Negative hydrostatic pressure is an unnoticed but significant source of contamination in tissue culture©

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INTRODUCTION

Plants are characterized by a negative hydrostatic pressure, brought about by transpiration and by capillary activity of xylem vessels (Taiz and Zeiger, 2010). Because of this, a stem that is being cut sucks up what is nearby. Often this is air but it may also be liquid. The diameter of the xylem vessels is 50-100 µm, so when the liquid contains bacteria (that are typically 0.5-5.0 µm), they will enter deeply into the tissue (Askari et al., 2014; De Klerk et al., 2014). To our knowledge, this alleged source of contamination has never been examined.

MATERIALS AND METHODS

Lily (*Lilium*) scales were detached from bulbs that were submerged in either water or 0.03% NaClO. It had been established before that 0.03% NaClO does kill all bacteria in liquid medium. After that, explants were cut from the scales and cultured for 12 weeks under standard conditions to regenerate bulblets (Figure 1; Aguettaz et al., 1990). Contamination was scored weekly: after 5 weeks hardly any additional contamination was observed.

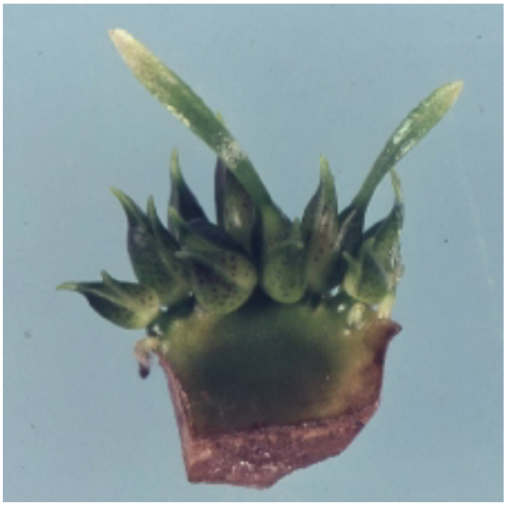

Figure 1. Bulblets regenerating from lily scale explants.

RESULTS

Detaching scales from bulbs that were submerged in a solution of acid fuchsin showed that the scales did suck up neighboring liquid (Figure 2). Detaching scales from bulbs submerged in sterilising liquid (0.03% NaClO) strongly reduced contamination (Figure 3). This shows that sucking up of liquid is a source of contamination. NaClO had no effect on the regeneration percentage and the number of regenerated bulblets but the weight of the regenerate bulblets was ca. 20% higher (Figure 4).

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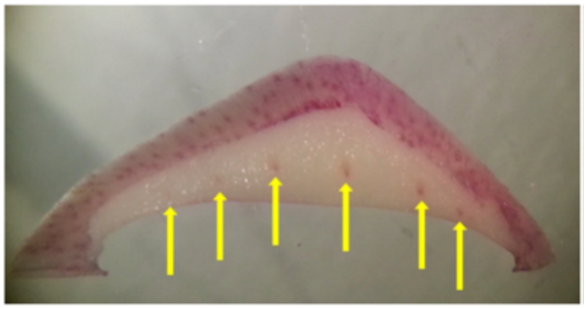

Figure 2. When scales were detached from bulbs submerged in a solution of acid fuchsine, the dye penetrated within seconds for ca. 1 cm into the scale. This demonstrates the occurrence of negative hydrostatic pressure in lily scales.

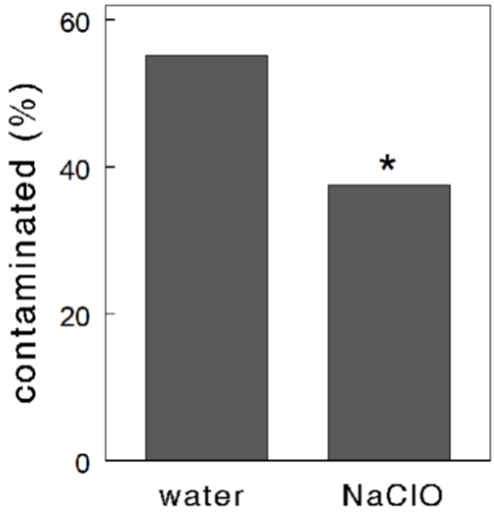

Figure 3. When scales were detached from bulbs submerged in 0.03% NaClO instead of water, contamination was strongly reduced.

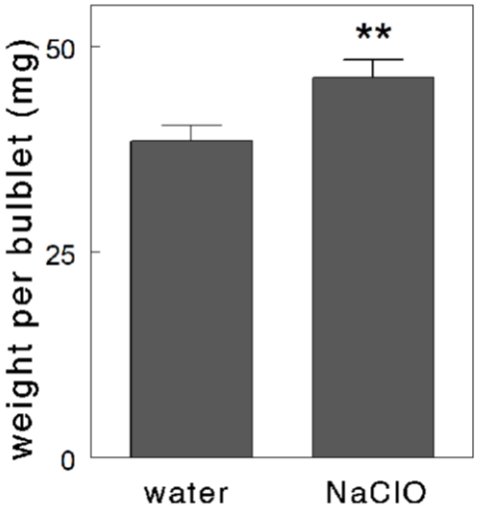

Figure 4. The bulblets regenerated from scale segments cut from scales that had been detached when submerged in 0.03% NaClO, had a significantly higher weight.

DISCUSSION AND CONCLUSION

In conclusion, the negative hydrostatic pressure is a major source of contamination. This problem can be easily overcome by detaching the scale from bulbs submerged in 0.03% NaClO. It should be noted that microorganisms are sucked up deep into the tissue so that they escape from the disinfectant during surface sterilization. It should also be noted that the extent of contamination depends on the presence of liquid at the cut surface and will be low when the explant is relatively dry. The same mode of contamination will occur in all plants and also when preparing conventional cuttings.

ACKNOWLEDGEMENTS

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