

RESPONSE OF DIFFERENT POTATO CULTIVARS TO THE PRESENCE OF *NACOBBUS ABERRANS*

Paola Lax^{1*}, María del Carmen Tordable², Javier Macagno², Paula Bima³, and Marcelo Edmundo Doucet¹

¹ Instituto de Diversidad y Ecología Animal (CONICET-UNC) and Centro de Zoología Aplicada, Facultad de Ciencias Exactas, Físicas y Naturales, Universidad Nacional de Córdoba, Rondeau 798, 5000 Córdoba, Argentina; ² Morfología Vegetal, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Estafeta Postal N° 9, 5800 Río Cuarto, Córdoba, Argentina; ³ Laboratorio de Biotecnología Vegetal, Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Av. Valparaíso s/n, Ciudad Universitaria, Casilla de Correo 509, 5000 Córdoba, Argentina. Corresponding author: plax@com.uncor.edu

ABSTRACT

Lax, P., M. del C. Tordable, J. Macagno, P. Bima, and M. E. Doucet. 2013. Response of different potato cultivars to the presence of *Nacobbus aberrans*. *Nematopica* 43:83-90.

Nacobbus aberrans occurs in temperate and subtropical areas of South and North America. In Argentina, the species is widely distributed although accurate knowledge of the host range is unknown for many populations. In this work the reproductive capacity of three Argentine populations of different geographical origin was evaluated on four potato cultivars (Spunta, Kennebec, Colorado, and Asterix); histological studies were conducted on root tissues of inoculated plants from each cultivar. Two *N. aberrans* populations from Córdoba province were not able to reproduce on those cultivars; no evidence of the nematode presence was detected in root tissues. The remaining population, which was from Catamarca province, induced gall formation in all cultivars. Histological studies showed hyperplastic parenchymatous tissue occupying the central cylinder of invaded roots. In the same zone, syncytia developed closely associated with females of the nematode. The results showed different responses of potato cultivars to the presence of the *N. aberrans* populations.

Key words: Argentina, false root-knot nematode, histology, *Nacobbus aberrans*, potato

RESUMEN

Lax, P., M. del C. Tordable, J. Macagno, P. Bima, and M. E. Doucet. 2013. Respuesta de diferentes cultivares de papa a *Nacobbus aberrans*. *Nematopica* 43:83-90.

Nacobbus aberrans está presente en áreas templadas y subtropicales de América del Sur y del Norte. En Argentina posee una amplia distribución y, por el momento, se desconoce el rango preciso de hospedadores de muchas poblaciones. En este trabajo se evaluó la capacidad de reproducción de tres poblaciones argentinas de diferente origen geográfico sobre cultivares de papa (Spunta, Kennebec, Colorado y Asterix) y se realizaron estudios histológicos en las raíces de esas plantas. Dos poblaciones de la provincia de Córdoba no fueron capaces de multiplicarse en papa; en los tejidos radicales no se detectaron evidencias de la presencia del nematodo. La otra población, proveniente de la provincia de Catamarca, indujo la formación de agallas en todos los vegetales. Los estudios histológicos mostraron tejido hiperplásico con características parenquimáticas ocupando el cilindro central de las raíces infectadas. En la misma zona, se desarrollaron los sincitios en estrecha relación con las hembras del nematodo. Los resultados mostraron diferencias entre cultivares de papa frente a las poblaciones de *N. aberrans* consideradas.

Palabras clave: Argentina, falso nematodo de la agalla, histología, *Nacobbus aberrans*, papa.

INTRODUCTION

The plant-parasitic nematode *Nacobbus aberrans* is present in temperate and subtropical regions of South and North America (Manzanilla-López *et al.*, 2002). It has a wide host range, including several horticultural crops, native plants and weeds. In some Southern American countries, the nematode is one of the major limiting factors affecting potato (*Solanum tuberosum*) production, causing yield losses that may range between 10.9 and 61.5% (Franco, 1994). Populations of the parasite are not only capable of attacking potato roots, where the life cycle can be completed, but can also infect the skin and, in some cases, the parenchyma of the tubers. This capability facilitates nematode dispersal when contaminated tubers are used as seed and results in reduce market value of seed potato.

