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Working

Semi-quantitative measure of roots colonization by arbuscular mycorrhizal fungi using standard light microscopy

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Plant Functional Ecology Lab

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Preparation

1

Preparing slides for observation

1. Prepare you slides as to put root segments parallel to one another on multiple from top to bottom of the slides, using multiple columns depending on root segment length. A 3x3 frame was used.

Notes: Roots were collected from living Acer saccharum seedlings from a greenhouse experiment.

For subsampling, roots were separated into 3 diameter categories, namely small, medium and large, in order to facilitate discoloration protocol, and cut into 1 cm long fragments. They were then ink stained following the ink and vinegar protocol (Vierheilig et al., 1998) and mounted in glycerol.

2 Start observation

Observe slides under light microscope at 200x. Start of observation is the first field to be filled by the length of a root from the left of slide, may it be the 1st or 3rd root of the column.

Notes: Higher or lower magnification can be used to identify structures

Record observations

3

Record the presence of fungal structures in the root.

If applicable, categorize structures observed and estimate the amount using a semi quantitative scale e.g. from 1 to 3

Notes: The scale is meant to enrich the presence data by obtaining more information on the degree of colonization, without being as time-consuming as an estimate percentage colonization (Zemunik et al. 2018).

Decision criterias differ as they depend on the colonization extent and are highly subjective. Preferably, A single observer should be in charge of measuring the complete sample, or a simpler presence-absence protocol should be used. (McGonigle et al.; 1990)

Observation pattern

4 Move field of view vertically in direction of other roots. Repeat count

Notes: count only in roots segment which fill the field of view.

5 Shift field of view horizontally, for a distance equal to the field, and so at the direct right of field just observed.

Notes: With this methods the same hyphaes can be counted more than once. However, a great number of observations ensures the objectivity of the method.(McGonigle et al.; 1990)

6 Repeat observation on roots moving vertically, and continue onward on the slide creating comb-like pattern over the roots.

Notes: If roots have sled vertical, a complete change in the field of view moving vertically is considered a distinct observation.

7 Continue until end of root column. If columns stayed well separated, start again from the first field to be filled by the length of a root from the left of column. If columns overlap, continue observations in pattern.

8 **Create a working sheet**

For purpose of analysis, all observations of different structures should have their own column, and rows carry each the unique identifier of the observation, of the individual and of other categories (e.g. diameter class).

Example:

ID	Type	Neg	Arb	Ves	Coil	Hyp.amf	Endo	Pres
M-FT8-NiR-AS-215	M			1	2	3		1
M-FT8-NiR-AS-215	M			1	1	3		1
M-FT8-NiR-AS-215	M				2		1	1
M-FT8-NiR-AS-215	M						1	1
M-FT8-NiR-AS-215	M						1	1
M-FT8-NiR-AS-215	M			1	1	3		1
M-FT8-NiR-AS-215	M						1	1
M-FT8-NiR-AS-215	M	1						0
M-FT8-NiR-AS-215	M						1	1

RECORD VARIABLES

9 go to step #3

scale and abbreviation used

ID: Specimen identifier

Type: Diameter categories, namely:

P: Small

M: Medium

G: Large

Arb: Scale from 0-3 on quantity of arbuscules observed, where

0: None

1: One arbuscule

2: Some (~2-4)

3: Many (~>4)

Coil: Scale from 0-3 on quantity of coils observed, where

0: None

1: One or two coils

2: Some (~3-6)

3: Many (~>6)

Ves: Scale from 0-3 on quantity of vesicles observed, where

0: None

1: One to three vesicles, small if many

2: Some (4-6)

3: Many (>6) and or very large vesicles

Hyp.amf: Scale from 0-3 on quantity of VAM hyphae (less than 2 µm diameter) observed, where

0: None

1: Isolated fragments

2: Many unbroken hyphae and/or at least two visible appressorium

3: Colonization of the major part of the cortex (approx 60% and over).

Endo: Scale from 0-3 on quantity of endophytes, or fungal structures other than VAM observed, where

0: None

1: Isolated fragments of hypeas (less or equal to 2 µm) or microsclerotia

2: Some fragments or microsclerotia

3: Many fragments and/or microsclerotia and/or more than 3 cells showing signs of parasitism (by chytrids fungi)

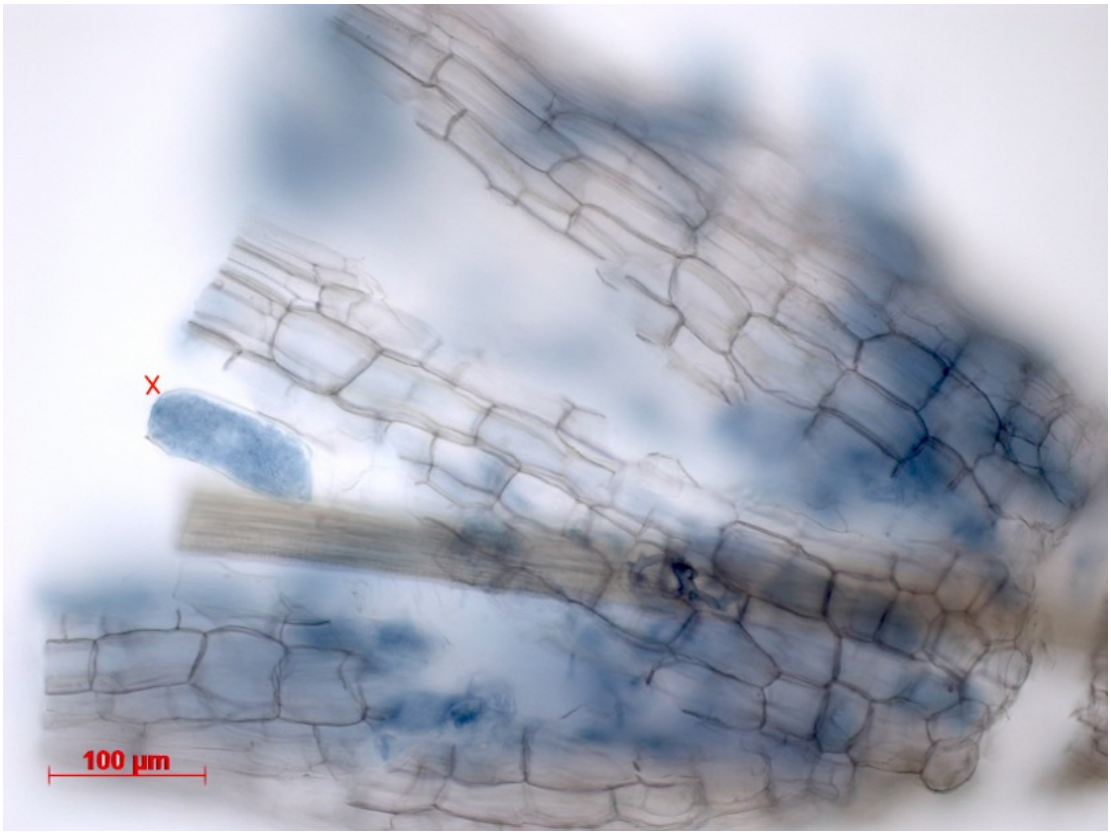
Neg: Negative, no fungal structure observed

