New technique for observing mycorrhizal fungi

T. Ishii[a] and K. Amanai

National Institute of Technology, Tokuyama College, Yamaguchi, Japan.

Abstract

Mycorrhizal fungi, particularly arbuscular mycorrhizal fungi (AMF) that infect almost all of the plants, play a very important role for the maintenance of natural ecology and the construction of safe, secure and sustainable (3S) crop production. The standard procedure to observe root colonization, however, is cumbersome, and various light microscopical techniques are required to clearly distinguish between AMF and root tissues. Recently, the fluorescein-labeled specific antibody against 24 kDa protein related to mycorrhizal symbiosis was developed. The new antibody was successfully used to detect various kinds of mycorrhizal fungi in fluorescence microscopic observation. In this study, we report on a new technique for observing mycorrhizal fungi by using a newly developed portable fluorescence microscope with the detection reagent.

Keywords: mycorrhizal fungi, mycorrhizal fungus-detection reagent, newly developed portable fluorescence microscope

INTRODUCTION

Mycorrhizal fungi form a symbiotic relationships with plants and have been known to contribute to environmental conservation and 3S food production (Ishii, 2014). Therefore, the observation of mycorrhizal infection is important for the evaluation of plant productivity. Generally, ectomycorrhizal infection is easily observed by the naked eye, but the observation of other mycorrhizal fungi, such as AMF, ericoid mycorrhizal fungi (ERMF) and orchid mycorrhizal fungi (OMF), is cumbersome. Conventional methods introduced by Phillips and Hayman (1970) require preparations before using light microscopical observation. Additionally, it is difficult to discriminate between the mycorrhizal fungi and root tissues.

Recently, Matsubara and Ishii developed a new reagent to detect a specific 24 kDa protein related to mycorrhizal symbiosis (patent pending). The 24 kDa protein has been reported to exist in various mycorrhizal fungi, including AMF (Ishii et al., 1999; Matsubara and Ishii, 2014), OMF (Matsubara et al., 2012; Matsubara and Ishii, 2014), ECMF (Matsubara and Ishii, 2014) and ERMF (Matsubara and Ishii, unpublished). For isolation and mass purification of the specific 24 kDa protein, we developed new preparative chromatographical techniques (Matsubara and Ishii, 2015).

In this study, we developed not only a portable fluorescence microscope that is possible to use even in the field for the observation of mycorrhizal fungi, but also a new technique for observing mycorrhizal fungi using the new portable fluorescence microscope with the detection reagent. Additionally, since only *Rhizoctonia solani*, which is the congeneric fungus of OMF, in typical plant pathogenic fungi reacted by the detection reagent, we investigated whether it is possible to distinguish between *R. solani* and AMF, using this technique.

MATERIALS AND METHODS

Since a specific 24 kDa protein related to mycorrhizal symbiosis exists in AMF, ECMF, ERMF and OMF, the fluorescein-labeled antibody against the 24 kDa protein is possible to detect every mycorrhizal fungus (Figure 1). Therefore, rootlets of various plants were cut in 1-2 cm from the apex. Then, 10 µL of the detection reagent was dropped onto each root, and then mycorrhizal root colonization was observed at wavelength of 472 nm using the newly developed portable fluorescence microscope (Joint development with Soma Optics, Ltd. and

[a]E-mail: aguaish@hotmail.com

Molecular Devices Corporation Japan) (Figures 1 and 2).