Nacobbus aberrans is widely distributed in Argentina (0-4500 m.a.s.l.), and parasitizes potato in the provinces of Catamarca, Tucumán, Salta and Jujuy (Doucet and Lax, 2005). The parasite has been recently detected attacking potato in the horticultural belt around Córdoba capital city (province of Córdoba). In Salta and Jujuy provinces, the nematode is closely associated with different varieties of Andean potato (*S. tuberosum* subsp. *andigenum*) (Lax *et al.*, 2006; 2008). Although several populations of *N. aberrans* have been detected in Argentina, the precise host range of many of them is still unknown. Some of these populations showed preference for certain hosts, revealing the existence of races/groups with different behaviour (Lax *et al.*, 2011).

Some potato cultivars used in the Andean region are particularly susceptible to *N. aberrans* attack (Jatala and Scurrah, 1975), demonstrating high production losses (Jatala and Bridge, 1990). Although several histopathological studies have been conducted to characterize symptoms induced by this nematode on different crops (Inserra *et al.*, 1983; Castillo and Marban-Mendoza, 1984; Tordable *et al.*, 2010), very little information is available regarding damage on potato (Finetti Sialer, 1990).

New potato cultivars, which are of growing interest in the market, have been recently introduced in Argentina (Gorostegui, 2005). The response of these and others cultivars commonly grown in the main producing regions of the country to local *N. aberrans* populations, is still unknown. The aim of the present work was to evaluate the reproductive capacity of three Argentine populations of this nematode on different potato cultivars and to analyze possible histological alterations in roots.

MATERIALS AND METHODS

The populations of *N. aberrans* employed in these studies were from the localities of Coronel Baigorria (CB), Río Cuarto (RC) (Río Cuarto department,

Córdoba province) and El Pucará del Aconquija (PA) (Andalgalá department, Catamarca province), and were found naturally parasitizing roots of quinoa (*Chenopodium album*), pepper (*Capsicum annuum*), and potato, respectively. Egg masses were extracted from infected roots, placed in Petri dishes containing distilled water, and maintained at room temperature until emergence of second-stage juveniles (J2) of the nematode.

Two potato cultivars commonly grown in the country (Spunta and Kennebec) and two other cultivars (Colorada and Asterix) that are becoming important in the main potato growing areas, were considered for the analysis. The plant material was obtained using *in vitro* multiplication of plants derived from meristem culture (Roca and Mroginski, 1993). Microplants (30 days old) were individually transplanted into pots containing approximately 100 g of sterile soil and vermiculite (1:2) and maintained at 21°C for 15 days to promote root growth. Plants were then transplanted to 200-cm³ pots (approximately 150 g of substrate) and 1.5 ml of water containing 100 mobile J2 (Initial population) was added to each pot on the roots. The tomato (*Lycopersicon esculentum*) cultivar Platense (obtained from seeds germinated in sterile soil) was used as a control (to confirm inoculum viability), using the same procedure as that described for potato. Six replicates per cultivar were performed at 21°C and 14-h light photoperiod. The experiment was repeated twice. Plants were watered as needed. After 90 days, plants were uprooted; roots were carefully washed with water and observed under a stereoscopic microscope for the presence of galls or signs of necrotic tissues.

Nematode extraction from the soil was performed using the centrifugal-flotation technique described by Jenkins (1964). Galls present in roots were counted. Egg masses were extracted and submerged in 1% sodium hypochlorite solution for 4 min (Hussey and Barker, 1973), and eggs were counted. Final nematode population was estimated for each plant by adding the number of eggs counted plus the nematodes extracted from the soil. Reproductive capacity was measured by calculating the Reproduction factor (RF = final population/initial population). Data on number of galls and RF were statistically compared using a Fisher's test ($P \leq 0.05$) with the aim of evaluating possible differences between potato cultivars. Analyses were performed with InfoStat program (InfoStat, 2002).

