

RESEARCH/INVESTIGACIÓN

HISTOPATHOLOGICAL ANALYSIS OF ROOTS OF *VITIS VINIFERA* CULTIVAR CABERNET SAUVIGNON INFECTED WITH *MELOIDOGYNE ETHIOPICA*

María del C. Tordable*¹, Paola Lax², Juan Carlos Magunacelaya³ and Marcelo Doucet²

¹Morfología Vegetal, Universidad Nacional de Río Cuarto, 5800 Río Cuarto. Córdoba, Argentina. ²Centro de Zoología Aplicada, Universidad Nacional de Córdoba, 5000 Córdoba, Argentina. ³Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso, Chile. Corresponding author: mtordable@exa.unrc.edu.ar

ABSTRACT

Tordable, M. del C., P. Lax, J. C. Magunacelaya and M. Doucet. 2012. Histopathological analysis of roots of *Vitis vinifera* cultivar Cabernet Sauvignon infected with *Meloidogyne ethiopica*. *Nematropica* 42:276-280.

Root tissues of grapevine cv. Cabernet Sauvignon from the locality of El Huique (VI Región, Chile), parasitized by *M. ethiopica*, were analyzed to evaluate the host-parasite association. Root segments with and without galls were fixed and processed following conventional techniques for optical microscopy. Galls in central and lateral positions relative to the root axis were observed. Central galls showed increase in the diameter of the central cylinder due to the presence of hyperplastic tissue. In addition, in this zone the nematode induced the formation of giant cells that were observed at different development stages: initial, differentiated and non-functional. Giant cells were embedded in the widest rays of secondary xylem, disrupting and reducing this tissue as well as the vascular cambium. In lateral galls, the central cylinder of the roots had hyperplastic tissue containing cavities communicated with the root periphery. The histopathological reaction observed in roots showed the susceptibility of the grape cv. Cabernet Sauvignon to the local *M. ethiopica* population.

Key words: Chile, giant cell, grapevine, histopathology, *Meloidogyne ethiopica*.

RESUMEN

Tordable, M. del C., P. Lax, J. C. Magunacelaya and M. Doucet. 2012. Análisis histopatológico de raíces de *Vitis vinifera* cultivar Cabernet Sauvignon infectadas con *Meloidogyne ethiopica*. *Nematropica* 42:276-280.

Con el objeto de evaluar la asociación hospedador-parásito se analizaron tejidos radicales de vid cv. Cabernet Sauvignon, provenientes de la localidad El Huique (VI Región, Chile), parasitados por *M. ethiopica*. Trozos de raíces con y sin agallas fueron fijados y procesados siguiendo técnicas convencionales para microscopía óptica. Se observaron agallas centrales y laterales en relación al eje de la raíz. En las centrales, se detectó aumento del cilindro central debido a la presencia de tejido hiperplásico. Además, en esta zona, el nematodo indujo la formación de células gigantes que se observaron en diferentes etapas de desarrollo: inicial, diferenciada y no funcional. Las células gigantes estaban inmersas en los radios más anchos del xilema secundario provocando interrupción y reducción de ese tejido como así también del cambium vascular. En las agallas laterales, en el cilindro central, se observó tejido hiperplásico en cuyo interior se encontraron cavidades que se comunicaban con la periferia de la raíz. Las alteraciones observadas mostraron la susceptibilidad del cultivar evaluado frente a la población local de *M. ethiopica*.

Palabras clave: célula gigante, Chile, histopatología, *Meloidogyne ethiopica*, vid.

INTRODUCTION

Grape (*Vitis vinifera*) is an economically important crop in Chile. Vineyards currently cover an area of about 190,000 ha, which is expected to expand over 20% within the next 10 years. Approximately 65% of the area is devoted to the cultivation of wine grape varieties, whereas the remaining area is cultivated with table grapes and cultivars for liquor production (SAG,

2005).

Chile has ideal conditions for grape cultivation due to optimal environmental factors and minor concerns with common pests; however, problems with plant-parasitic nematodes are increasing (Aballay *et al.*, 2009). The main nematodes found in grapevines in Chile are *Xiphinema index* (Magunacelaya *et al.*, 2004), *Pratylenchus vulnus*, *Tylenchulus semipenetrans* (Di Vito *et al.*, 2009), *Mesocriconema xenoplax*, and

