DEC 19

Technology of the month – DEC 2019

Improved contrast of Acid Fuchsin stain using UV fluorescence microscopy

Long term storage in acidified glycerol solution of RKNs infected tomato roots

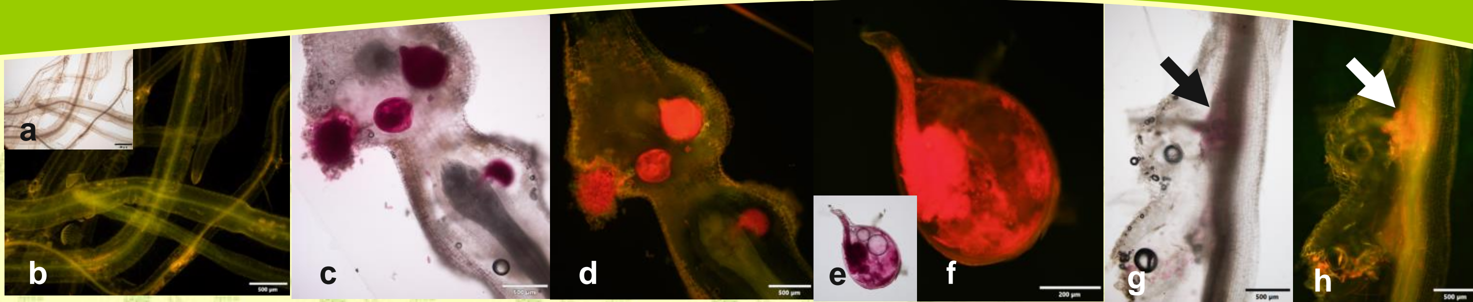

Fig. 1. (a) and (b) Acid Fuchsin (AF) stained control tomato roots; (c) and (d) Freshly stained AF *Meloidogyne* sp. infected roots; (e) and (f) Female *Meloidogyne* sp. nematode only; (g) and (h) stained AF *Meloidogyne* sp. juveniles in tomato roots stored in acidified glycerol solution for more than 3 months. Images (a), (c), (e) and (g) were captured under light microscopy and (b),(d), (f) and (h) under UV fluorescence using the U-MWIB2 filter cube (BP460-490/510IF/DM505) with an Olympus CMOS DP74 camera .

Acid Fuchsin (AF) stain is used for the detection of plant pathogenic nematodes within host roots under light microscopy. Nematodes within host roots appear magenta in colour due to the stain penetrating the impermeable cuticle during the heat treatment step in the staining protocol i.e. boiling in AF solution. AF stained nematode infected plant root samples are stored for a short period in acidified glycerol solution or observed as soon as possible under the microscope. However, if stored for a longer period in acidified glycerol solution, the stain within the nematodes decreases in intensity leading to a loss of contrast between the nematodes and plant roots. AF is a red fluorochrome (Em^{max} 630nm) under 'green light' excitation when UV fluorescence is used. If the microscope is not equipped with a 'green light' excitation filter, AF can be excited under 'blue light' exhibiting a dull red fluorescence signal at pH values below 6. UV fluorescence under 'blue light' excitation was used as an alternative means to observe AF destained *Meloidogyne* sp. infected tomato root samples stored for a long period in acidified glycerol solution.

Method: Tomato (*Solanum lycopersicum* cv Tiny Tom) infected with *Meloidogyne* sp. nematodes (RKNs) and uninoculated roots were washed in tap water to remove any excess potting mix and stained with AF using the 'Staining Roots for Plant-Parasitic Nematode Visualization' protocol (refer to Further Reading section). Freshly stained root samples and root samples stored in acidified glycerol solution for more than 3 months were viewed under light and fluorescence microscopy. Hand sectioned root samples were placed in some drops of acidified glycerol or 50% (v/v) glycerol solution on glass microscope slides with a cover slip and observed under light and UV fluorescence (Short Arc Mercury Hg lamp HBO).

Microscopy: An Olympus BX51 microscope with an Olympus digital colour and monochrome CMOS DP74 camera with the Olympus cellSens Standard software Version 2.2 was used to capture images under light and UV fluorescence. A U-MWIB2 filter cube (blue excitation Bandpass (BP)460-490nm/ Emission 510nm Interference filter (IF)/ Dichromatic mirror (DM) 505nm) was used to detect AF as a dull red fluorochrome and the root cell walls appeared green/yellow due to possible autofluorescence.

UV fluorescence under 'blue light' excitation and a longpass (green and red) emission filter can be used to view AF stained *RKN*s within tomato roots (a – h). AF root samples that were stored in acidified glycerol solution for a longer period were not ideal for image capture due to loss of contrast under light microscopy (g; black arrow). However, when samples were viewed under UV fluorescence/WIB2 long pass 510IF filter cube, contrast was improved where nematodes displayed a dull red fluorescence against the plant root cell walls in green/yellow fluorescence (h; white arrow). For specificity, AF stained samples could be viewed using a 'green light' excitation with red emission filter to confirm the presence of a red fluorochrome with AF stained nematodes due to the dye's excitation maximum being at 543nm (if possible).

Advantages: Use of UV fluorescence with a blue light excitation with a green and red emission filter (long pass 510IF filter) enables scientists/microscopists with no/limited access to a confocal laser microscope or a 'green light excitation filter to observe fluorochromes that can be excited by this filter and emit within the 510 to 700 nm range. In addition, autofluorescence can be used to improve contrast under such conditions (b).

Further Reading: Bruni & Tosi (1980) Protoplasma 102: 343-347; Byrd et al. (1983) J Nematol. 15(1): 142-143; Hals (1977) Scand. J. Dent. Res. 85: 542-548; Marshall & Johnsen (2017) Philos Trans R Soc Lond B Biol Sci. 372(1724): 20160335; Reichman (2017) https://www.chroma.com/sites/default/files/HandbookofOpticalFilters.pdf; Staining Roots for Plant-Parasitic Nematode Visualization Protocol https://www.plantpath.iastate.edu/tylkalab/content/staining-roots-plant-parasitic-nematode-visualization

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