Pres: Presence, at least one fungal structure observed

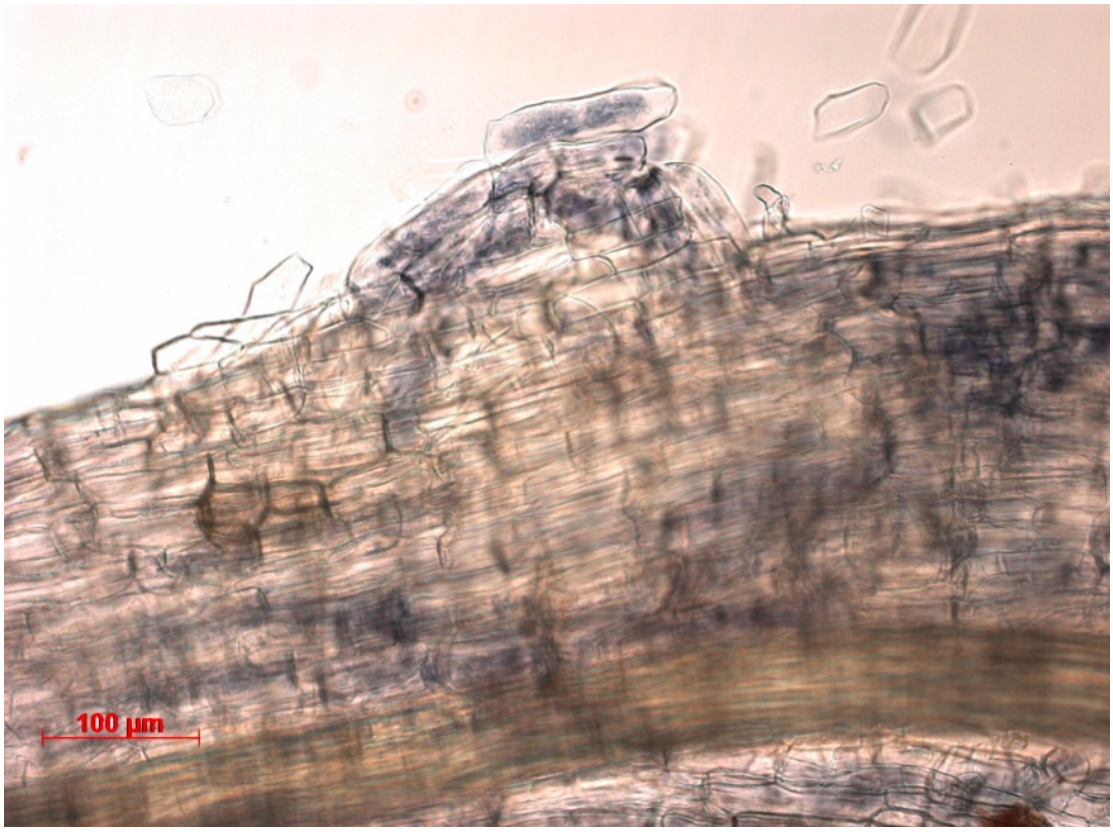
N.B.: Debris, or everything clearly outside the root, even if root broken apart, was not taking into consideration for colonization data.