several species of *Meloidogyne* (Magunacelaya and Dagnino, 1999; Carneiro *et al.*, 2007). *Meloidogyne* spp. are found associated with root systems of grape plants, being especially harmful to wine grape cultivars, such as Cabernet Sauvignon, Chardonnay, Merlot and Shiraz (Aballay *et al.*, 2009). The most important species of root-knot nematodes affecting grapevine in Chile is *M. ethiopica* (Carneiro *et al.*, 2007). In recent years, the nematode has been detected in the main grapevine producing areas of the country. It is widely distributed over a *ca* 1000 km area, from Copiapó valley north of Santiago (*ca* 800 km) to Talca (*ca* 250 km) south of Santiago (Carneiro *et al.*, 2007). Chile is the only country in the world where *M. ethiopica* has been detected causing severe yield losses of grapes; it appears to be rather common especially in sandy soils (Magunacelaya, 2005; Magunacelaya, 2010). The implementation of a plant certification program appears necessary to avoid spread of the nematode across the country.

The few histopathological analyses conducted on grapevines parasitized by *Meloidogyne* spp. were focused on *M. javanica* (Doucet *et al.*, 1984; Vovlas *et al.*, 1978) and *M. incognita* (Hafez and Sundararaj, 2000; Khan *et al.*, 2009); some differences related primarily to damage in the root tissues were observed in the investigations.

The cultivar Cabernet Sauvignon is widely grown in Chile and has shown some tolerance to *M. ethiopica* (Magunacelaya, 2010). To date, this host-parasite association has not been evaluated for histopathological characteristics. Therefore, the aim of the present work was to evaluate the histological alterations induced by a population of *M. ethiopica* in the grape cultivar Cabernet Sauvignon.

MATERIALS AND METHODS

Root samples of grape cv. Cabernet Sauvignon were taken from a field naturally infested with *M. ethiopica* at El Huique, municipality of Santa Cruz (VI Región, Chile). Samples were gently washed with water to remove adhering soil particles; roots undergoing secondary growth (with and without galls) were cut (segments about 5 mm long) under a stereoscopic microscope and fixed in FAA (ethanol: glacial acetic acid: formaldehyde: water; 50:5:10:35 v/v). The roots were then dehydrated in a graded series of ethyl alcohol and xylene baths and embedded in histowax. Serial transverse and longitudinal sections 8-10 μ m thick were obtained using a rotatory microtome. Sections were stained with triple staining (hematoxylin-safranin-fast green) and mounted in Depex (Johansen, 1940; O' Brien and Mc Cully, 1981). Previously dewaxed sections were subjected to PAS (Periodic Acid - Schiff's Reaction) test and analyzed under bright field microscopy (Harris and Oparka, 1994). Photographs were taken with a Carl Zeiss Stemi SV6 stereoscopic microscope equipped with a Canon digital camera and

a Carl Zeiss Axiophot optical microscope; images were captured with an AxioCam HRC camera and processed with AxioVision 4 software.

RESULTS AND DISCUSSION

Histological sections of healthy control roots exhibited the two typical zones, cortex and central cylinder, the latter showing development of secondary vascular tissues (Fig. 1A), with wide rays of xylem and phloem parenchyma (Fig. 1B). They had periderm as protective tissue and in some samples, the epidermis was partially detached (Fig. 1A).

Macroscopic examination of infected roots showed two types of galls: a) round galls (approximately 5 mm diameter), which developed centrally with respect to the axis of the root body in different sectors along the parasitized roots (Fig. 1C) or at the tips of the roots (Fig. 1D); and b) laterally oriented and elongated (approximately 3-5 mm long) or round (3 mm diameter) galls, consisting in lateral protuberances eccentric with respect to the root axis (Fig. 1E, F).

Round central galls. The cortical zone had a few cell layers and the central cylinder diameter was significantly increased due to the presence of hyperplastic parenchymatous tissue located in the periphery of vascular tissues (Fig. 2A). Nematode feeding site establishment induced the formation of giant cells in tissues of this region (Fig. 2B). Three to 7 cells per feeding site (Fig. 2C) were embedded in the secondary xylem, especially in the widest parenchymatic rays. During their formation, feeding sites incorporated cells of xylem tissue, disrupting and reducing that tissue and vascular cambium (Fig. 2C, D). Giant cells represented initial, differentiated and non-functional stages of development. At the initial stage, cells were slightly hypertrophic (about 50 μ m along the long axis), whereas cells surrounding vascular tissues did not exceed 20 μ m in length, with an increase in the cytoplasmic density and secondary vacuolization and containing several nuclei (Fig. 2D). The differentiated giant cells reached about 150 μ m in transverse section along the long axis. They had dense, vacuolated cytoplasm, with fewer starch grains (Fig. 3A) compared with control roots (Fig. 1B). Each cell had several nuclei (with 5 to 12 nuclei observed per plane of section); the nuclei were scattered or clustered together (Fig. 2E, F). In some sectors, cell walls were thick and of irregular internal contour due to the presence of localized, digitiform wall ingrowths of several sizes. Cell walls maintained their cellulosic characteristic (PAS+), being evident in areas adjacent to xylem vessel elements (Fig. 2F) and on walls between giant cells (Fig. 3A). Non-functional cells showed progressive loss of the cytoplasm and nuclei, keeping the characteristics and chemical composition of their walls. Anterior region of females appeared completely surrounded by giant cells in some sectors (Fig. 3B). Increased volume of female body produced damage to adjacent tissues and the root

