

Short communication

A modified procedure for staining arbuscular mycorrhizal fungi in roots

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Summary – Zusammenfassung

Roots were cleared by boiling in 10% KOH and rinsed several times with tap water. Thereafter roots were boiled in the staining solutions. The staining solutions consisted in different dyes (trypan blue (0.05%), anilin blue (0.05%) and acid fuchsin (0.01%)) which were dissolved in usual household vinegar (5% acetic acid). For best contrast, trypan and anilin blue stained roots were destained with tap water, whereas acid fuchsin stained roots were destained with vinegar. All fungal structures were stained and clearly visible.

Eine modifizierte Methode zur Anfärbung von arbuskulären Mykorrhizapilzen in Wurzeln

Nach Entfärbung durch Kochen in 10%igem KOH wurden die Wurzeln mehrmals mit Leitungswasser gewaschen und daraufhin in den Färbelösungen gekocht. Diese bestanden aus den verschiedenen Farbstoffen Tryphanblau (0.05%), Anilinblau (0.05%) und Säurefuchsin (0.01%), die in gewöhnlichem Haushaltessig (Essigsäure 5%) gelöst waren. Nach der Anfärbung wurden die mit Tryphanblau oder Anilinblau angefärbten Wurzeln mit Wasser und die mit Säurefuchsin angefärbten Wurzeln mit Essig entfärbt. Alle Pilzstrukturen waren angefärbt und deutlich zu erkennen.

1 Introduction

Arbuscular mycorrhizal (AM) fungal structures are stained with trypan blue (Phillips and Hayman, 1970; Koske and Gemma, 1989), anilin blue (Nicolson, 1959) or acid fuchsin (Gerdeman, 1955). Trypan blue for example is dissolved in lactophenol (Phillips and Hayman, 1970) or in an acidic glycerol solution (Koske and Gemma, 1989). Recently, Mauler-Machnik and Nass (1990) developed a simple technique with a dye/25% acetic acid solution for staining fungal pathogens in wheat leaves. We studied whether a dye/vinegar (= 5% acetic acid) solution can be applied for staining AM fungi in roots.

2 Materials and methods

Bean, cucumber and ryegrass were inoculated with either of the three AM fungi *Glomus mosseae*, *G. intraradices* or *Gigaspora margarita*. Plants were harvested 7 weeks after inoculation. After removal of the growing substrate (sand:turf:soil/2:2:1) roots were rinsed with tap water and cleared by boiling for 3 min in 10% (w/v) KOH (Phillips and Hayman, 1970). After rinsing several times with tap water, the cleared roots were boiled for another 3 min in the following solutions: trypan blue (0.05%)/vinegar (5%), anilin blue (0.05%)/vinegar (5%) or acid

fuchsin (0.01%)/vinegar (5%). Thereafter roots were destained for 20 min with tap water or vinegar (5%).

3 Results

In all plants tested fungal structures of the three AM fungi, *G. mosseae*, *G. intraradices* and *Gi. margarita* were stained by the tested dyes (trypan blue, anilin blue, acid fuchsin) when dissolved in vinegar.

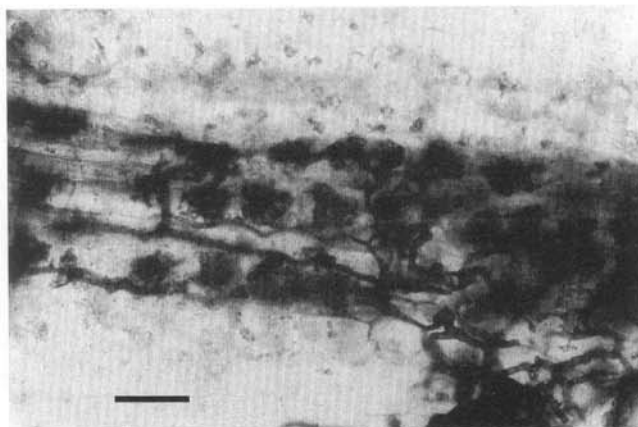


Figure 1: Bean root colonized by *Glomus intraradices*. The root was stained with a trypan blue/vinegar solution (Bar = 10 µm).

Abbildung 1: Mykorrhizierung von Bohnenwurzeln durch *Glomus intraradices*. Die Färbung erfolgte mit einer Tryphanblau/Essiglösung (Balken = 10 µm).

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When roots were stained with trypan or anilin blue the contrast was better after destaining with tap water, whereas after staining with acid fuchsin a better contrast was observed after destaining with vinegar.

4 Discussion

The proposed modifications for staining AM fungi in roots give excellent staining results with all fungi tested. Vinegar, an inexpensive and nontoxic compound can replace chemicals as lactophenol (Phillips and Hayman, 1970) and the acidic glycerol solution (Koske and Gemma, 1989) used for dissolving the dyes. Moreover, an acidification step with HCl necessary so far after root clearing with KOH can be omitted.

The method provides a simple technique for staining AM fungi and may also be applicable for staining other root colonizing fungi.

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