Photo example

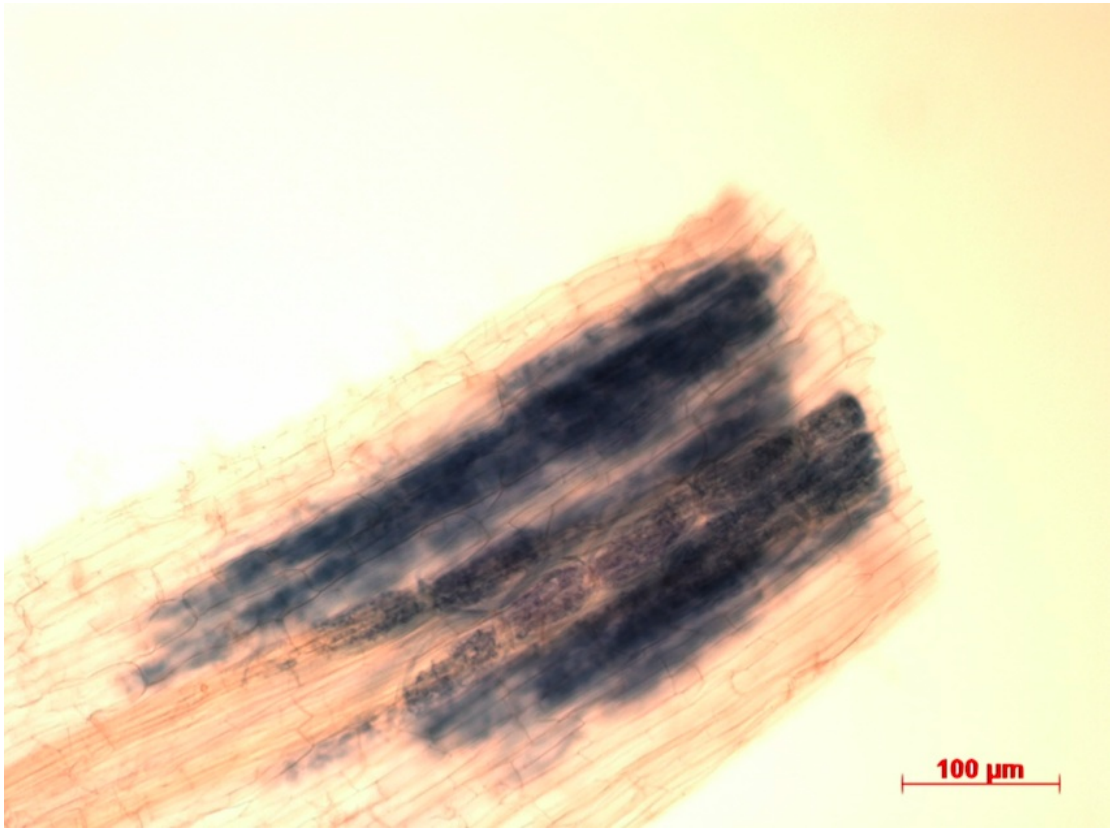
10

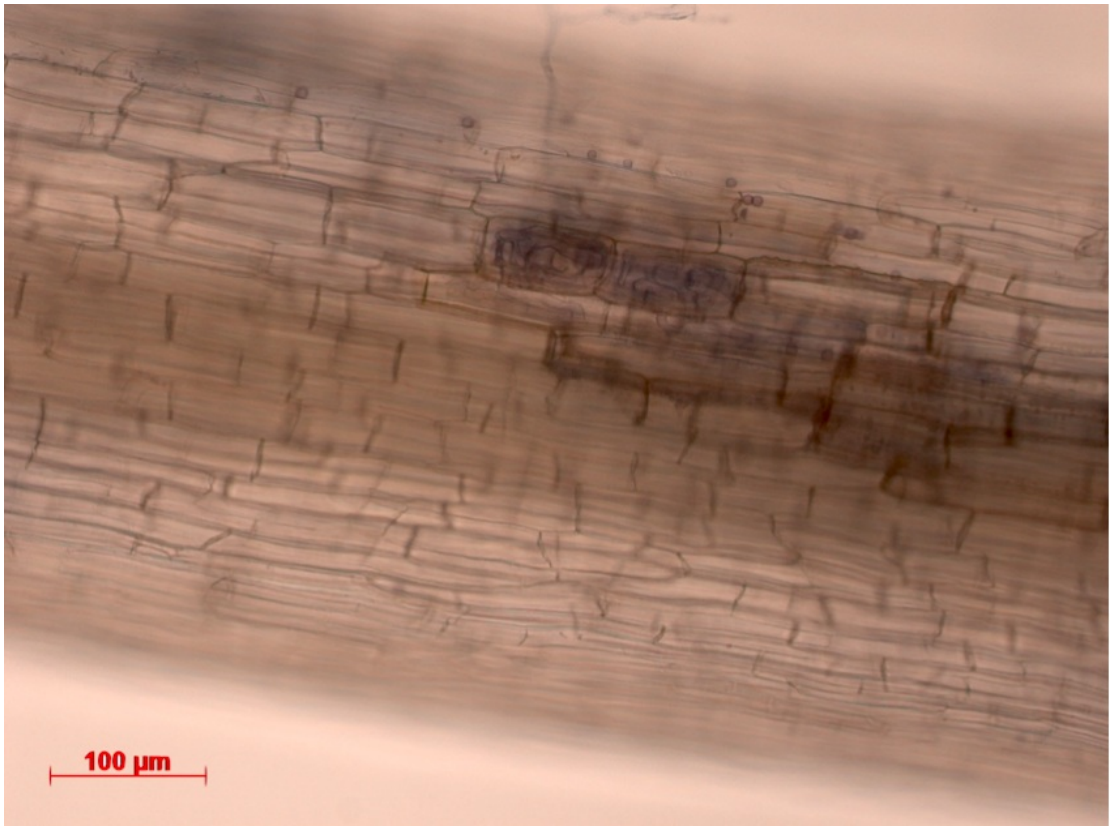


Arbuscule 1