For histopathological studies, root fragments of the different cultivars inoculated (with and without galls) were cut into small segments about 5 mm long and fixed in FAA (formol 10%, ethyl alcohol 96° 50%, glacial acetic acid 5%, distilled water 35%). They were dehydrated in a graded series of ethyl alcohol and xylene baths and embedded in Histowax (Leica Microsystems, Germany). Serial transverse and longitudinal sections 8-10 µm thick were obtained using a rotatory microtome. Sections were stained with triple staining (hematoxylin-safranin-fast green) and

final mounting was performed with Depex (Sigma-Aldrich, Germany) (Johansen, 1940; O' Brien and Mc Cully, 1981). Photographs of galls were taken with a Carl Zeiss Stemi 2000 stereoscopic microscope equipped with a Canon digital camera. Micrographs were obtained with a Carl Zeiss Axiophot microscope; images were captured with an AxioCam HRC camera and processed with AxioVision 4.3 software.

RESULTS

Evaluation of nematode multiplication

All the populations of *N. aberrans* parasitized tomato, which confirmed the viability of the inoculum used; the mean number of galls was: 3 (PA), 5 (CB) and 12 (RC), and mean RF values were 8.7, 10.5, and 26.6, respectively. Neither of the populations from Córdoba infected potato cultivars (Number of galls = 0; RF = 0), whereas the nematodes from El Pucará del Aconquija attacked and multiplied on all potato cultivars (Table 1).

Table 1. Number of galls (Galls) and Reproduction Factor (RF) of *Nacobbus aberrans* population from El Pucará del Aconquija on different potato cultivars.

Potato	Galls	RF
Spunta	12 a	28.5 a
Colorada	3 b	6.8 b
Kennebec	4 b	5.6 b
Asterix	4 b	9.1 b

Means in the same column followed by different letter indicate significant differences ($P \leq 0.05$).

Histopathological studies

Tomato cultivar Platense (control). The tomato cultivar was parasitized by all three *N. aberrans* populations. The development of galls on taproot and lateral roots was observed (Fig. 1 A), as a consequence of alterations affecting mainly the central cylinder. Hyperplastic tissue, part of the enlarged female body and feeding sites were observed in this zone (Figs. 1 B, C). All these alterations caused reduction, displacement and fragmentation of vascular tissues (Figs. 1 D, E). Damage in the cortex was mainly related to the increased volume of female body; wide areas with ruptured cells were observed (Fig. 1 B), including some the endodermis. In the galls induced by PA population, juveniles were also found in the cortex (Fig. 1 F). Feeding sites were of variable shape and size, and were adjacent to the vascular tissues. Cells of these tissues were either modified and incorporated to the sites (Fig. 1 G) or were crushed and/or broken

(Fig. 1 H). The cytological characteristics of syncytia were similar in the three populations; they had dense, vacuolated cytoplasm of fibrillar texture; in some syncytia, starch was observed (Figs. 1 I). Nuclei were spherical or elongated and hypertrophied, and up to about 14 μm in diameter in the PA population, which also had lobulated nuclei (Fig. 1 H). Cell walls were thin (PA=2-3 μm) or more thickened (approximately 6 μm in CB and RC); all of them were cellulosic and partially fragmented, allowing the fusion of neighbouring cytoplasms. Syncytial cells, which in some sectors remained in contact with the nematode female, were crushed and ruptured due to the increased body volume.

Potato cultivars. Río Cuarto and Coronel Baigorria populations. None of the cultivars showed external symptoms of the attack of these two populations (Fig. 2 A). No feeding sites or evidence of the nematode presence was observed in tissues; the typical root morphology, cortex and central cylinder, were observed. Both were organized and composed of unaltered tissues (Fig. 2 B).