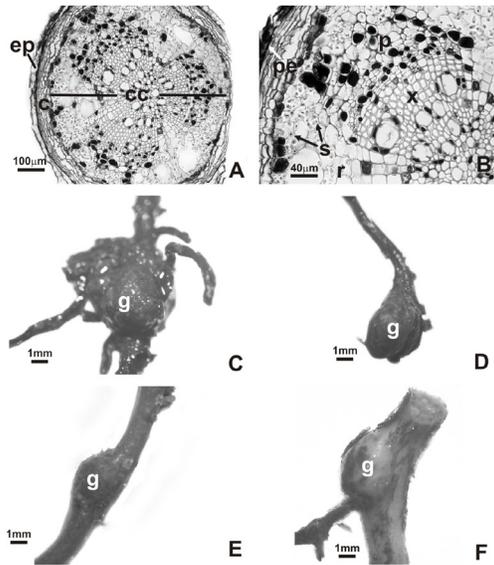


Fig. 1. Association between *Meloidogyne ethiopica* and *Vitis vinifera* cv. Cabernet Sauvignon. A: transverse section of healthy root. B: detail of A. C: round central gall. D: round terminal gall. E: elongated lateral gall. F: round lateral gall. Abbreviations. c: cortex; cc: central cylinder; ep: epidermis; g: gall; p: phloem; pe: periderm; r: rays of secondary vascular tissues; s: starch; x: xylem.

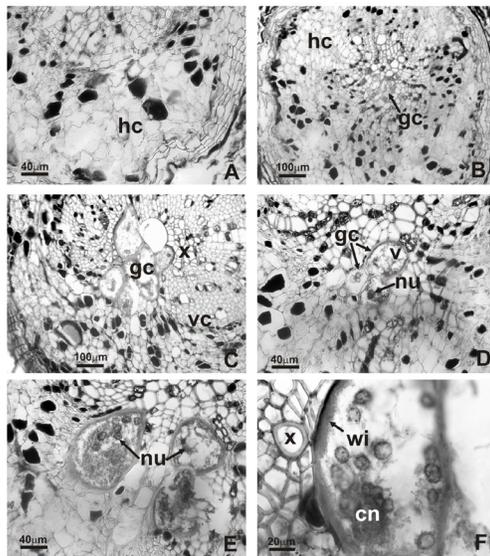


Fig. 2. *Meloidogyne ethiopica* - *Vitis vinifera* association. Transverse sections of round central galls. A: hyperplastic cells in the central cylinder. B: hyperplastic cells and giant cells in the central cylinder. C: giant cells embedded in the xylem and reduced vascular cambium. D: giant cells at initial stage of differentiation. E: differentiated giant cells. F: detail of giant cell adjacent to the xylem with wall ingrowths and clustered nuclei. Abbreviations. cn: clustered nuclei; gc: giant cell; hc: hyperplastic cells; nu: nucleus; v: vacuole; vc: vascular cambium; wi: wall ingrowths; x: xylem.

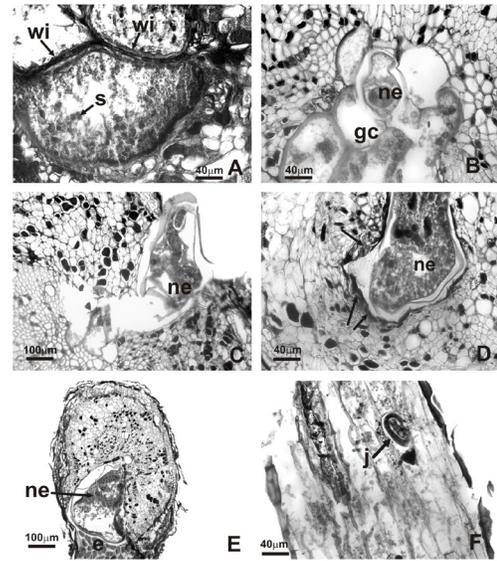


Fig. 3. *Meloidogyne ethiopica* - *Vitis vinifera* association. Transverse section of round central galls. A: detail of adjacent giant cells with wall ingrowths (PAS+). B: detail of giant cells and nematode. C: general view of a female in the gall. D: detail of crushed and broken cells (arrows) near the nematode. E: female with egg mass on the gall surface. F: juvenile on a lateral root. Abbreviations. e: eggs; gc: giant cell; j: juvenile; ne: nematode; s: starch; wi: wall ingrowths.

