

Plant-Nematode co-cultures in the screening of sustainable nematicides against soil-dwelling parasitic nematodes of plants

Co-culturas planta-nemátode para a despistagem de nematicidas sustentáveis contra nemátodes fitoparasitas do solo

Jorge M. S. Faria^{1,*}, Cláudia S. L. Vicente^{1,2}, Leidy Rusinque¹,
M. João Camacho^{1,2} & Maria L. Inácio^{1,3}

¹ INIAV, I.P., Instituto Nacional de Investigação Agrária e Veterinária, Quinta do Marquês, 2780-159 Oeiras, Portugal

² MED, Mediterranean Institute for Agriculture, Environment and Development, Institute for Advanced Studies and Research, Évora University, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal

³ GREEN-IT Bioresources for Sustainability, Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa (ITQB NOVA), Av. da República 2780-157 Oeiras, Portugal

(*E-mail: jorge.faria@iniav.pt)

<https://doi.org/10.19084/rca.28556>

ABSTRACT

The diseases caused by plant parasitic nematodes are still a serious constraint to modern global crop production. An increasing number of active compounds in commercial nematicidal formulations is being banned from use by common policies of pest management. Farmer communities report a low efficiency for the replacement pesticides, which reflects on crop yield and productivity. Novel sustainable biopesticides are urgently needed to cope with global food demands while respecting the most recent environmental policies. Plant-nematode co-cultures offer a stable biotechnological screening tool able to assess the active compound's nematicidal activity and its effect on host tissues, simultaneously, in an easily accessible system that simulates natural infection. These systems are being developed and optimized at the Nematology laboratory of INIAV. Preliminary results were obtained for co-cultures of *Solanum lycopersicum* with the nematode *Meloidogyne ethiopica* and *S. tuberosum* with *Globodera pallida*. Future studies will target other plant parasitic nematodes, e.g., the root lesion nematodes (*Pratylenchus penetrans*) in transgenic roots of potato and the pinewood nematode (*Bursaphelenchus xylophilus*) on *in vitro* pine shoots (*Pinus* sp.).

Keywords: agroecosystems, biopesticides, *in vitro* cultures, nematicide screening, soil nematodes

RESUMO

As doenças causadas pelos nemátodes fitoparasitas permanecem uma considerável limitação à produção agrícola global. Um crescente número substâncias activas, utilizadas em formulações nematicidas comerciais, têm sido proibidas por políticas comuns de gestão de pragas, devido a preocupações ambientais e de saúde humana. Muitas vezes, os pesticidas de substituição demonstram uma menor eficiência no campo, o que se reflete num decréscimo do rendimento e da produtividade agrícola. O desenvolvimento de novos biopesticidas de alta eficiência para o combate de pragas e doenças das culturas é urgente e permitirá dar resposta adequada à crescente necessidade de produtos agrícolas, respeitando as novas políticas ambientais. As co-culturas planta-nemátode permitem uma despistagem simultânea da eficiência nematodocida de um composto e da sua influência nos tecidos vegetais do hospedeiro, num sistema facilmente acessível que simula a infecção natural. O laboratório de Nematologia do INIAV encontra-se a desenvolver estes sistemas e foram já obtidos resultados preliminares para as co-culturas de *Solanum lycopersicum* com o nemátode *Meloidogyne ethiopica* e *S. tuberosum* com *Globodera pallida*. Em ensaios futuros outros nemátodes fitoparasitas serão avaliados, como os nemátodes das lesões da raiz (*Pratylenchus penetrans*) em raízes transgénicas de batata e do nemátode da madeira do pinheiro (*Bursaphelenchus xylophilus*) em rebentos *in vitro* de pinheiro (*Pinus* sp.).

Palavras-chave: agroecossistemas, biopesticidas, culturas *in vitro*, despistagem de nematicidas, nemátodes do solo

INTRODUCTION

Plant parasitic nematodes (PPNs) are small roundworms that greatly affect crops in modern agriculture. They belong to the phylum Nematoda and are characterized by the presence of the stylet, a piercing mouthpart that aids in plant parasitism. Root-knot nematodes (RKN), of the genus *Meloidogyne*, and cyst nematodes (CNs), of the genus *Globodera* and *Heterodera*, are the most economically important soil-dwelling PPNs, responsible for heavy losses to farmers. Recently, the damaging effects of PPN diseases have increased dramatically, due to globalization and monoculture mass production, requiring more effective means of pest management.

Pest management is usually performed through non-chemical means, relying on natural host resistance or on cultural controls, and chemically, using nematicides. Nematicide screening is mainly performed using *in vitro* direct contact assays or *in vivo* screening. However, direct contact assays are unable to reflect phytotoxicity to the host or compound biotransformation and metabolization, while *in vivo* screening is an extremely lengthy and unpractical process, beset by environmental and genetic variations.