El Pucará del Aconquija population. All four cultivars exhibited galls of approximately 3 mm in diameter (Fig. 3 A), which often contained more than one female (up to three in Colorada and Asterix) (Fig. 3 B). Hyperplastic parenchymatous tissue occupied the central cylinder in modified roots. Syncytia developed in the same zone, closely associated with females. The anterior portion of the nematode was always surrounded by syncytial cells (Fig. 3 C); the remaining part of the body was embedded in cortical parenchyma in some sectors, whereas in others, it was in contact with the syncytium. In both cases, as in the control, the surrounding cells were crushed and/or broken. Feeding sites were adjacent to vascular tissues (Fig. 3 C), occupying and modifying part of the xylem cells, causing reduction, fragmentation and displacement of tissue. In the cultivar Spunta, broken conductive xylem elements were also observed (Fig. 3 D); the phloem was also reduced and detached to the periphery of the gall, losing contact with the xylem (Fig. 3 E). In the cultivars Colorada and Spunta, the syncytia were composed of several hypertrophic cells that reached a size similar to that of syncytial cells of the control (80-100 μm , along the major axis) and presented themselves in variable shapes (Fig. 3 F); in Asterix and Kennebec, these characteristics were not observed because the walls were too fragmented, hindering individualization of syncytial cells (Fig. 3 G). In all the cultivars, affected cells showed dense cytoplasm. Some showed a low number of small vacuoles (Fig. 3 E) while others showed even greater vacuolization (Fig. 3 F); cells also contained starch (Fig. 3 G). Nuclei were hypertrophied, spherical or oval, and were up to 12 μm in diameter (Spunta and Asterix). Cell walls were cellulosic and somewhat thickened (4-6 μm thick) with respect to the control (2-3 μm thick). In