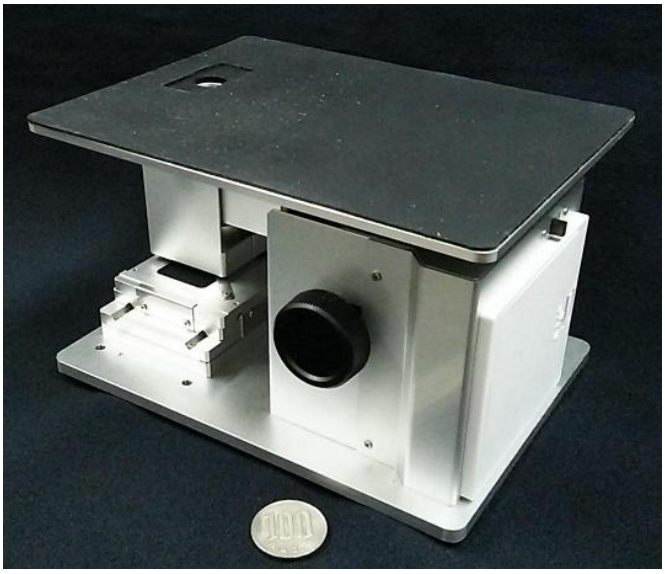

Figure 1. A newly developed portable fluorescence microscope and mycorrhizal fungus-detection reagent.

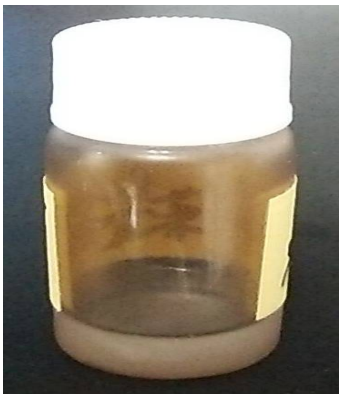

Figure 2. Mycorrhizal root colonization was easily observed even in the field and had been photographed using camera-equipped mobile phones.

RESULTS AND DISCUSSION

Using the portable fluorescence microscope and the mycorrhizal fungus-detection reagent, mycorrhizal root colonization by AMF and ERMF was clearly observed (Figures 3-8). Although *R. solani* shows the fluorescence using the detection reagent, the distinction of AMF and *R. solani* is very easy because no septa in AMF hyphae were observed (Figure 3). Interestingly, it was clearly demonstrated that even the roots of *Brassica rapa* and *Cyperus microiria* that have been known to be non-mycorrhizal plants (Wang and Qiu, 2006) can form AMF symbiosis (Figure 7). Under environmental stress conditions or after herbicide application, however, AMF symbiosis in these plants is frequently observed (Ishii, 2014).

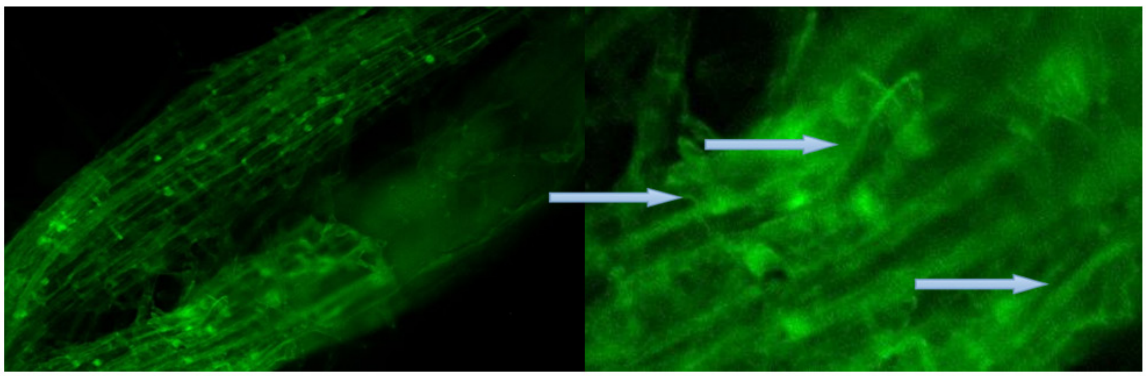

Figure 3. AMF symbiosis in chrysanthemum roots. Right (enlarged view): there are no septa in AMF hyphae (see arrows). Therefore, the distinction of AMF and *R. solani* was very easy.

