JOURNAL OF THE TENNESSEE ACADEMY OF SCIENCE 72(3–4):63–64 JULY–OCTOBER 1997

USE OF POLYETHYLENE GLYCOL TO PRESERVE PLANT TISSUES CULTURED IN VITRO

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ABSTRACT—A technique for preservation of plant tissues cultured in vitro involves fixation with formalin-acetic acid-alcohol followed by infiltration with polyethylene glycol 400. Plant tissues removed from culture and preserved at successive stages of an experiment can be compared directly, enabling documentation of developmental changes that may occur over time. Results also can be compared among experiments repeated over time. Tissues infiltrated with polyethylene glycol remain hydrated during short-term removal from their aqueous environment for photographic sessions or other data-collection manipulations. Tissues can be stored in polyethylene glycol for several months or longer.

Quantitative data from plant tissues cultured in vitro frequently must be recorded at prescribed intervals or at a particular developmental stage. Similar constraints exist for photographing cultures to provide permanent visual records. However, manipulation of cultures cannot always be conducted at a prescribed time. Adaptation of a method that utilizes infiltration of tissue with polyethylene glycol 400 (Graham et al., 1987) makes it possible to preserve explants and cultured tissues and, subsequently, remove them from storage for manipulations and visual comparisons. Polyethylene glycol 400 enables tissues to remain hydrated while temporarily removed from storage in polyethylene glycol 400. Intact specimens ≤1-cm thick can be preserved.

MATERIALS AND METHODS

The technique has been tested on tobacco and celery callus cultures. The procedure for preservation is as follows:

1. Fix specimens ≤1-cm thick in 50% formalin-acetic acid-alcohol (Johansen, 1940).
2. Allow callus to stand in three successive changes of 50% aqueous ethanol to eliminate formaldehyde and acetic acid. The time required for tissue penetration will depend on its thickness.
3. Immerse callus in a 1:1 mixture of 50% aqueous ethanol and polyethylene glycol 400 and let stand for 24 h.
4. Incubate uncapped specimen bottles at 60°C just until the odor of ethanol is no longer detectable.
5. Transfer callus into final storage solution, 80% v/v polyethylene glycol 400 (water as solvent), and recap bottles.

RESULTS AND DISCUSSION

Polyethylene glycol allows the general shape, size, and surface features of explants and cultured plant tissues to be preserved (Fig. 1). It prevents dehydration and associated shrinking and hardening of tissue surfaces. Tissues infiltrated with polyethylene glycol, unlike tissues infiltrated with formalin-acetic acid-alcohol alone, remain hydrated during exposure to air. Toxicity of polyethylene glycol is low, and it does not support mold growth on tissues during storage (Windholz, 1983). However, the association between tissue surfaces and polyethylene glycol gives surfaces a glossy appearance when removed from the storage solution.

Specimens can be easily preserved at any arbitrary point or any stage in their development. Thus, data collection or photography can be delayed until enough specimens have been collected to make the task efficient. Tissues from early and middle stages of an experiment can be preserved for direct comparison with specimens left in culture for several months longer. The direct, side-by-side comparison of tissues removed from culture at successive stages of an experiment enables developmental changes to be clearly documented. Preservation also makes it possible to visually compare tissues from experiments replicated over time.

ACKNOWLEDGMENTS

Support or technical assistance from the following individuals is gratefully acknowledged: E. T. Graham; W. A. Skroch; C. E. Anderson; T. P. McCracken; J. M. Stoop.

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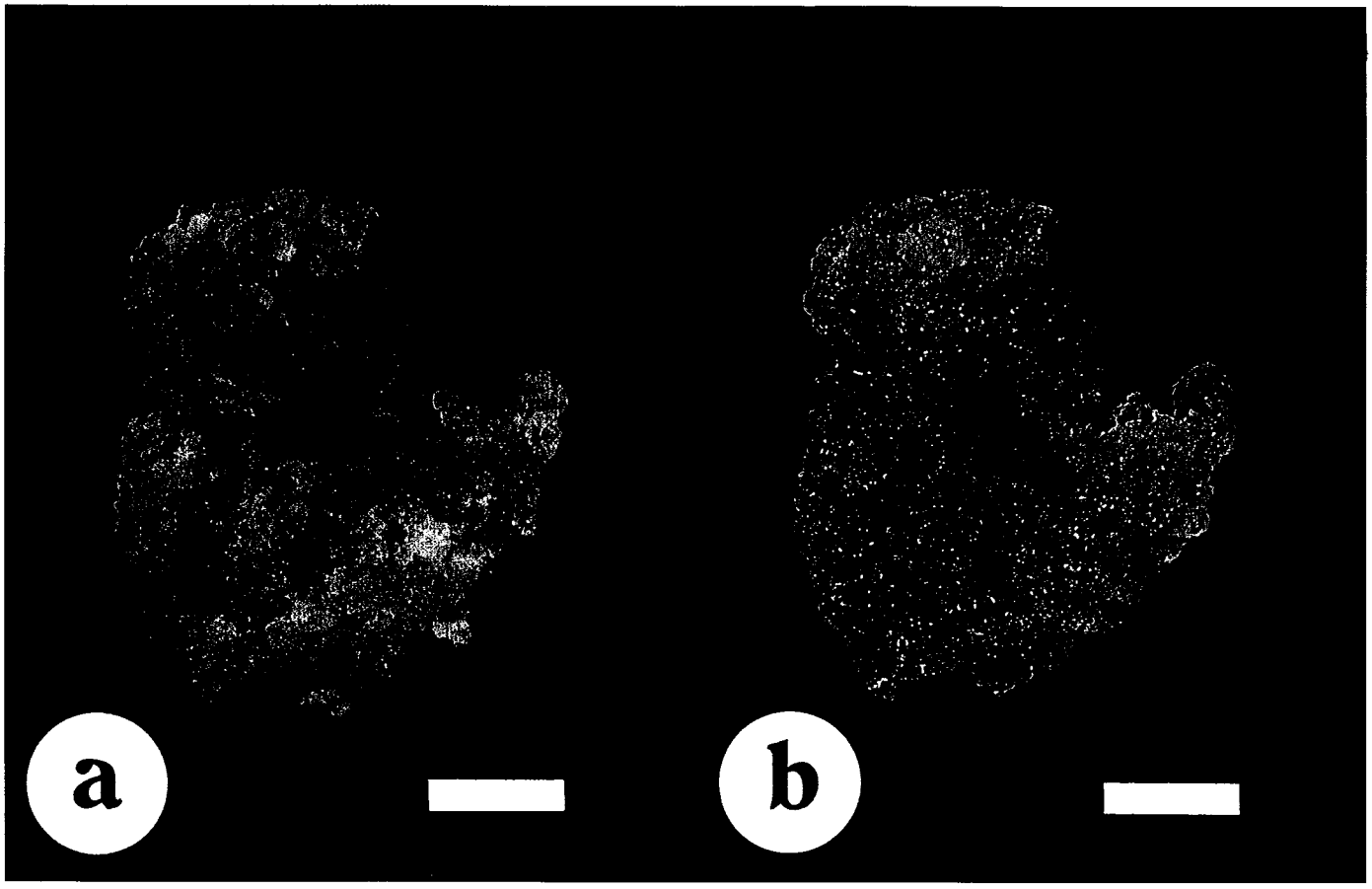

FIG. 1. Tobacco callus (a) removed directly from culture and (b) fixed with formalin-acetic acid-alcohol, infiltrated with polyethylene glycol 400, and stored in 80% polyethylene glycol. The reduced contrast in "b" is due to loss of pigmentation during the fixation process. Scale bars = 5 mm.