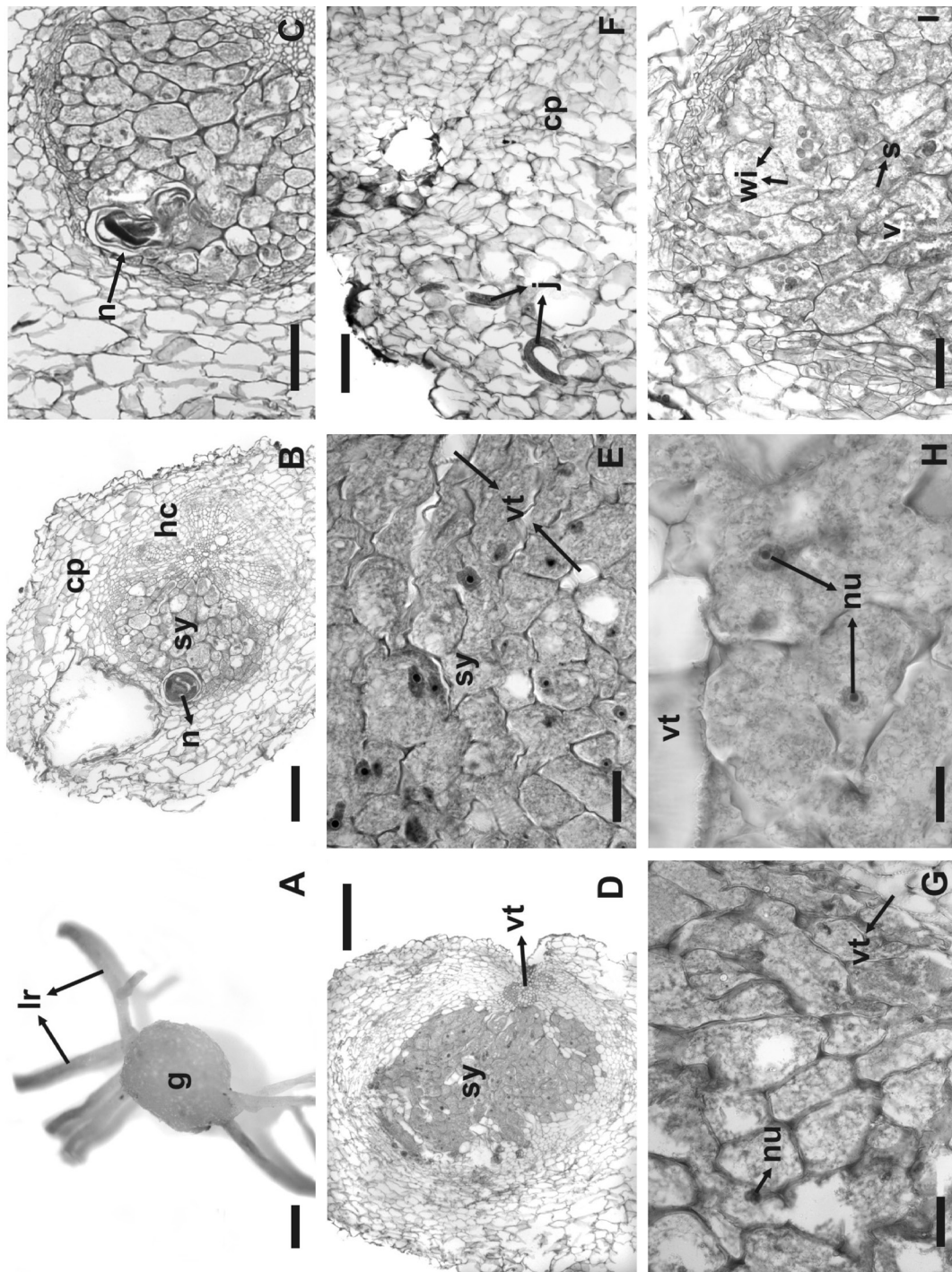


Fig. 1. Anatomical changes induced in tomato roots by three populations of *Nacobbus aberrans*: El Pucará del Aconquija (A, D, E, F, H), Coronel Baigorria (B, C, I) and Río Cuarto (G). A) External view of gall; B, C) Transverse section of gall with hyperplastic tissue, syncytia and nematode; D, E) Transverse section showing syncytia and reduction of the vascular tissues; F) Sector of gall with juveniles in the cortex; G, H) Detail of sector showing alterations in vascular tissues; I) Detail showing syncytial features. Abbreviations: cp: cortical parenchyma; g: gall; hc: hyperplastic tissue; n: nematode; nu: nucleus; s: starch; sy: syncytium; v: vacuole; vt: vascular tissues; wi: wall interruption. Scale bars: A = 1 mm; B, D = 200 µm; C, F = 100 µm; E, G, I = 40 µm; H = 20 µm.

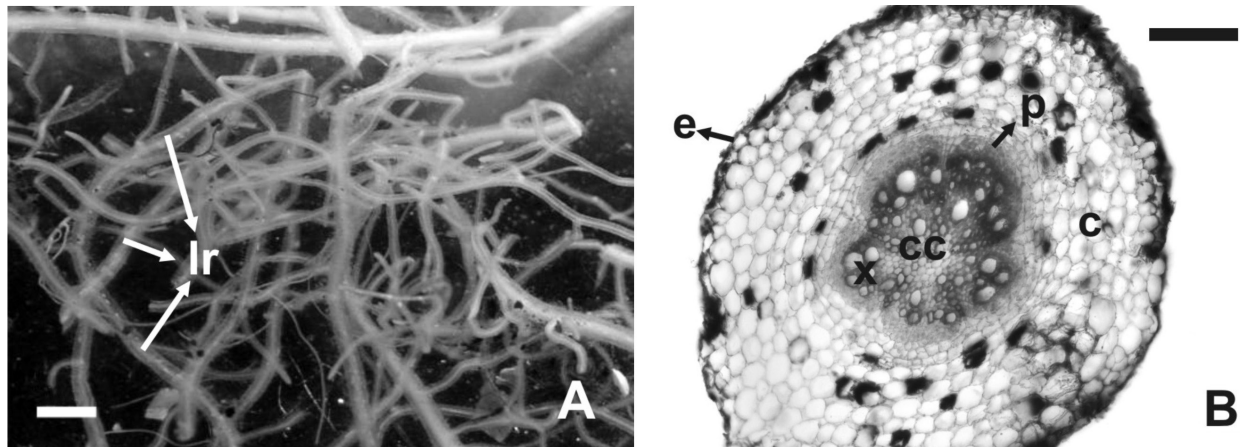


Fig. 2. Potato roots inoculated with second-stage juveniles of *Nacobbus aberrans* from Coronel Baigorria and Río Cuarto. **A**) External view of lateral roots (Spunta cultivar); **B**) Transverse section of roots with development of secondary growth (Asterix cultivar). Abbreviations: c: cortex; cc: central cylinder; e: epidermis; Ir: lateral root; p: phloem; x: xylem. Scale bars: A: 1 mm; B = 100 µm.