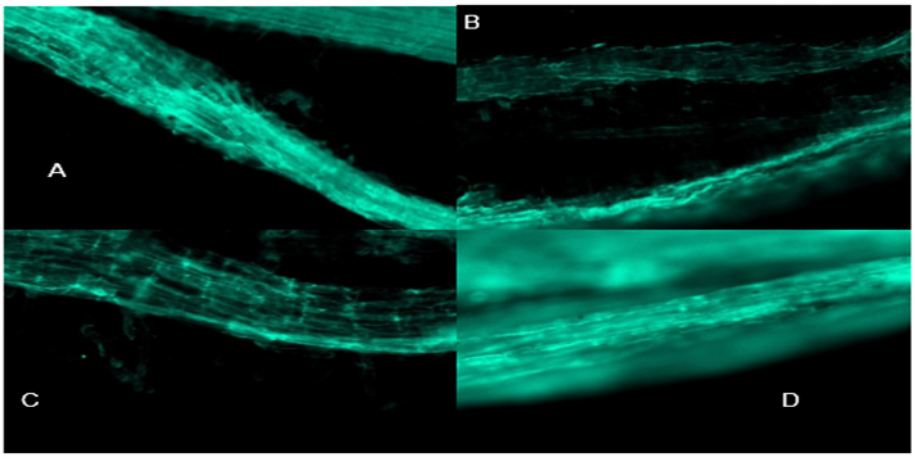

Figure 4. AMF symbiosis in several kinds of plant roots. A: corn, B: soybean, C: cucumber, D: lettuce.

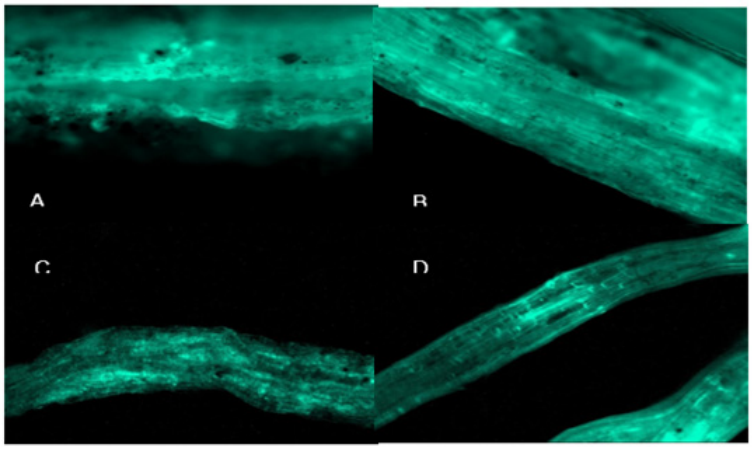

Figure 5. AMF symbiosis in several kinds of plant roots. A: carrot, B: garlic, C: peppermint, D: *Pelargonium* sp.