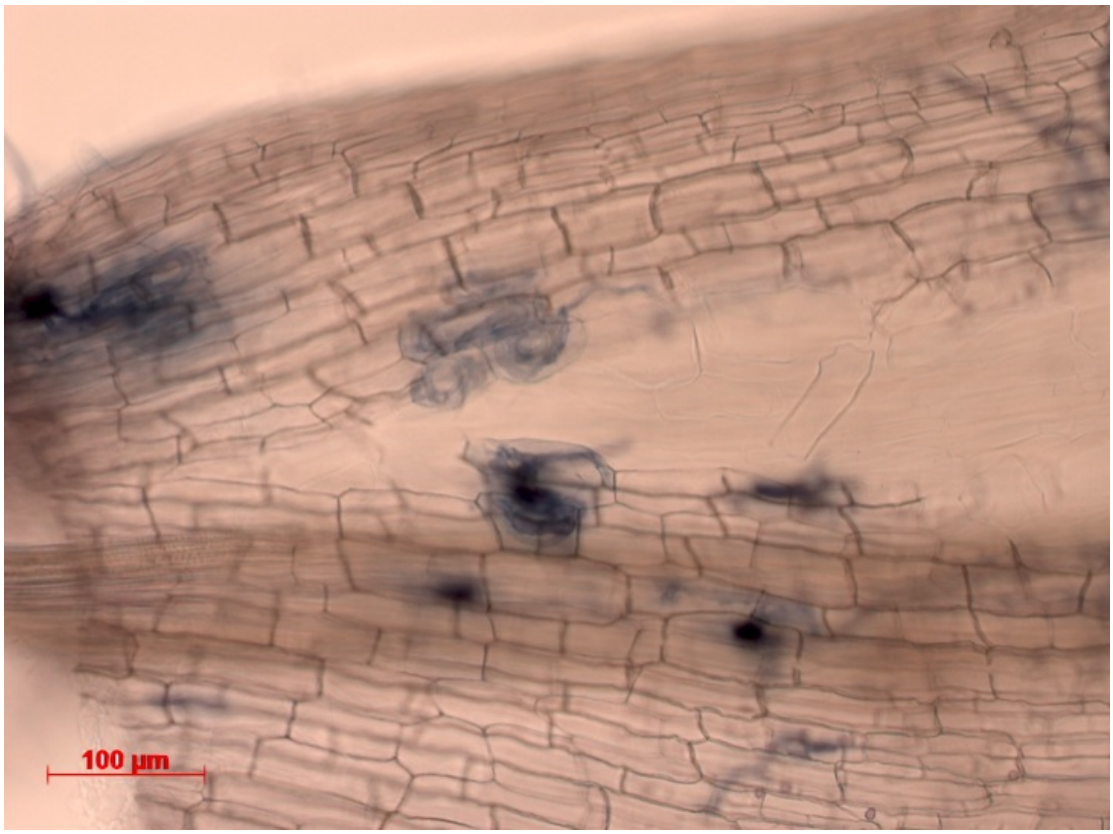


Arbuscules 2

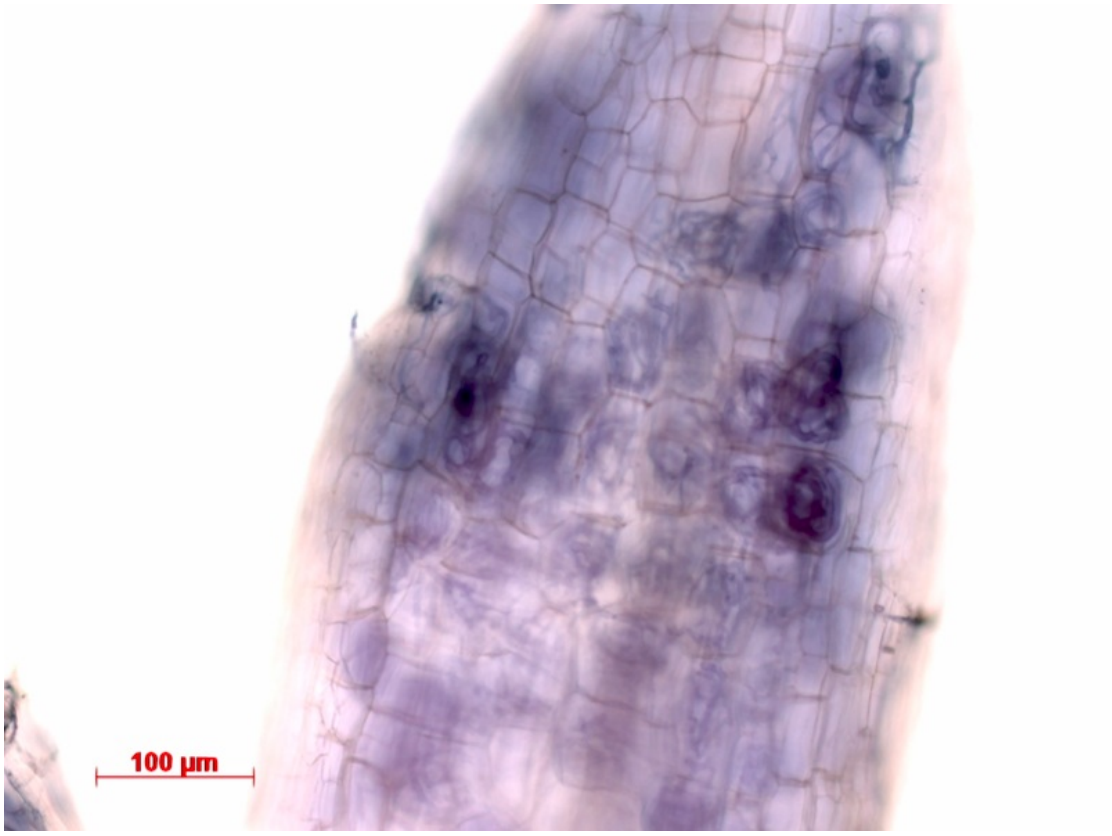