An innovative screening procedure was developed based on *in vitro* cultures of transgenic roots with soil PPNs (Faria *et al.*, 2014), and validated as being able to evaluate nematicidal activity and toxicity to the host, simultaneously, in an easily accessible biotechnological system (Faria *et al.*, 2015). *In vitro* co-cultures are refined screening systems, kept under controlled nutritional and environmental conditions, with defined and easily manipulated biological parameters (Faria *et al.*, 2016). Furthermore, co-cultures need fewer resources, in terms of space and time, offering additional advantages to conventional *in vivo* systems for large-scale screening of nematicidal compounds.

In the present work, *Solanum lycopersicum* and *S. tuberosum* transgenic roots were established as *in vitro* culture hosts for *Meloidogyne ethiopica* and *Globodera pallida*. These plant - parasite biotechnological systems will be used to obtain aseptic phytoparasitic nematodes in large amounts and to futuressly test biopesticide compounds.

MATERIAL AND METHODS

Tubers of *S. tuberosum* cv. Agria and fruits of *S. lycopersicum* cv. Cerasiforme were obtained locally. Samples were washed with commercial bleach 20% (v/v), for 10 min, and surface sterilized by immersion in ethanol 96% (v/v) for 10 min. Under asepsis, potatoes and tomatoes were rinsed 3 times in ultrapure sterile water, the outer 2 cm portion was removed, and the central piece divided into segments with approximately 0.5 cm of thickness.

Transgenic roots were induced by inoculating the aseptic potato or tomato sections with *Rhizobium rhizogenes* ARqual strain, according to the previously described protocol (Faria *et al.*, 2014). Transgenic roots were then maintained in semi-solid Schenk and Hildebrandt (SH) medium supplemented with 30 g L⁻¹ sucrose and 8 g L⁻¹ agar at pH = 5.6. Plates were kept at 24±1°C, in darkness, under regular subculturing.

Co-cultures were established according to Faria *et al.* (2014). For *M. ethiopica*, egg masses were handpicked from infected tomato plants from the *in vivo* nematode collection of INIAV's Nematology Lab (Rusique *et al.*, 2022). The eggs were extracted with 0.52% (v/v) sodium hypochlorite (NaOCl) solution and the debris removed with a 47% (w/v) sucrose solution. Afterwards, second stage juveniles (J2) were hatched from the eggs in moist chambers and surface sterilized in 70% ethanol for 2 min, centrifuged for 2 min at 500 g, and finally rinsed in sterile water (4 times) to eliminate traces of ethanol. For *G. pallida*, cysts were provided by the cyst nematode collection of Nematology Lab of INIAV. Cysts were extracted from the soil samples using the Fenwick can method, eggs and J2 were recovered from cysts and surface sterilized as described above. Approximately 50 *M. ethiopica* juveniles or *G. pallida* eggs were added to *S. lycopersicum* or *S. tuberosum* transgenic roots, respectively, and kept for 2 months for infection and female maturation.

RESULTS AND DISCUSSION

For *in vitro* infected tomato sections, transgenic roots began emerging from etiolated seedling hypocotyls, from the seeds that immediately

germinated of the tomato sections. After a month, transgenic roots were already evident (Figure 1A). These roots were excised and periodically subcultured (Figure 1B and C). The aseptic *M. ethiopica* J2 added were identified in the vicinity of tomato roots and after approximately 2 weeks the first indications of root galling were detected (Figure 1D).

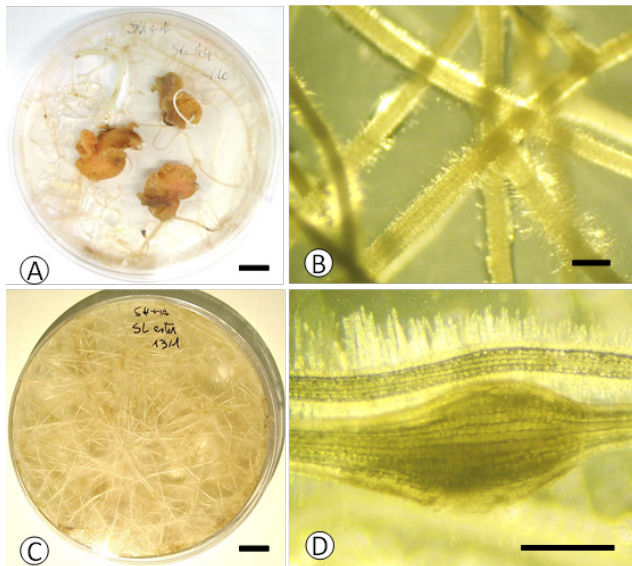


Figure 1 - (A) Visual aspect of transgenic roots developing from aseptic tomato sections, (B) microscopic aspect of a tomato transgenic root, (C) transgenic roots culture in semi-solid SH culture medium and (D) a root gall encasing a maturing *Meloidogyne ethiopica* female. Bar = 1 cm for A and C; and 1 mm for B and D.