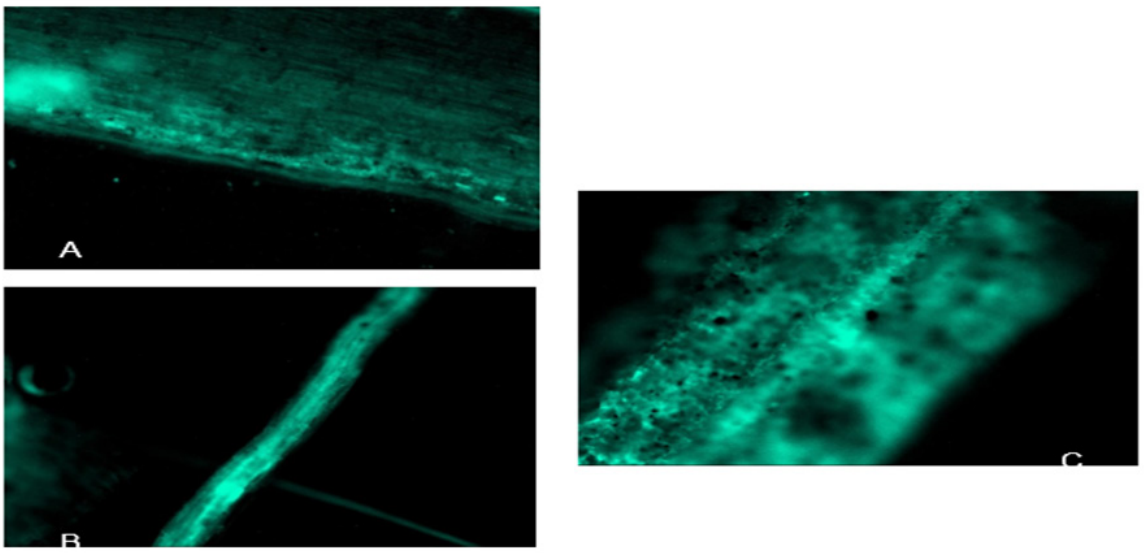

Figure 6. AMF symbiosis in several kinds of plant roots. A: *Leucaena leucocephala*, B: *Duchesnea chrysantha*, C: trifoliate orange.

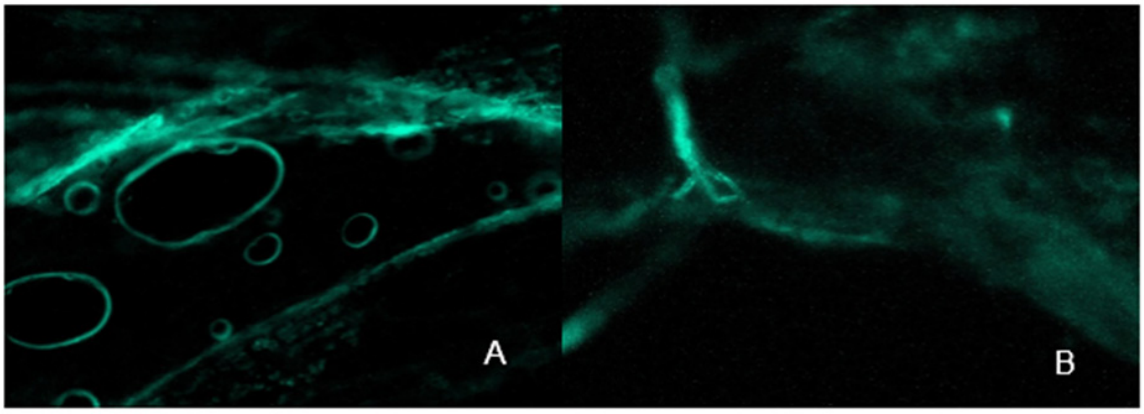

Figure 7. AMF symbiosis in several kinds of plant roots. A: *Brassica rapa* var. *perviridis*, B: *Cyperus rotundus*.

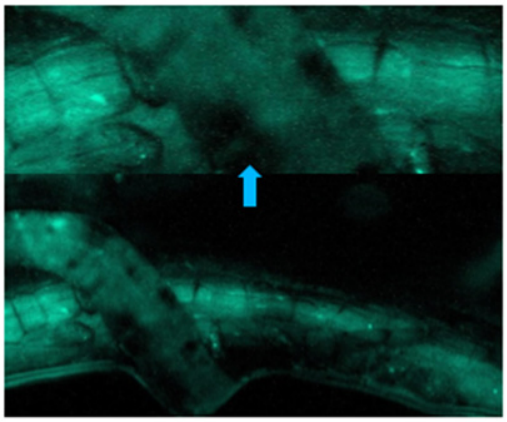

Figure 8. ERMF symbiosis in blueberry roots. Above (enlarged view): hyphal complexes occupying the cell.

In blueberry roots, a typical feature of ERMF such as hyphal complexes occupying the cell or coiled hyphae in the cell was clearly detected (Figure 8).

Thus, our new technique for observing mycorrhizal fungi is very simple; the only operation required before observation is the addition of 10 µL of the detection reagent. Moreover, mycorrhizal root colonization can be easily observed even in the field and was photographed using camera-equipped mobile phones. Therefore, the technique will contribute to the promotion of nature conservation, and 3S crop production or organic farming which does not hold good condition without the presence of mycorrhizal fungi.

ACKNOWLEDGEMENTS

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