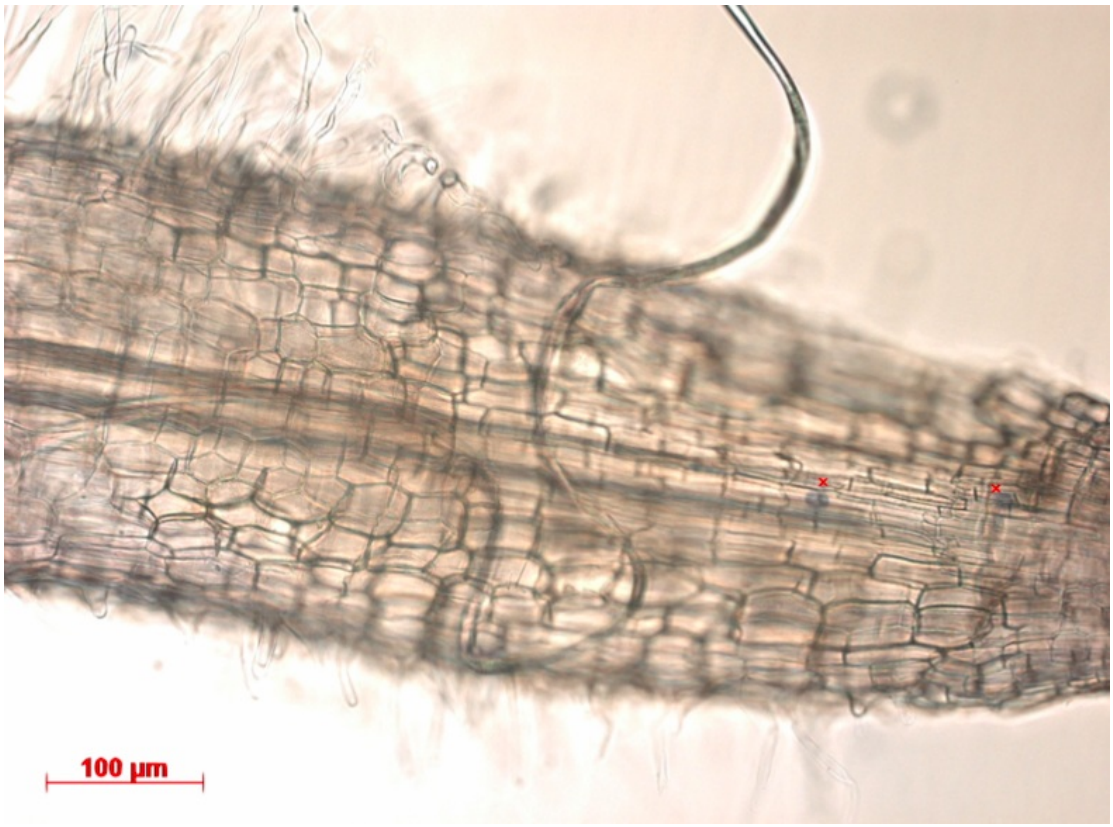
coil 1



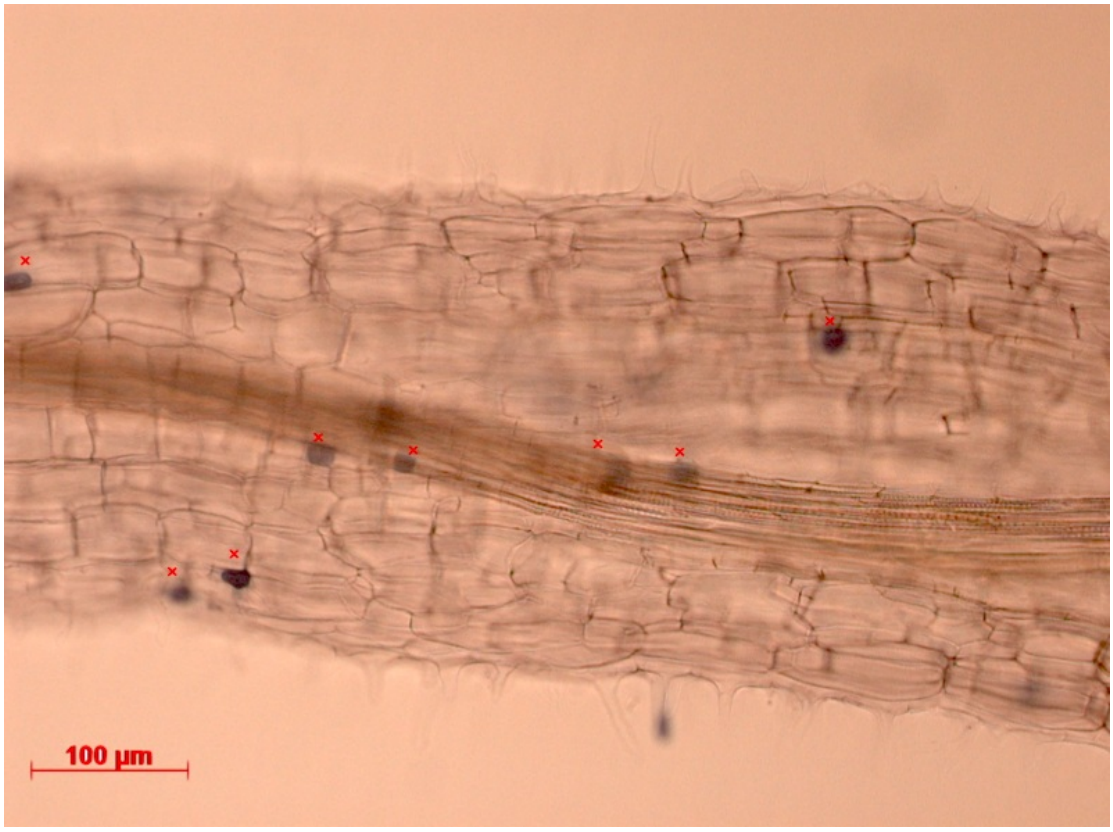
coil 2



coil 3

