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Stamp Pad Ink, an Effective Stain for Observing Arbuscular Mycorrhizal Structure in Roots

Panna Das and Highland Kayang

Laboratory of Microbial Ecology, Centre for Advance Studies in Botany,
School of Life Sciences, Mawkynroh Umshing, North Eastern Hill University, Shillong 793 022, India

Abstract: We report the use of stamp pad ink for staining arbuscular mycorrhizal structure in the roots. The technique of staining the root is modified from the standard method for optimal visualization of arbuscular mycorrhizal structure. The brand of stamp pad ink used is economically feasible and also its easy availability may boost the research activity of arbuscular mycorrhizal fungi.

Key words: Arbuscular mycorrhizal fungi · staining · stamp pad ink

INTRODUCTION

The modifications to standard clearing and staining procedures of mycorrhizal roots have been proposed for poor contrast images and safety reasons [1]. In an attempt to eliminate some of the hazardous effect and cost of these chemicals, a procedure [2] for staining of arbuscular mycorrhizal fungi in roots has already been made to stain arbuscular mycorrhizal fungi with ink and vinegar. We report the use of stamp pad ink could be adapted for staining arbuscular mycorrhizal structure in the roots.

MATERIALS AND METHODS

Ten seedlings of healthy root system of *Michelia champaca* Linn. of 40-50 cm heights were collected from nursery of Social Forestry Division, Upper Shillong, Meghalaya. The seedlings were six months old grown on soil collected from the nearby areas of the nursery in the polythene containers size of 12x20cm.

The method is modified from standard procedures [2-5]. The roots were normally cleared in 10% (w/v) KOH kept overnight at room temperature. The roots were then bleached in alkaline hydrogen peroxide in the water bath at 70°C for 15 minutes. The roots were acidified with vinegar and roots were directly stained from this solution with Faber Castell black stamp pad ink. Adding 30 ml of ink in 70ml of distilled water does the preparation of the suitable concentration of the stamp pad ink. The root material was kept in staining solution at 90°C for 1 hour and rinsed the samples with two changes of tap water followed by keeping immersed in tap water for 5-20 minutes. The stained roots were mounted in lacto glycerol. The light microscopy of the samples was observed under Leitz Wetzlar Germany 513467.

RESULTS AND DISCUSSION

The staining of arbuscular mycorrhizal fungi with the Faber Castell stamp pad ink shows excellent results (Fig. 1a-d). The intraradical hyphae of arbuscular mycorrhizal fungi exhibit modifications dependent on their locations within the cortex. The intraradical hyphae hypertrophic hyphae, called vesicles (Fig. 1a), intracellular hyphae (Fig. 1b) and intracellular hyphae with many ramifications, known as arbuscules (Fig. 1c and d) are visible.

The method with black stamp pad ink still further reduces health risks. The method [2] reporting several pen inks are not available in the South Asian countries and also the ink used in this method is used for rubber stamp. The low cost availability of this stain is also reliable to carry out experimental research and teaching exercises in the developing country like India. Moreover, this very simple technique often results in high quality images of visible competitive interactions at early stages of the plant growth by arbuscular mycorrhizal fungal colonization and symbiosis that may

Corresponding Author: Dr. Panna Das, Laboratory of Microbial Ecology, Centre for Advance Studies in Botany, School of Life Sciences, Mawkynroh Umshing, North Eastern Hill University, Shillong 793 022, India

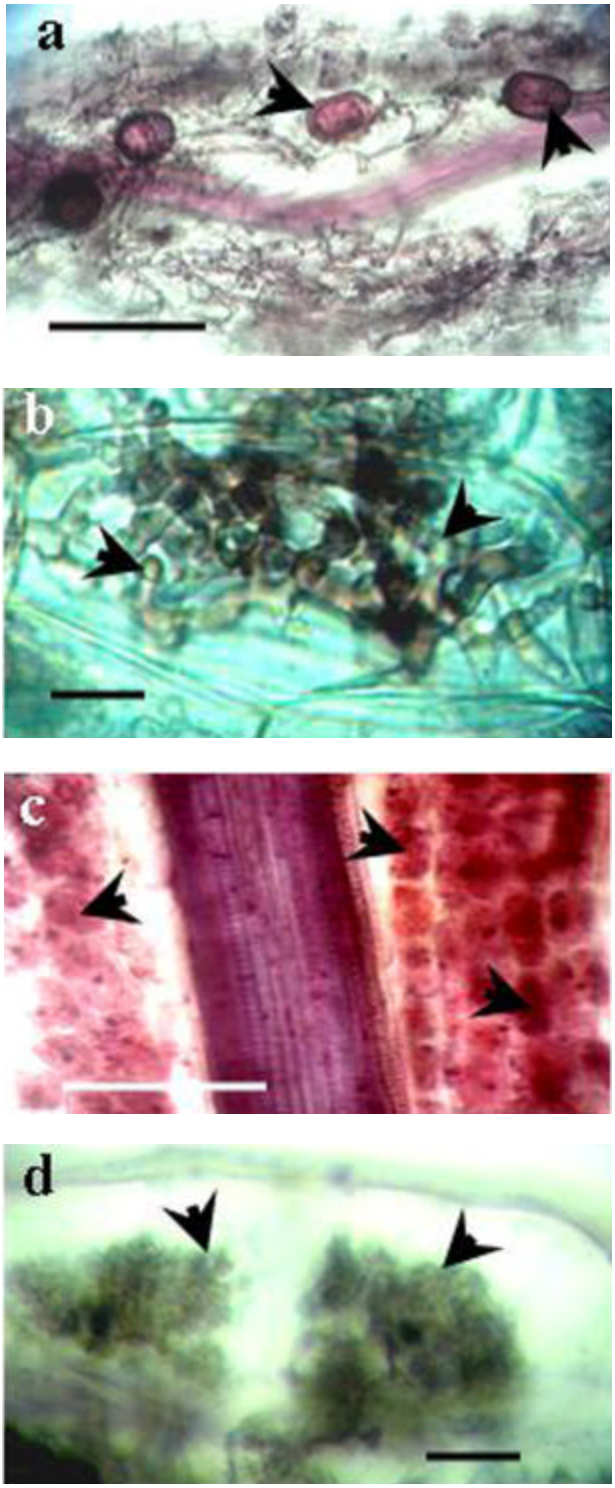

Fig. 1: Light photomicrographs (a-b) of arbuscular mycorrhizal colonization of roots of *Michelia champaca* L. stained with Faber Castell stamp pad ink. (a) Portion of root showing vesicles. Bar = 40 µm. (b) The intracellular hyphae exhibiting finger-like projections. Bar = 25 µm. (c) Arbuscules colonizing the segment of the root. Bar = 50 µm. (d) Distinctly visible arbuscules. Bar = 25 µm

Photomicrographs of Fig. c and d were taken after two weeks of staining of roots

be used in a routine way for staining of other root colonizing fungi in different plant species.

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