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Tissue Culture of Grape Hyacinth

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Abstract. **Laboratory exercises are described to familiarize students with procedures for preparing a sterile nutrient medium and for culturing bulb scales of grape hyacinth (*Muscari* spp.) *in vitro*.**

Micropropagation by plant tissue culture is the development of new plants in an artificial medium under aseptic conditions from very small pieces of plants, such as embryos, seeds, stems, shoot tips, root tips, callus, single cells, and pollen grains (1, 5). Tissue culture techniques are becoming important for rapid multiplication and maintenance of a wide range of heterozygous plants (2). These techniques can be applied to ensure disease free conditions and establish pathogen-free plants, and to isolate genetically unique cultivars (1). A clear idea of some *in vitro* methods can be realized by the student in the following laboratory exercise. It involves perparation of a medium and culture of bulb scales of grape hyacinth, *Liliaceae* family, a plant that responds rapidly in culture and is thus very suitable for a laboratory experiment in a one-term course in horticulture or plant science.

This laboratory exercise has been designed for 2 periods, followed by a 4-week observation period.

First laboratory period. During the first period, a nutrient culture medium is prepared. Stock solutions of the macro and micro salts of Murashige and Skoog (3) may be prepared by the instructor in advance of the laboratory (Table 1) and stored in a refrigerator. Prepackaged MS salt mixes (provided by Gibco Inc. in USA, or Gibco Canada) may be used. Before using, solutions should be allowed to adjust to room temperature. Iron-containing solutions should be stored in amber-colored bottles (4). Preparation of the iron stock solution (Fe $SO_4 \cdot 7H_2O$) with a chelating agent (sodium ethylenediaminetetraacetate) should prevent precipitation of iron; chelated iron also is readily translocated in plant tissues.

Each student should prepare one liter of nutrient medium by referring to Tables 1 and 2. The pH should be adjusted to 5.5 before the medium is solidified with 2.2 g/liter Gelrite (Kelco, Division of Merck & Co. Inc), an agar substitute. Glass bottles (e.g., 120 ml) with plastic autoclavable covers provide suitable culture vessels. Nutrient medium (e.g., 50 ml) should be added to each, and the medium should be autoclaved for 20–25 min at 120°C and 1 kg/cm^2.

Second laboratory period. The second period involves culturing sections of grape hyacinth (*Muscari armeniacum* Baker) bulb scales (explants) in sterile conditions on the prepared medium. Because plant parts have microorganisms on their external surfaces, they must be disinfected before transferring to the sterile nutrient medium. After removing the outer surface layers from a grape hyacinth bulb, the individual scales may be pulled away (Fig. 1). These should be washed gently with soapy water and then rinsed for 30 min in running tap water. Surface steriliaztion is accomplished by submerging the washed bulb scales for 20 min in 0.6% sodium hypochlorite, then rinsing the scales once in sterile double distilled water. As an extra precaution against contamination, bulb scales should be submerged in 0.1% mercuric chloride for 10–15 sec and rinsed 3 times with sterile double distilled water.

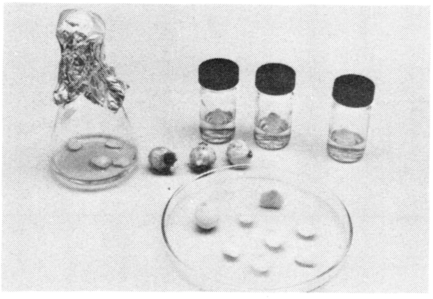

Fig. 1. Bulbs of grape hyacinth with and without the outer surface layer and cut bulb scales that will be the cultured explant. Liquid medium and semi-solid medium culture vesels are illustrated.

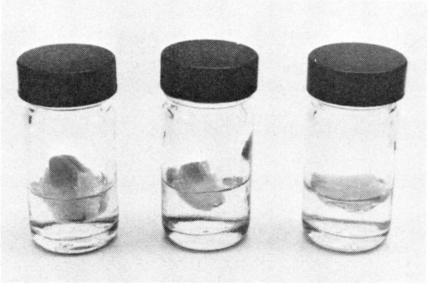

Fig. 2. Swelling of explants after 10–14 days. Explants were placed on the medium in an upright, inverted, and sideways orientation.

Ideally, explants should be prepared in the sterile environment of a laminar flow transfer chamber, but other simple transfer cabinets can be used. Each culture vessel should be numbered so that specific treatments can be identified: for example, the orientation of the tissue in the medium, the environmental regime in which the tissue is to be kept (photoperiodic regime, light intensity and quality, temperature). Records of dates of planting and subcultures also should be kept.

Table 1. Stock solutions of Murashige and Skoog (3) macro and micro salts.

Chemical constituent		Amount of chemical required per liter for a 1×10^{-3} M stock solution (g)	Volume of stock solution required for one liter of medium (ml)
Macronutrients			
NH_4NO_3		165.0	10
KNO_3		190.0	10
$CaCl_2 \cdot 2H_2O$		44.0	10
$MgSO_4 \cdot 7H_2O$		37.0	10
KH_2PO_4		17.0	10
Micronutrients			
$FeSO_4 \cdot 7H_2O$	for one stock	2.78 +	
Na_2EDTA	solution	3.72	10
H_3BO_3		0.62	10
$MnSO_4 \cdot H_2O$		1.69	10
$ZnSO_4 \cdot 7H_2O$		0.86	10
KI		0.083	10
$Na_2MoO_4 \cdot 2H_2O$		0.025	10
$CuSO_4 \cdot 5H_2O$		0.0025	10
$CoCl_2 \cdot 6H_2O$		0.0025	10

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Table 2. Constituents of the nutrient medium to be added to Murashige and Skoog's (full strength) salts (3) to provide a 1 liter aqueous solution.