For potato sections, transgenic roots began emerging from small cell clusters formed in the surface of the section. Within two months root primordia were discernible, and in the following weeks transgenic roots began growing throughout the culture medium (Figure 2A, B and C). The added aseptic *G. pallida* eggs hatched within the first week and J2 were observed thrusting their stylet at the root epidermis. After approximately one month the first maturing females were identified on wounds in the root (Figure 2D).

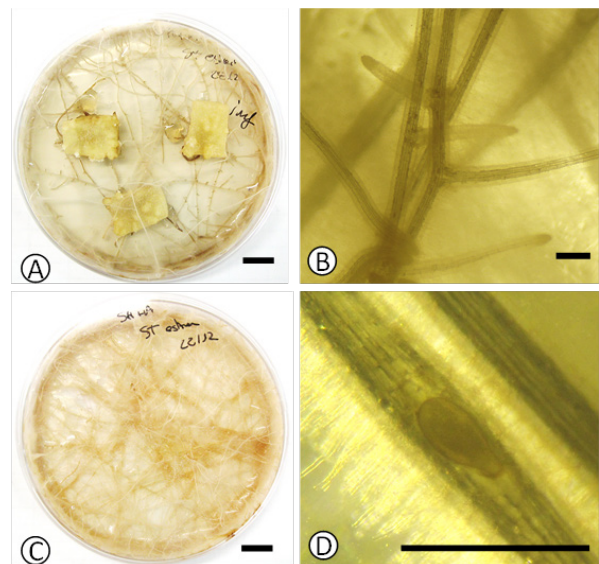


Figure 2 - (A) Visual aspect of transgenic roots developing from aseptic potato sections, (B) microscopic aspect of a potato transgenic root, (C) transgenic roots culture in semi-solid SH culture medium and (D) a maturing *Globodera pallida* female. Bar = 1 cm for A and C; and 1 mm for B and D.

CONCLUSIONS

Transgenic roots offer many advantages for plant-parasitic nematode research. At the Plant Nematology Lab of INIAV, they will be used to maintain a reference collection of PPN races, to obtain high quantities of aseptic PPNs for nematocide screening and as novel biotechnological tools to enhance procedures of biopesticides screening.

ACKNOWLEDGEMENTS

We would like to thank Dr. Esther Menéndez (Universidad de Salamanca) for providing *R. rhizogenes* ARqual strain. This research was partially funded by the Fundação para a Ciência e a Tecnologia (FCT/MCTES) through national funds, under LISBOA-01-0145-FEDER-028724 and PTDC/ASP-PLA/0197/2020 projects.

REFERENCES

- Faria, J.M.S.; Rodrigues, A.M.; Sena, I.; Moiteiro, C.; Bennett, R.N.; Mota, M. & Figueiredo, A.C. (2016) - Bioactivity of *Ruta graveolens* and *Satureja montana* Essential Oils on *Solanum tuberosum* Hairy Roots and *Solanum tuberosum* Hairy Roots with *Meloidogyne chitwoodi* Co-cultures. *Journal of Agricultural and Food Chemistry*, vol. 64, n. 40, p. 7452–7458. <https://doi.org/10.1021/acs.jafc.6b03279>
- Faria, J.M.S.; Sena, I.; Moiteiro, C.; Bennett, R.N.; Mota, M. & Figueiredo, A.C. (2015) - Nematotoxic and phytotoxic activity of *Satureja montana* and *Ruta graveolens* essential oils on *Pinus pinaster* shoot cultures and *P. pinaster* with *Bursaphelenchus xylophilus* in vitro co-cultures. *Industrial Crops and Products*, vol. 77, p. 59–65. <https://doi.org/10.1016/j.indcrop.2015.08.045>
- Faria, J.; Sena, I.; Maleita, C.; da Silva, I.; Ascensão, L.; Abrantes, I.; Bennett, R.; Mota, M. & Figueiredo, A.C. (2014) - Characterization of structure, growth and production of volatiles of *Solanum tuberosum* hairy roots and *Solanum tuberosum* hairy roots/*Meloidogyne chitwoodi* in vitro co-cultures. *Planta Medica*, vol. 80, n. 16, art. P2B85. <https://doi.org/10.1055/s-0034-1394962>
- Rusique, L.; Nóbrega, F.; Cordeiro, L.; Lima, A.; Andrade, S. & Inácio, M.L. (2022) - Root-Knot Nematode Species Associated with Horticultural Crops in the Island of Azores, Portugal. *Horticulturae*, vol. 8, n. 2, art. 101. <https://doi.org/10.3390/horticulturae8020101>