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Preparing and Staining Mycorrhizal Structures in Dry Bean, Sweet Corn, and Wheat Using a Block Digester

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ABSTRACT

The use of safe staining techniques in the evaluation of mycorrhizal colonization is critical to the continued understanding of this important symbiosis. Several procedures being utilized currently involve regulated and/or toxic chemicals. The integration of unregulated and nontoxic chemicals into these procedures is important to alleviate potential dangers currently used chemicals pose. We eliminated all regulated reagents by combining portions of several previously published staining and root preservation procedures. A block digester for plant tissue digestion was used as a heating unit and proved to be easier to use, quicker and more reliable than either a water bath or a circulating air oven. Optimum clearing time in KOH varied from 8 to 10 min and 30 to 40 min for wheat, sweet corn, and dry bean roots,

respectively. We also successfully used both drying and freezing of roots for storage prior to staining. These modified procedures were quick and easy and provided reliable temperature control and excellent staining while protecting individuals and the environment from toxic chemicals.

INTRODUCTION

The use of preservation and staining techniques to evaluate arbuscular mycorrhizal fungi (AMF) is an important and common aspect of mycorrhizal research. Present knowledge in staining techniques is solid; however, many standard techniques require the use of chemicals that are regulated by COSHH (Control of Substances Hazardous to Human Health Regulations, 1988). Recent research efforts have made it possible to recommend alternative materials that are safer and more convenient to use and reduce the use of compounds such as phenol and trypan blue (Koske and Gemma, 1989; Grace and Stribley, 1991). Staining techniques also vary in equipment used and overall time efficiency. We attempted to find a fast, safe, and high quality staining procedure to study wheat (*Triticum aestivum* L.), corn (*Zea mays* L.), and dry bean (*Phaseolus vulgaris* L.) root mycorrhizal colonization. To manage heating time and temperature control, we utilized a plant tissue digestion heating block as a heat source and to reduce regulated chemical use, we combined staining techniques from Koske and Gemma (1989) and Grace and Stribley (1991). We also used simplified preservation procedures from Hetrick et al. (1989) and Mayer et al. (1991). We found these combinations beneficial and believe others conducting similar research may benefit from our studies.

DISCUSSION

Preservation

Root samples were preserved for future analysis by either drying or freezing. Dry bean roots from a greenhouse study were cleaned and dried (Hetrick et al., 1989) at 65°C and stored in paper sampling bags. Prior to staining, samples were placed in petri dishes over night and rehydrated in water. Wheat and corn root samples collected during a field study were cleaned and placed in plastic zip-lock bags and frozen in a conventional household freezer (-20°C) (Mayer et al., 1991). Roots remained in the freezer an average of one week. Prior to staining, roots were allowed to thaw. The use of these preservation techniques was fast, simple, and eliminated the use of regulated fixing chemicals that can be harmful to the environment.

Block Digester

Heating equipment such as circulating air ovens and water baths are commonly

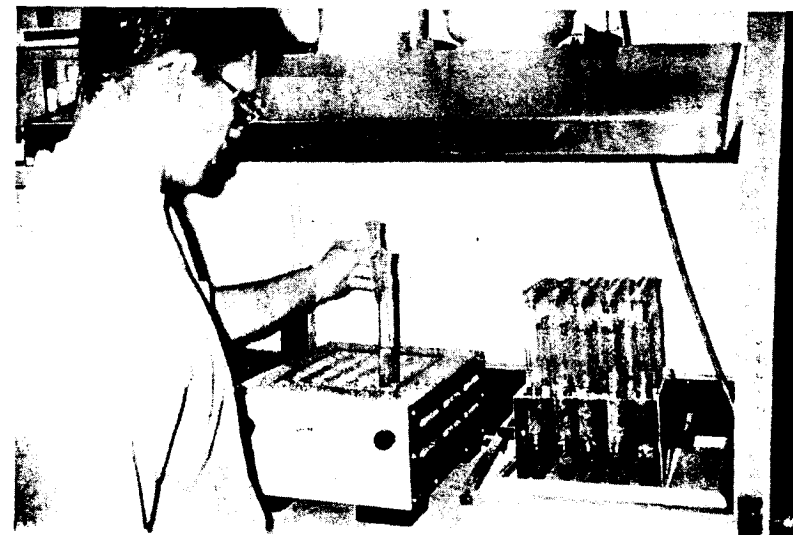


FIGURE 1. Clearing of cortex cells and staining of mycorrhizal structures in dry bean, sweet corn, and wheat roots using a block digester was quicker, more reliable, and provided more precise regulation of temperature and time than a water bath or a circulating air oven.

air oven temperature was difficult to calibrate and the water bath was small, had poor temperature control, and the time required to preheat water and heat up the samples was too long and inconvenient.

A standard plant tissue analysis block digester with temperature control was used as our heating unit (Figure 1). Most labs have access to plant tissue analysis block digesters and consequently to a handy heating unit. The digester was found to be easier to use, required less time, and was more reliable than either the oven or water bath. The temperature control was easy to calibrate and to maintain at a constant temperature. Thus the block digester proved to be a quick, easily managed, and efficient means of heating root samples for clearing and staining. More root samples could be stained using the block digester than using the small water bath. The 110 mL block digestion tubes were used to clear, acidify, stain, and destain the root samples. The block digester temperature was calibrated at 90°C for clearing and staining. The unit was easily placed in a ventilation hood to reduce potential problems with caustic KOH fumes.