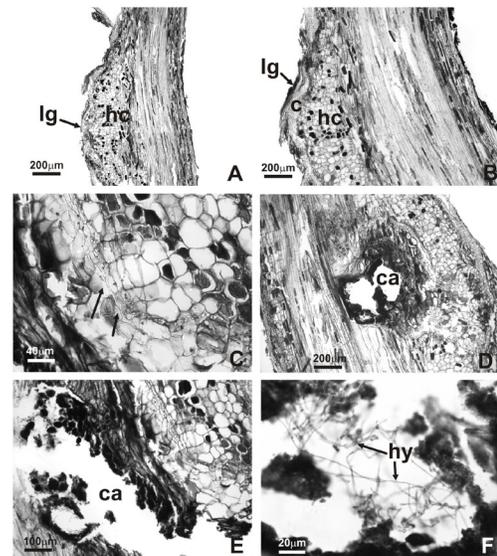


Fig. 4. *Meloidogyne ethiopica* - *Vitis vinifera* association. Transverse sections of lateral galls. A and B: view of the lateral gall with hyperplastic tissue. C: detail of alterations produced on the cortical layers (arrows). D: internal cavity in the gall. E: cavities communicated with the surface of the gall. F: detail of a sector of the cavity with fungal hyphae. Abbreviations. ca: cavity; hc: hyperplastic cells; hy: hyphae; lg: lateral gall.

in general (Fig. 3C); crushed and broken cells were observed, some of them with strengthening of cell walls (Fig. 3D). Females with egg masses were detected (Fig. 3E). Juveniles and septate fungal hyphae were observed on cortical layers of lateral roots located near the galls (Fig. 3F).

Lateral elongated or round galls. These galls showed hyperplastic parenchymatous tissue that developed in the central cylinder of the roots (Fig. 4A, B) and then moved to the periphery, to cortical layers whose cells might be crushed and broken (Fig. 4C). Cavities surrounded by cells with thick, crushed or broken walls (Fig. 4D) and communicated with the root periphery were observed in this parenchyma (Fig. 4E). No nematodes were detected in these galls. Inside the cavities analyzed, amorphous, unidentified material and branched, septate fungal hyphae were observed (Fig. 4F) and these non-functional galls may have favored the entry of other pathogens, such as the fungi detected.

Histopathological analysis of central galls showed that changes induced by the nematode in the cortex and central cylinder of parasitized roots led to the development of giant cells, reduction of vascular tissues and increase in diameter of the central cylinder due to the presence of hyperplastic parenchymatous tissue. Increased body size of females also caused crushed and broken cells in the cortex and central zone of galls. The latter characteristic agrees with findings of Hafez and Sundararaj (2000) in *V. vinifera* cv. Thompson parasitized by *M. incognita* in that anomalies in root tissues consisted mostly in the presence of hyperplastic and hypertrophied cells located in the stelar region. By contrast, in *V. vinifera* cv. Muscatel, galls produced by *M. javanica* resulted from an increase in cortical parenchyma (Doucet *et al.*, 1984).

In the present study, reduction of vascular tissues also involved the vascular cambium, affecting the formation of secondary vascular tissue. In the analysis of the hybrid VRe2 (*V. vinifera* x *V. rotundifolia*), parasitized by *M. javanica*, Vovlas *et al.* (1978) indicated that very marked alterations of the central cylinder, especially the presence of feeding sites, disrupted the root vascular tissue.

The characteristics of the differentiated giant cells: dense, vacuolated cytoplasm, with several single or clustered nuclei, is consistent with giant cells type A described in *V. vinifera* cv. Muscatel parasitized by *M. javanica* (Doucet *et al.*, 1984). Also in *V. vinifera* cv. Thompson parasitized by *M. incognita* (Hafez and Sundararaj, 2000), each cell had several nuclei located in the center or near the periphery. In *V. vinifera* cv. Muscatel parasitized by *M. javanica* (Doucet *et al.*, 1984), walls with more or less irregular thickenings were observed in the giant cells, which is in agreement with the present findings. Regarding the internal ingrowths in the giant cell walls, it should be noted that they were PAS positive, confirming the presence of total polysaccharides (Harris and Oparka, 1994). This characteristic suggests the specialization of these cells

as transfer cells in sectors adjacent to the xylem vessels (Doucet *et al.*, 2003) and between giant cells.