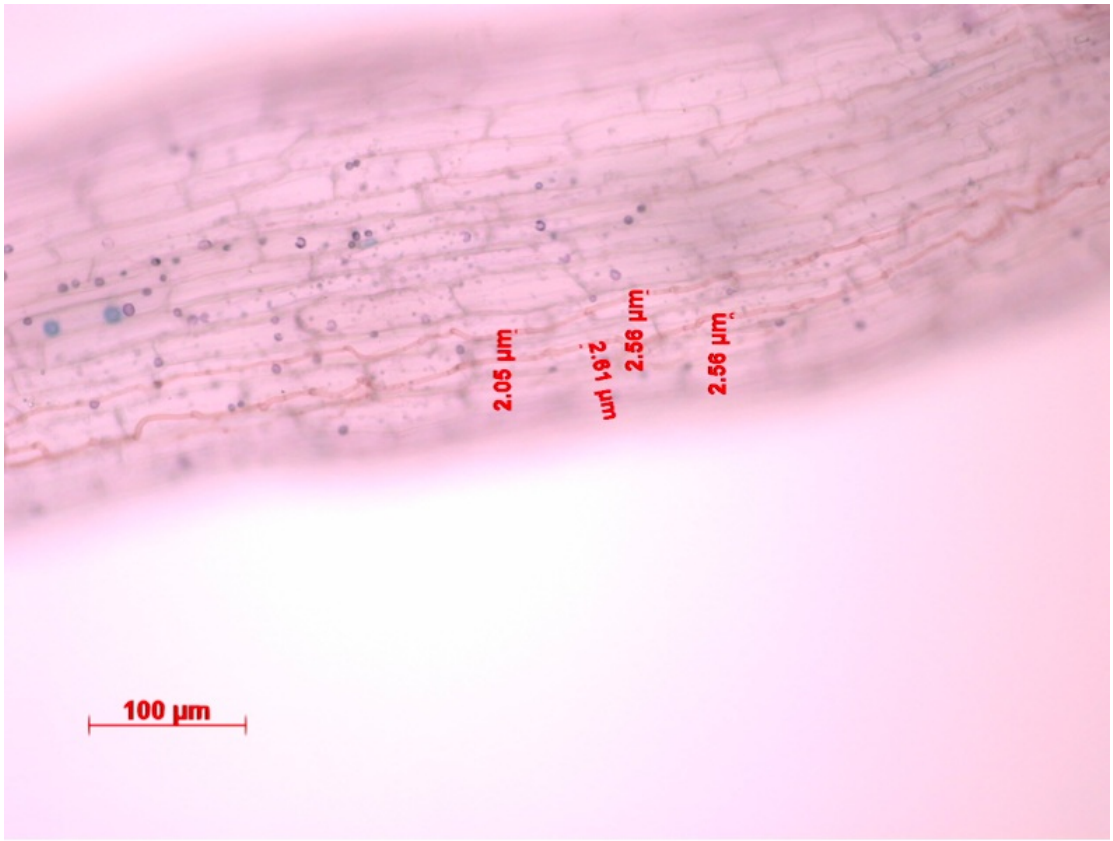


Vésicule 1

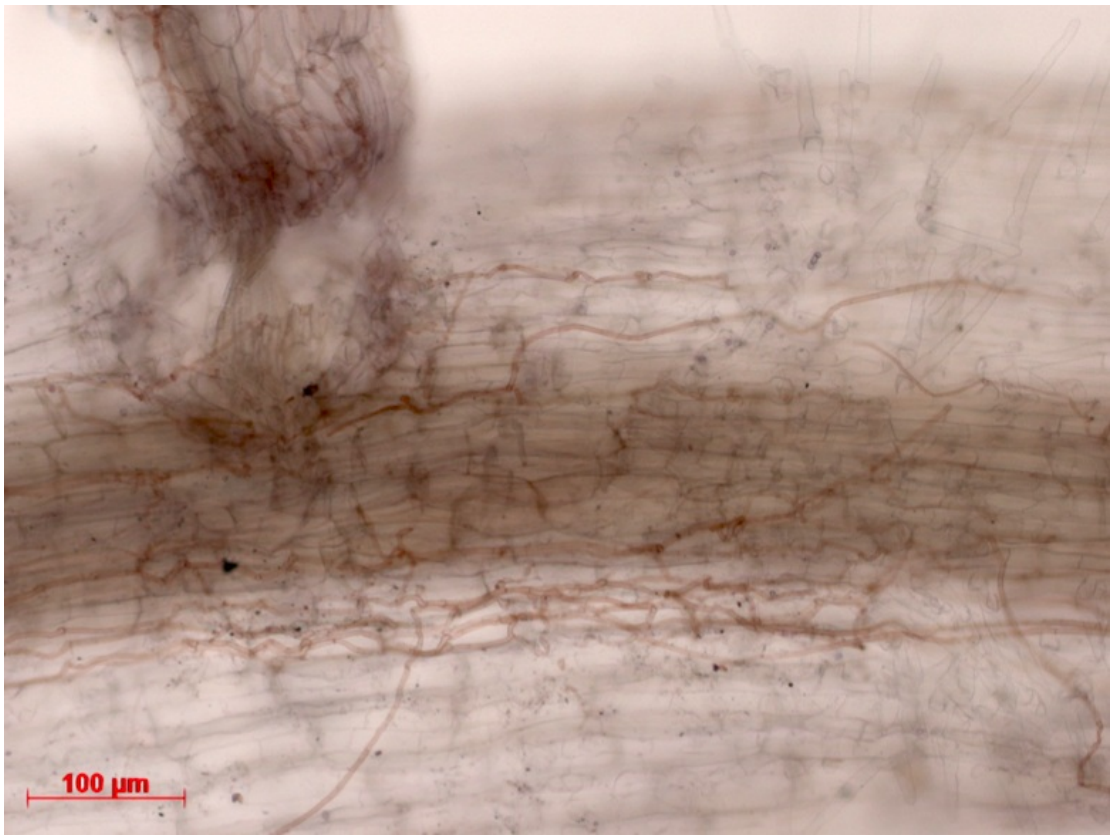


Vésicule 2

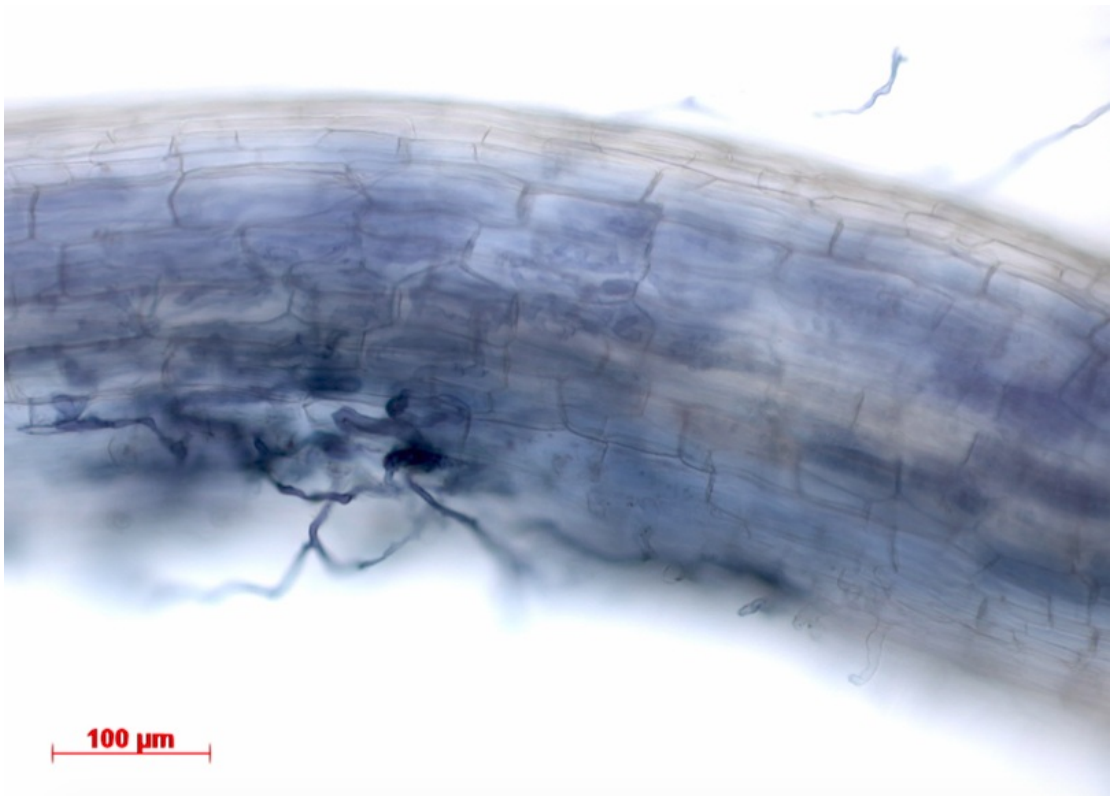
Vésicule 3



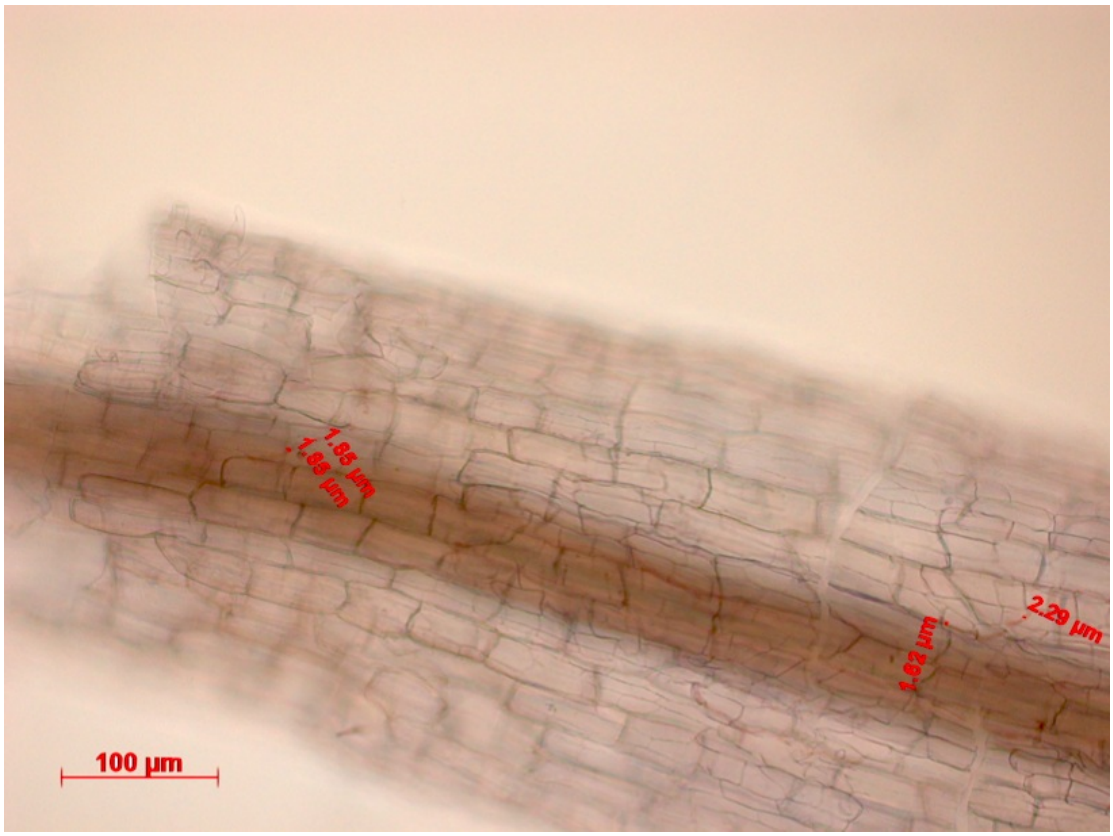
Hyphes 1



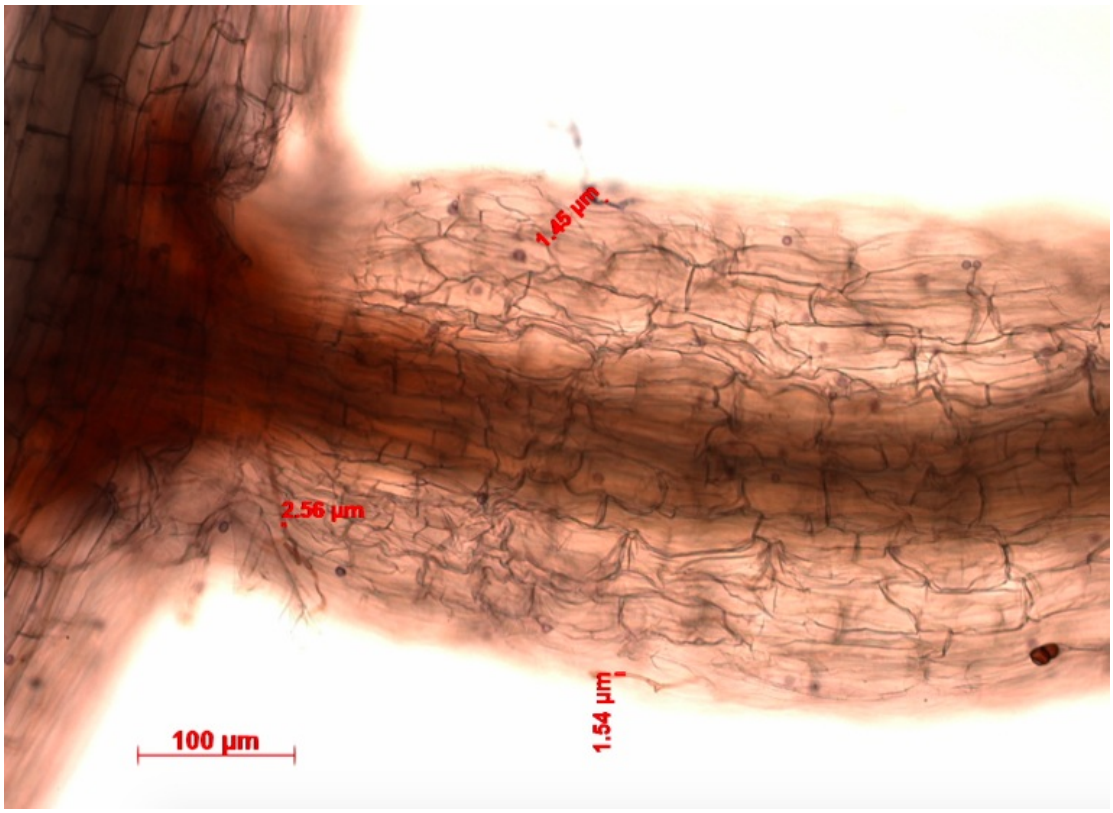
Hyphes 2



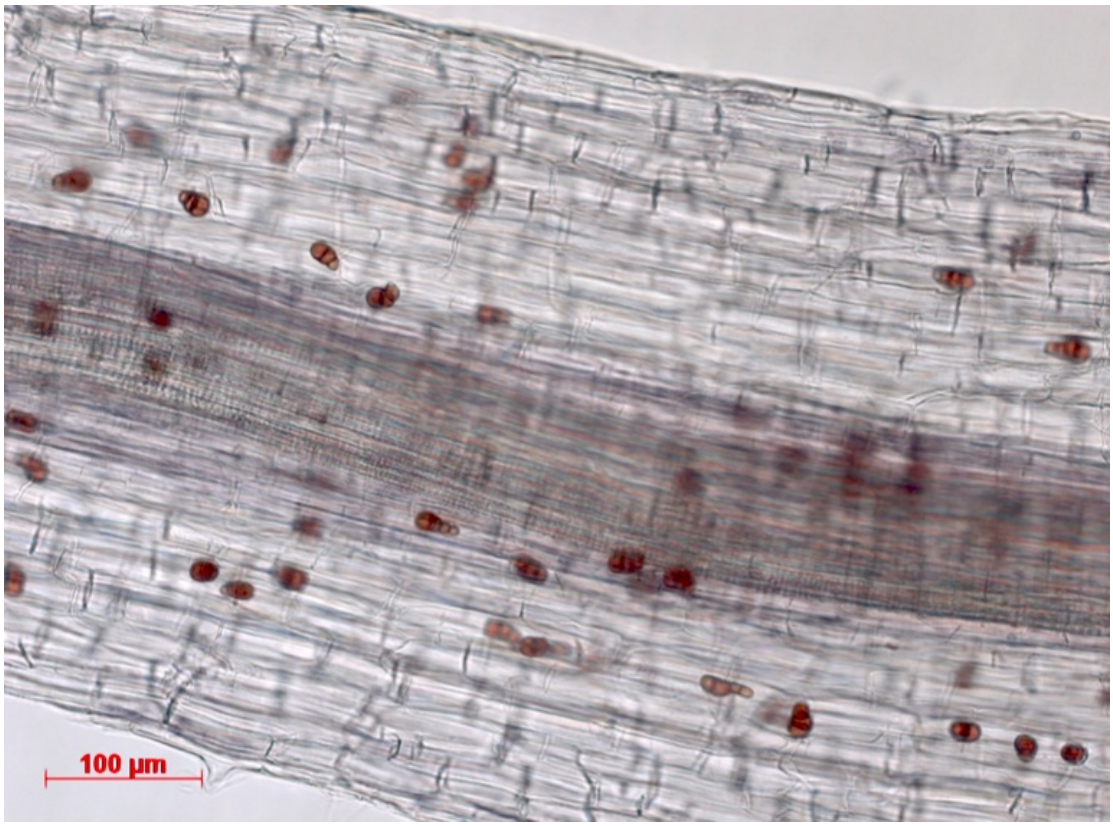
Hyphes 3



Endophytes 1



Endophytes 2



Endophytes 3