Root Clearing and Acidification

Except for the block digester and the variable time required for clearing for

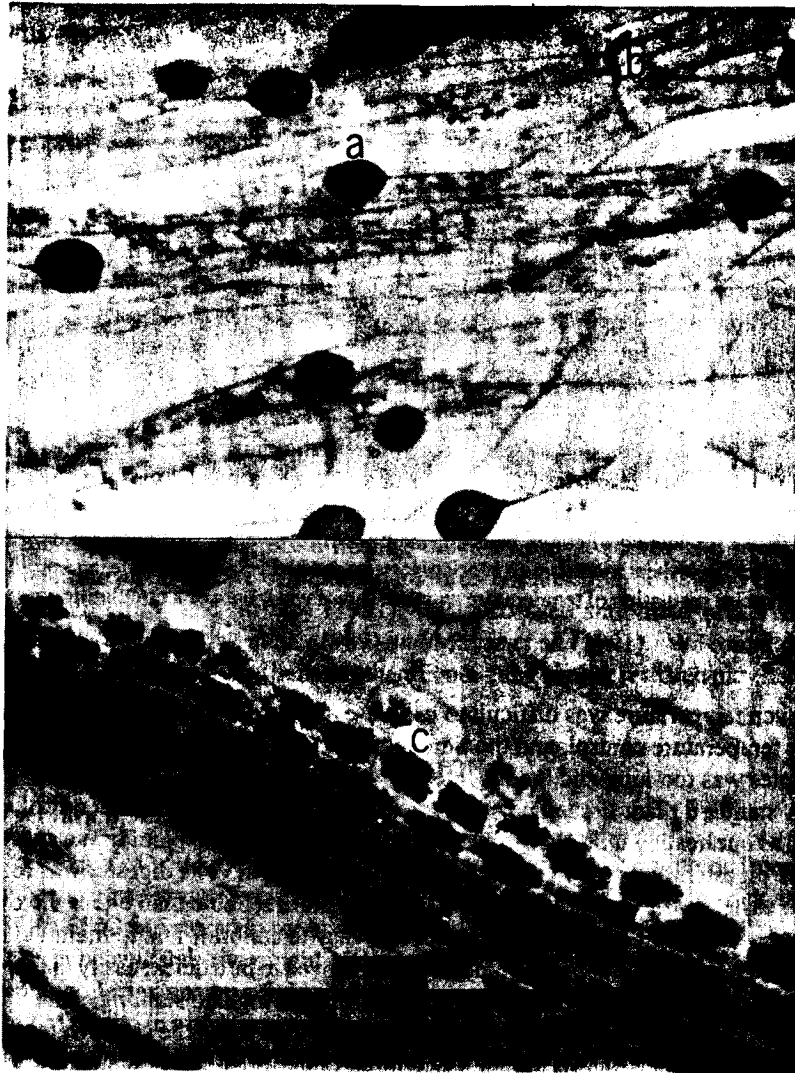


FIGURE 2. Mycorrhizal vesicles (a), intercellular hyphae (b), and arbuscules (c) in sweet corn roots cleared with KOH and stained with aniline blue using a block digester.

Gemma (1989). Root samples were placed in 110 mL block digestion tubes and submerged in approximately 30 mL of KOH (2.5%). Tubes containing root samples were placed in a block digester preheated to 90°C. It took approximately 8 to 10 min for the samples to heat up to 90°C. The time each of the three crop roots spent clearing in the KOH at 90°C varied from 30 to 40 min for dry beans, 20 to 25 min for corn, and 8 to 10 min for wheat. The wheat root cortex cells were more susceptible to degradation by KOH than either corn or dry bean roots. Degradation of the wheat cortex cells resulted in their separation from the steele, making it difficult to recognize mycorrhizal structures. This could be controlled by limiting time in KOH. Corn root cortex cells remained intact even after extensive clearing in the KOH (up to 90 min), but such extensive clearing resulted in poor staining of mycorrhizal structures (also controlled by limiting time in KOH). After the completion of root clearing, KOH was decanted. Each sample was rinsed three times with water to remove excess KOH and acidified with excess 2% HCL (30 mL).

Staining and Destaining

Because of its low toxicity and unregulated status and its proven ability to be a good stain for viewing mycorrhizal structures (Figure 2), aniline (cotton) blue was used to stain mycorrhizal structures within the root samples (Grace and Stribley, 1991). The 2% HCl from acidification was first decanted and a 0.05% aniline blue/acidified glycerol stain was added to each sample. Wheat, corn, and dry bean root samples were submerged in the staining solution and heated for 10 to 15 min at 90°C. After staining was complete, the stain solution was decanted and approximately 30 mL of an acidified glycerol destain (500 mL glycerol, 450 mL H₂O, 50 mL 1% HCl) (Koske and Gemma, 1989) was added to each sample. The samples were stored in the dark in the digestion tubes and the destain solution until percent colonization was determined (generally 1-2 days).

CONCLUSIONS

Preservation of the roots by either drying or freezing precluded the use of chemical preservatives. The use of a common plant tissue block digester as a heating unit was quicker, easier, and more reliable, convenient and consistent than either the water bath or the circulating air oven. The use of aniline blue as a stain was convenient because it is low in toxicity and unregulated, requires no special discarding regulations, stains mycorrhizal structures effectively and makes determination of percent colonization reliable. The roots of wheat, corn, and dry bean required different times in heated 2.5% KOH for effective clearing. This clearing time was important due to the effects of the KOH on the integrity of the cortical cells and stainability.

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