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A Method to Preclude Moisture Condensation in Plated Tissue Cultures

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SUMMARY

Excessive condensate normally accumulates in in vitro-illuminated petri dishes containing plant tissue cultures, causing a variety of problems. A dark-colored rubber net-mesh placed over the petri dishes prevented such condensation, even when charcoal-supplemented media are used under high light intensity in a growth chamber.

INTRODUCTION

Petri dishes containing agar-solidified media are commonly used in microbiology as well as in plant and animal tissue culture. However, the illumination employed to satisfy the photosynthetic light requirements of higher plant tissues frequently leads to the accumulation of condensate under the petri dish lids. This problem is compounded by the need to seal the rim of covered dishes during the standard several-week tissue incubation periods to prevent desiccation of the medium and to maintain asepsis. During these periods, condensate accumulates and eventually drips onto the medium. The resultant pools of moisture not only alter the environment of plant tissues in situ, but may cause the spread of any contaminant microorganisms from small, isolated colonies otherwise available for selective removal. Free moisture has also been implicated in the development of vitreous tissues (von Arnold and Eriksson 1984). Moreover, this condensate restricts both the visual examination of cultures and their photography through the covered petri dish. Stacking one or two empty petri dishes atop the dish containing the tissues is common practice and sometimes helps reduce the formation of condensate. However, the use of charcoal-supplemented media (Amerson and others 1988) nullifies any benefit this practice might have; evaporation of water from the illuminated (and therefore slightly warmed) black substrate presumably accounts for the unusually large amounts of condensate that accumulate.

The inexpensive and simple method described in this article completely precludes the accumulation of condensate even in charcoal-supplemented media and under conditions of high light intensity.

MATERIALS AND METHODS

We observed that condensate will characteristically not accumulate contiguous to any black (magic marker) lettering used for tissue treatment identification on the petri dish lid surface. This led to the suspicion that light absorption by the dark lettering, and the resultant local and slight warming of the petri dish plastic lid 2 to 3 mm on either side of the ink line, precluded the accumulation of condensate. Therefore, 115 mm^2 sheets of a black, soft-rubber mesh (Softliner, Nalle Plastics, Austin, TX) were placed directly upon Parafilm-sealed plastic petri dishes in a 20 °C growth chamber under 100, 150, and 250 micro-Einsteins meter^{-2} second^{-1} ($\mu Em^{-2} s^{-1}$) cool-white

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fluorescent illumination. The diamond-shaped mesh network consisted of 6 × 10 mm openings bordered by a 2 mm-thick mesh. Petri dishes contained loblolly pine (*Pinus taeda* L.) adventitious micropropagules on nutrient media with and without 1 percent (w/v) activated charcoal. A second group of plates containing similar propagules was similarly illuminated, either directly or by having two empty petri dishes stacked on top of each other.

All plates were examined for the presence of condensate or free water following a 2 week incubation. Light intensities incident upon the petri dish lid surfaces were measured with and without the intervening Softliner.

RESULTS AND DISCUSSION

Plates containing medium with or without activated charcoal and illuminated (all intensities) with no intervening empty petri dishes showed a large amount of condensate on both the inner surface of the lid and the medium surface, the latter having dripped from the lid. Plant tissues in plates containing the charcoal-supplemented medium were thus obscured from view. Plated media stacked with empty dishes were less wet than were the directly illuminated plates. All plates covered with the Softliner mesh were free of water on all inner surfaces. The mesh reduced light intensity on the petri dish lids 40 percent; light intensities effective for in vitro micropropagation of *Pinus* (Amerson and others 1988) and other genera (Diner 1990) could therefore be maintained employing incident illumination of at least 150 $\mu E\ m^{-2}\ s^{-1}$. Pine propagules in plates covered by the Softliner grew and developed normally.

The use of Softliner or a similar product should benefit research in plant tissue culture employing tissue containers that otherwise would accumulate unwanted condensate from the agar-solidified, aqueous medium. A mesh color other than black may prove advantageous under circumstances of extreme tissue temperature-sensitivity where conversion of radiant to thermal energy by the Softliner would be less efficient.

LITERATURE CITED

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