Constituent	Amount
Thiamin HCl	1 mg
Nicotinic acid	1 mg
Pyridoxine HCl	1 mg
Myo-inositol	50 mg
Sucrose	30 g
Adenine sulphate	62.50 mg
Naphthaleneacetic acid (NAA)	2 mg
6-Benzylaminopurine (BA)	5 mg
Gelrite	2.2 g

Table 3. Response of bulb scale segments in darkness and light (30 μmol s^{-1} m^{-2} cool-white fluorescent) at 25 °C. Data determined 4 weeks after start of cultures.

Orientation of bulb scale segments	No. of shoot primordia		Root formation		Callus formation	
	Dark	Light	Dark	Light	Dark	Light
Upright	3.5 (white)	21.5 (green)	Good	Scant	+	+
Inverted	1.0 (white)	20.5 (green)	Good	Scant	+	+
Sideways	15.0 (white)	26.5 (green)	Good	Scant	+	+

Experiments that we have conducted in student classes indicate that 25°C is a more suitable temperature than 20°, that more shoot or bulblet primordia are formed in light (e.g., 30 μmol/ $s^{-1}m^{-2}$ cool-white fluorescent) than in darkness, and that root formation can be improved in darkness (such results can be tabulated as in Table 3). The bulb scales will start to swell and produce shoot or bulblet primordia in 10 to 14 days at 25° (Fig. 2), and a total period of 4 weeks is sufficient for callus formation, shoot or bulblet and root formation, depending on hormonal concentrations. A higher auxin: cytokinin ratio will promote callus and root formation. addition of one g/liter activated charcoal to the medium promotes good bulblet formation.

The laboratory outline allows for study of tissue regeneration and differentiation *in vitro*. Students could carry their studies further by culturing parts of the inflorescence primordia taken from the bulb (that is, perianth members, ovaries or anthers), or, the leaf primordia, all of which are amenable to culture. The constituents of the medium, particularly the phytohormones, could be varied. Also, a liquid rather than a semi-solid medium may be used. Small organs, such as the flower parts, are more amenable to culture in a liquid than on an agar medium. If time allows, subculturing culd be done to enhance bulblet and root formatioon before transferring to a soil mix. Subsequent growth and development of the bulbs could then be studied. Fig. 3 illustrates good shoot formation after 6 to 8 weeks in culture. Fig. 4 illustrates good bulblet production after 8 to 12 weeks on a medium containing charcoal.

This laboratory work should illustrate how undifferentiated tissue can be induced to produce callus and or root and shoot or bulblet formation. Using a binocular dissecting microscope, differences can be seen clearly between the formation and growth of individual roots, shoots, and bulblets. The apical meristem on the shoot or bulblet primordium is exposed initially, but it is then gradually enfolded within the leaf primordia and the developing basal shoot or bulb structure.

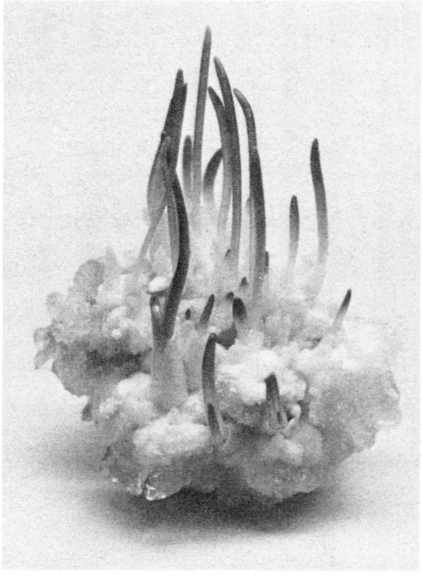

Fig. 3. Shoot production that might be expected after 6 to 8 weeks in culture. This tissue was removed from the culture environment for photographing.

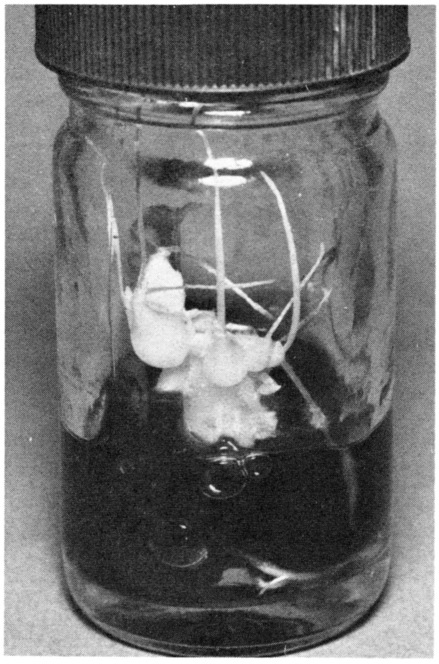

Fig. 4. Bulblet production that can be obtained after 8 to 12 weeks in culture when activated charcoal is incorporated in the original medium.

Literature Cited

1. Hartmann, H.T. and D.E. Kester. 1983. Plant propagation, principles and practices. 4th ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.
2. Hussey, G. and A. Falavigna. 1980. Origin and production of *in vitro* adventitious shoots in the onion, *Allium cepa* L. J. Expt. Bot. 31:1675–1686.
3. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497.
4. Murashige, T. 1973. Sample preparations of media. In: P.F. Kruse and M.C. Patterson (eds.) Tissue culture, methods and applications. Academic Press, New York.
5. Reinert, J. and Y.P.S. Bajaj (eds). 1977. Applied and fundamental aspects of plant cell, tissue and organ culture. Springer-Verlag, Berlin, Heidelberg, New York.