The wide distribution of *M. ethiopica* in Chile is probably the result of infested grapevine seedlings; nurseries are severely infected by the nematode and commercialization is allowed (Carneiro *et al.*, 2007). Therefore, examination of seedlings and soil are necessary prior to crop planting. Also, a comparison of the different cultivars and rootstocks of grapes used in Chile to evaluate their response to this nematode should be undertaken, especially of those genotypes that have shown tolerance or resistance to other *Meloidogyne* spp.

ACKNOWLEDGEMENTS

This work was financially supported by the Secretaría de Ciencia y Técnica (Universidad Nacional de Río Cuarto and Universidad Nacional de Córdoba) and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Pontificia Universidad Católica de Valparaíso.

LITERATURE CITED

- Aballay, E., P. Persson, and P. Martensson. 2009. Plant-parasitic nematodes in Chilean vineyards. *Nematopica* 39:85-97.
- Carneiro, R. M. D. G., M. R. A. Almeida, E. Cofcewicz, J. C. Magunacelaya, and E. Aballay. 2007. *Meloidogyne ethiopica*, a major root-knot nematode parasitising *Vitis vinifera* and other crops in Chile. *Nematology* 9:633-639.
- Di Vito, M., J. C. Magunacelaya, T. Ahumada, and F. Catalano. 2009. Relationships between initial population densities of *Meloidogyne ethiopica* and growth of *Vitis vinifera* grape in pots. *Nematologia Mediterranea* 37:105-108.
- Doucet, M. E., E. L. Ponce de León de, and E. Bustos. 1984. Alteraciones histológicas en *Vitis vinifera* variedad moscatel introducidas por *Meloidogyne javanica* (Treub, 1885) Chitwood, 1949. *Revista de Investigaciones Agropecuarias INTA* 19:107-113.
- Doucet, M. E., M. del C. Tordable, and E. Lorenzo. 2003. Response of the soybean cultivar Pioneer 9501 to *Heterodera glycines*. *Nematologia Mediterranea* 31:15-20.
- Hafez, S. L. and P. Sundararaj. 2000. Host parasite interaction of *Meloidogyne incognita* and *Vitis vinifera* under green house conditions. *International Journal of Nematology* 10:71-74.
- Harris, N. and K. J. Oparka. 1994. *Plant Cell Biology. A Practical Approach.* Oxford University Press Inc., New York.
- Johansen, D. A. 1940. *Plant Microtechnique.* Mc Graw-Hill Book Co. Inc., New York.
- Khan, A., F. M. Bilqees, and N. Khatoun. 2009.

- Histopathology of grapevine roots infected with root-knot nematode *Meloidogyne incognita*. Pakistan Journal of Nematology 27:155-158.
- Magunacelaya, J. C. 2010. Estrategias de Manejo de nematodos fitoparásitos de importancia agrícola en Chile. Revista Frutícola 3:24-35.
- Magunacelaya, J. C. and E. Dagnino. 1999. Nematología Agrícola en Chile. Universidad de Chile, Facultad de Ciencias Agronómicas, Serie Ciencias Agronómicas N°2.
- Magunacelaya, J. C., F. Lamberti, and M. T. Ahumada. 2004. On the occurrence of *Xiphinema index* Thorne & Allen in Chile. Nematologia Mediterranea 32:235-236.
- Magunacelaya, J. C., M. T. Ahumada, and H. Pacheco. 2005. Aspectos generales de manejo de nematodos fitoparásitos de importancia agrícola en viñedos de Chile. Red Agrícola:17-20.
- O' Brien, T. P. and M. E. Mc Cully. 1981. The study of plant structure: principles and selected methods. Termacarphi PTY Ltd., Melbourne, Australia.
- SAG. 2005. Catastro Vitícola Nacional. Ministerio de Agricultura. Servicio Agrícola y Ganadero (SAG). Departamento de Protección Agrícola, Subdepartamento Viñas y Vinos. Santiago, Chile.
- Vovlas, N., R. N. Inserra, and G. P. Martelli. 1978. Modificazioni anatomiche indotte da *Xiphinema index* e *Meloidogyne incognita* in radici di un ibrido di *Vitis vinifera* x *V. rotundifolia*. Nematologia Mediterranea 6:67-75.

Received:

14/V/2012

Accepted for publication:

4/IX/2012

Recibido:

Aceptado para publicación: