Simple methods to detect fungi in plant tissues

No staining

Cut fresh hand sections of infected plant tissue, observe under brightfield with condenser aperture half-closed. If fungal hyphae are not readily identified this way, they are often autofluorescent, with autofluorescence wavelengths distinct from the surrounding plant tissue.

Clearing agents

If fungal structures are obscured by surrounding plant tissues, it is usually because the plant tissue has refractile or dark-staining cell contents. Tissue clearing aims to digest these cell contents while leaving the cell walls relatively intact, although the tissue is often rather soft and fragile afterwards. Glycerol (=glycerine) is often added as it has a higher refractive index than water and will reduce visibility of plant cell walls. Common clearing agents include:

KOH – 2-10%
chloral hydrate – 5% to saturated solution
lactophenol (see recipe below – just omit the cotton blue)
lactic acid – anything up to 100%
Hoyer's solution – another mixture of chloral hydrate, glycerol and lactic acid
alkaline hydrogen peroxide – 0.5% NH_4OH and 0.5% H_2O_2 v/v in water can be used to bleach dark tissues after clearing if the clearing agents do not remove very dark phenolics or if the tissue begins to disintegrate before dark compounds have been cleared

Toluidine blue

0.05% toluidine blue
0.2% sodium benzoate, pH 4.4.

Cut fresh hand sections of infected plant tissue, stain for 1-2 minutes, rinse in water – avoid over-staining. This works well in harder tissues, either moderately lignified or at least tissue not very degraded by fungal enzymes. Fungal hyphae appear pink-purple within cells. Plant cell walls will be varying shades of blue in lignified tissues.

Trypan blue or acid fuchsin

0.05% trypan blue in lactoglycerol (25% lactic acid in 50% glycerol or 1:1:1 lactic acid:glycerol:water)
0.05% acid fuchsin in lactoglycerol

Clear tissue pieces in 10% KOH, with heating or autoclaving if necessary. Rinse in water – slightly acidified water (1% HCl) may enhance staining. Stain in trypan blue-lactoglycerol. Fungal structures appear blue, as well as lignified or suberised plant cell walls. Longer clearing and bleaching reduces staining of plant tissues but may not completely eliminate it.

Acid fuchsin is an alternate stain that may work better with heavily lignified tissues that stain strongly in trypan blue.

Stained roots can be stored in 2:1 water:glycerol with 0.1% sodium azide to stop bugs growing. Note that glycerol = glycerine.

Lacto(phenol) cotton blue

phenol	20 g – preferably omitted, very toxic
cotton blue	50 mg
glycerol	40 ml
lactic acid	20 ml
deionized water	20 ml

The formulation above makes aout 100 ml of solution. Cotton blue is very similar to aniline blue and can be used almost interchangeably with it if no cotton blue is available.

Fresh tissue can be immersed directly in the stain and left until the surrounding tissue is clear and fungal hyphae and other structures are visible – they will be stained blue. If the surrounding plant tissue is very dense it can be heated in this stain to enhance penetration. Dark compounds can be bleached by first soaking in 10% or stronger KOH then applying the stain.

Phenol is very toxic, this must all be carried out in a fume hood. In most cases, this stain works just as well if phenol is omitted. The ratios of the other components can be adjusted as required for specific tissues.

Schiff's reagent

Pretreating plant tissues with bleach or KOH then staining with Schiff's reagent (the commercially available stain is cheap and works fine) may stain fungal tissues pink against a clear or at least paler background. Denser or darker tissues may be first cleared before applying the stain.

The standard PAS (Periodic acid-Schiffs) staining procedure may be used instead:

1. 0.5% periodic acid (0.2-1.0%) – 5-10 min – vary as necessary
2. Wash in running tap water – 5-10 min – can use metabisulfite here
3. Rinse in distilled water.
4 Schiff's reagent – 15 min – avoid overstaining
5. Wash in running tap water – 5-10 min – do not over-rinse or stain will be too diffuse

Fungal stuctures will be dark pink, hopefully against a pale background of plant tissue.

Wheatgerm agglutinin, concanavalin A plus fluorescent tag

20 μM WGA-Alexa488 or ConA-Alexa633 (in water); store in aliquots in freezer
dilute to 2 μM in water before use

Cut fresh hand sections of infected plant tissue, stain for 5-20 minutes, rinse in water. These are very specific and very bright tags for most fungi in plant tissue. Choice of fluorescent tag will depend on major autofluorescence in the infected tissue.

Autofluorescence of plant tissue can often be blocked by staining in 0.05% toluidine blue or crystal violet. Otherwise, the plant tissues can be stained with calcofluor white, congo red or propidium iodide as a contrast to the Alexa488 fluorescence.

If staining is patchy or does not label fungal material well, the plant tissue will need to be permeabilised. KOH, lactophenol, chloral hydrate, strong detergents, lactic acid at various concentrations and sometimes with heating or autoclaving, are all methods of pretreating the plant tissue which will allow the fungal stain to penetrate into it. Most of these treatments will also substantially reduce plant tissue autofluorescence.