Colorada and Spunta egg masses embedded in tissues were observed (Fig. 3 H). In Asterix there were septate fungal hyphae in the cavities occupied by females and inside the syncytia (Fig. 3 I).

DISCUSSION

Because of the variability in host-parasite relationship observed among certain populations of *N. aberrans*, the existence of races/groups within the species was considered (Inserra *et al.*, 1985; Costilla, 1990; Manzanilla-López *et al.*, 2002). The present results confirm such variability, even between populations of the nematode on the same cultivar. Such is the case of tomato, used here as control plants. In the galls induced by the Catamarca population, syncytial cytoplasms were very dense, with a low number of vacuoles, and had more hypertrophied and lobulated nuclei than the syncytia produced by the other two populations of the nematode.

Recent studies conducted by Lax *et al.* (2011) showed that the two populations from the province of Córdoba (RC and CB) were able to multiply on tomato, sugar beet (*Beta vulgaris*) and pepper, but not on potato (Spunta cultivar); hence, they belong to the “sugar beet group”, according to the classification of Manzanilla-López *et al.* (2002). The inability of these populations to reproduce on potato was again reflected in the results of our studies wherein we attempted to infect previously untested cultivars. Histological analysis failed to demonstrate nematode-induced alterations in any portion of the root anatomy.

On the other hand, all the potato cultivars were efficient hosts for the population from the province of Catamarca; RF values ranged between 5.6 (Kennebec) and 28.5 (Spunta), indicating high susceptibility. The differential host test performed on this population

showed that it belongs to race 1 (Lax *et al.*, 2011), according to Castiblanco *et al.* (1999), due to its capacity to develop on tomato, pepper, sugar beet, and potato.

Histopathological analysis of potato cultivars showed that the galls were result of numerous infections because they usually hosted more than one female (up to three in the cultivar Colorada and Asterix). In addition, hyperplastic tissue occupying the central cylinder was observed, this damage being more abundant in Spunta and Colorada; the same phenomenon was described in pepper, sugar beet (Tordable *et al.*, 2007), and tomato (Tordable *et al.*, 2010) parasitized by the populations from Córdoba (CB and RC).

Likewise, the characteristics of the syncytia of the central zone in general were consistent with those described for other associations of the nematode with different plants of agricultural importance (Inserra *et al.*, 1983; Vovlas *et al.*, 2007), and with those of susceptible potato cultivars (Finetti Sialer, 1990). However, the galls analyzed in this work did not show the accumulation of starch grains in cells surrounding feeding sites, as indicated by Finetti Sialer (1990).

Although in all syncytia the cell walls showed interruptions, in Asterix and Kennebec the walls were highly fragmented, hindering individualization of cells. In the cultivars Spunta, Asterix, and Kennebec, syncytial cytoplasm was characterized by the presence of starch, which is one of the characteristics of feeding sites induced by *N. aberrans* and might be indication of intensive metabolic activity of syncytial cells (Souza, 2001). This nutrient reserve might also be used by the nematode until the reproductive stage (Schuster *et al.*, 1964).

Because not all *N. aberrans* populations show similar behaviour with the same plant species, not even with the same cultivar, further studies involving other

