conditions, the juveniles will become females that can produce fertile eggs parthenogenetically, in unfavourable the conditions juveniles will become males, which leave the plant.

The eggs of *Meloidogyne* spp. are within a gelatinous matrix lay for the female. Eggs get between 50 to 100 µm length and 20 to 50 µm width. Each egg contains a nematode that will produce the first moult with the appropriate conditions and becomes a second stage juvenile.

Figure 8. Sexual dimorphism in *Meloidogyne* spp. male (a) and female (b)
The duration of *Meloidogyne* spp. life cycle will depend on the environmental conditions, especially temperature, because they are poikilothermic organisms. The relation between temperature and time to complete a life cycle depend of the relation *Meloidogyne* species – host plant. Once the cycle is finished, new J2 will be released to the soil and they will need a suitable plant host to infect (Agrios, 2005).

Two different methods were used to carry out the PCR analysis for the identification of every population of *Meloidogyne* spp. used for the screen experiments. With the same individual tomato plants used for the collecting of inoculum, pieces from infested roots were removed and maintained at 4 ºC.

Females and egg masses were hand hatched from the galls of tomato roots, juvenile of second stage were obtained from the suspensions previously prepared for inoculum.

DNA was extracted from the samples adding glass beads (Sigma) to the tube (Eppendorf) and agitating for 5 minutes on the vortex. The amplifications were carried out in volumes of 25 µL containing 10 µL of DNA suspension in buffer TRIS pH 8.0 and 12.5 µL of Master Mix (mix of dNTPs, Taq DNA polymerase and buffer) and 2.5 µL of the corresponding primer synthesised by Operon Technologies (Alameda, CA, USA) (Zijlstra *et al.*, 2000)

**Table 13.** Nucleotide sequence of primer used for each SCAR-PCR. Pairs shown were used in PCR reactions using two primers specifically detect a single species. RAPD, specie corresponding to the sequence, name sequence of SCAR primer, size of marker of SCAR.

<table>
<thead>
<tr>
<th>RAPD marker</th>
<th>Specie identified</th>
<th>Name of SCAR primer</th>
<th>Sequence of SCAR primer</th>
<th>Size of SCAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPA-06&lt;sub&gt;1200&lt;/sub&gt;</td>
<td><em>Meloidogyne incognita</em></td>
<td>Finc</td>
<td>ctctgccAATGAGCTGTCC</td>
<td>1200</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rinc</td>
<td>ctctgccTCACATTAAG</td>
<td></td>
</tr>
<tr>
<td>OPA-01&lt;sub&gt;700&lt;/sub&gt;</td>
<td><em>Meloidogyne javanica</em></td>
<td>Fjav</td>
<td>GGTGCGCGATTGAAGACTGAGC</td>
<td>670</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rav</td>
<td>caggcccttcAGTGGAACTATAC</td>
<td></td>
</tr>
</tbody>
</table>
Other extractions were carried out by the protocol described of López-Llorca & co. (2010) with some modifications, for the DNA extraction from tomato roots.

Twenty root grams were collected from each RKN population; roots were frozen using liquid nitrogen and later were lyophilized and conserved at -20ºC until their use.

Each sample was pulverized using a porcelain pestle with liquid nitrogen. 100 µL of glass bead (Sigma) to homogenize the material. Then 6500 µL of buffer of extraction (Tris-HCl 100 mM pH 8.4; NaCl 1,4 M; EDTA 25 mM pH 7.5; cetyl trimethylammonium bromide (CTAB) at 2% and polyvinylpyrrolidone (PVP) at 2%. Roots with buffer and glass beads were hatched at 65 ºC for 1 hour with agitation. After the hatch period, 375 µL of phenol and other 375 µL of isoamyl alcohol-chloroform (24:1) were added in each tube and were centrifuged at 14000 rpm for 10 minutes. The supernatant was collected in a new tube of 1.5 mL, 375 µL of isoamyl alcohol-chloroform (24:1) were added and the mix was centrifuged again at 14000 rpm for 10 minutes. Again, the supernatant was collected in a new tube of 1.5mL where 700 µL of isopropanol were placed and the mix was centrifuged for 10 minutes at 14000 rpm. The supernatant was decanted and sample was washed two times with 500 µL of ethanol at 70% at -20 ºC. In the second washed, sample was left for 2 hours at -20 ºC for the DNA precipitation, was centrifuged at 11000 rpm for 5 minutes, the ethanol of the walls was decanted and the rests of ethanol were vaporized in gas extraction chamber.

The precipitation were dissolved in 200 µL of TNE (Tris-HCl 10 mM, EDTA 1 mM, NaCl 150 mM, pH 8.0). The extract was hatched with 1 µL of Ribonuclease A (Sigma) at 37 ºC for 30 minutes. Steps of isoamyl alcohol-chloroform previously described were repeated and 1mL of absolute ethanol at -20 ºC was added to the last supernatant, DNA precipitated during 2 hours. Then was centrifuged at 14000 rpm for 10 minutes. Ethanol was decanted and when the precipitates were completely dry, 50 µL of free nucleases water (Sigma) were added. Samples were maintained at 4 ºC to their quantification and later use. The completeness of DNA was conducted by electrophoresis in agarose gen at 0.8 %, colouring the gel with GelRed (Biotools). On the gel was included a sample of tomato DNA as control to discard the hybridization of the chains.
Figure 10. Picture of gel resulted from the PCR procedure. DNA markers of known weight (M), tomato control DNA and primers to *M. incognita* (1), DNA tomato plant inoculated with *M. incognita* and his primers (2), negative control for *M. incognita* primers (3), tomato control DNA and primers to *M. javanica* (4), DNA tomato plant inoculated with *M. javanica* and his primers (5), negative control for *M. javanica* primers (6).
9. Annex 4 – Procedures

Hussey and Barker’s method (1973)

For the collection of nematode populations were used tomato plants cv. Durinta maintained in the glasshouse of Agròpolis. After the fulfilment of two generation, tomato plants were removed from pots and the roots were washed with water. Eggs were extracted using Hussey and Barker’s method (1973) as follows, the cleaned roots were ground by a blender and macerated with NaClO 0.225% solution during 30 seconds. Then, roots were maintained in the solution for 10 minutes for tissue degradation. After that, roots were passed through 100, 200 and 500 mesh sieves. The eggs retained in the 500 mesh sieve were collected and deposited in Baermann trays to collect them. Deposit of water with the eggs suspension from the Baermann was maintained during three weeks, time for the egg hatching. Then J2 were collected and counted by using optic microscopes with an Hawksley chamber. The collections of J2 nematodes were maintained at 9ºC until their use.

The same procedure was used to the eggs extraction for the evaluation of all the assays with NaClO at 0.5%.