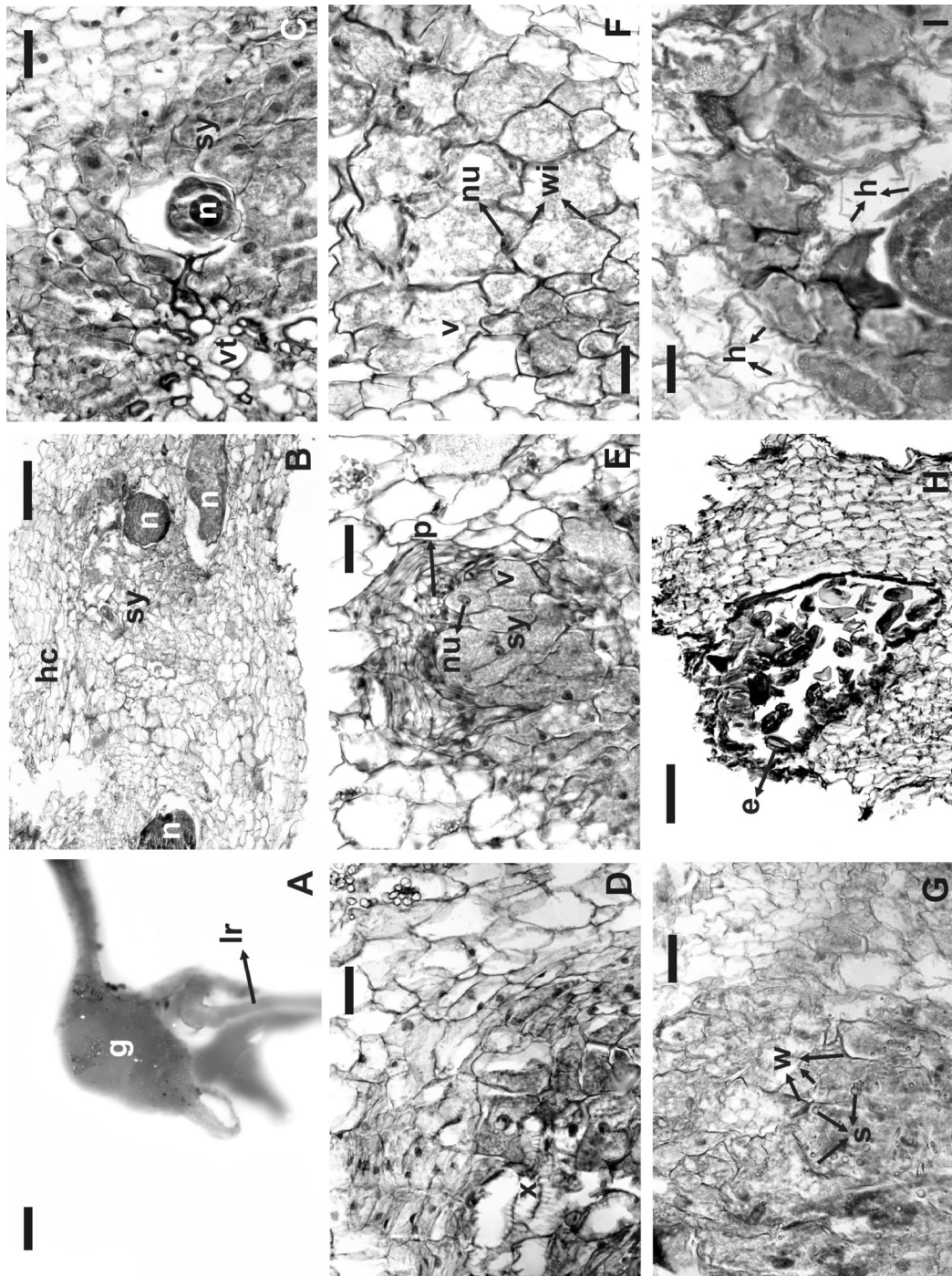


Fig. 3. Anatomical changes induced by *Nacobbus aberrans* (El Pucará del Aconquija population) on potato cultivars: Colorado (A, D, F, G), Asterix (B), Spunta (C, E, I) and Kennebec (H). A) External view of gall; B) Transverse section of galls with hyperplastic tissue, syncytia and nematodes; C) Detail of sector showing the syncytium and the anterior portion of a female; D) Detail of broken conductive elements of xylem; E) Transverse section with syncytia and phloem reduced and withdrawn to the periphery; F, G) Detail of different sectors showing syncytial features; H) Egg mass; I) Detail showing fungal hyphae. Abbreviations: e: egg; g: gall; h: hyphae; hc: hyperplastic cell; lr: lateral root; n: nematode; nu: nucleus; p: phloem; s: starch; sy: syncytium; v: vacuole; vt: vascular tissues; w: wall; wi: wall interruption; x: xylem. Scale bars: A = 1 mm; B = 200 µm; C, D, E, F, G, I = 40 µm; H = 100 µm.

important potato cultivars in the country are needed. Considering other local populations of the nematode is equally important to contribute to the selection of the most appropriate cultivars for use in rotation schemes.

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