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In Vitro Propagation of Hyacinth[1, 2]

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Abstract. **Numerous bulblets were obtained directly from flower bud tissue of *Hyacinthus orientalis* L. cvs. Anna Marie and Delft Blue on medium containing Murashige and Skoog salts, vitamins, glycine, *i*-inositol, sucrose, and agar plus 3 mg/liter 6-benzylamino purine (BA) and 0.3 mg/liter naphthaleneacetic acid (NAA), both in darkness and in light. Root formation occurred when individual bulblets were separated and transferred to a medium containing 0.1 mg/liter NAA. Over 90% of bulblets of both cultivars grown on this medium were successfully transferred to a soil mixture.**

Under normal conditions hyacinth bulbs produce very few offset bulblets. Propagation using scooping and scoring produces about 20–50 bulblets per bulb (4, 12). An efficient *in vitro* propagation system would accelerate this propagation rate (4).

In vitro propagation of bulb- and corm-forming plants has been achieved using explants from bulb scales, leaves, shoot tips, and inflorescences (2, 3, 5, 8, 9, 15). Regeneration of hyacinth by tissue culture has been achieved with varying degrees of success (4, 10, 13, 14). Pierik and Post (10) have found that regeneration of hyacinth bulblets from scale explants could result in 240–300 bulblets from 1 bulb but 10–20% of the cultures become infected with microbial contamination. In this paper we describe a method for *in vitro* propagation of hyacinths using explants from immature flower buds.

Callus and bulblet formation from flower bud and scape explants. Bulbs of 'Anna Marie' and 'Delft Blue' hyacinths, 6 cm in diameter, were planted in pots containing a mixture of 2 spagnum peat: 2 perlite: 1 soil (v/v/v) and placed in a cold room maintained at a constant temperature of 8° C until the chilling dormancy requirement had been satisfied. Individual flower buds (4–5 mm in length) and scape were excised from the center of the bulb and disinfested for 10 min under vacuum in a solution of 0.5% sodium hypochlorite (v/v) plus 0.1% Tween 20 (v/v). The flower buds were bisected longitudinally and the individual halves were cultured separately. The scape was sliced into cross sections 2 mm long and each section was placed in culture.

The basal medium contained Murashige and Skoog salts (7), and the following in mg/liter; *i*-inositol, 100; glycine, 2.0; nicotinic acid, 0.5; pyridoxine·HCl, 0.5; thiamine·HCl, 0.4; sucrose 30,000; and Bacto agar, 8,000. The pH of all media was adjusted to 5.7 ± 0.1 and medium was distributed (25 ml each) into 25 × 150 mm culture tubes. Media were autoclave sterilized for 15 min at 121°C and cooled as slants. All cultures were normally incubated at 26° and except where noted under 16 hr illumination at 1.3 klux (Cool White fluorescent lamps).

Freshly excised flower bud and scape explants were cultured on a medium containing the basal constituents plus BA (0.1, 0.3, 1.0, and 3.0 mg/liter) and NAA (0.1, 0.3, 1.0, and 3.0 mg/liter). Explants cultured on medium without BA and NAA failed to form callus or differentiate bulblets.

After 2 weeks, white compact callus was induced from 'Delft Blue' and 'Anna Marie' flower bud explants on media with NAA at 1 or 3 mg/liter and BA at 0.1 or 0.3 mg/liter. Callus initiation occurred more rapidly in darkness than in light; however, after 6 weeks in culture no difference in callus initiation could be determined. Callus recultured from these explants did not initiate bulblets under any of the culture conditions examined.

For both 'Delft Blue' and 'Anna Marie', bulblets were initiated on the cut surface of the cultured flower bud after 6 weeks on medium with 3.0 mg/liter BA and 0.3 mg/liter NAA (Fig. 1). Bulblet formation was greatest from flower buds cultured on medium with 3.0 mg/liter BA and 0.3 mg/liter NAA whether the cultures were grown in light or in darkness (Table 1). The rate of bulblet formation from flower bud explants of 'Delft Blue' was greater in light than in darkness, while multiplication of 'Anna Maria'

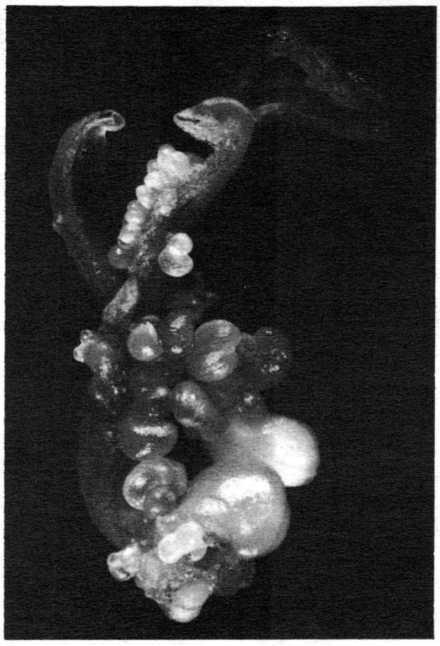

Fig. 1. Bulblet initiation from cultured 'Anna Marie' flower buds on medium containing BA (3 mg/liter) and NAA (0.3 mg/liter).

Table 1. Bulblet formation from cultured 'Anna Marie' and 'Delft Blue' flower buds on media with varying concentrations of BA and NAA.

BA (mg/liter)	NAA (mg/liter)	No. of bulblets[z]			
		Anna Marie		Delft Blue	
		Light	Dark	Light	Dark
1	0.1	13 ± 1[y]	14 ± 3	13 ± 1	18 ± 5
	0.3	13 ± 1	13 ± 1	21 ± 3	23 ± 3
3	0.1	15 ± 1	18 ± 3	19 ± 4	14 ± 4
	0.3	20 ± 2	48 ± 3	48 ± 7	30 ± 6

[z]5 to 10 explants per treatment
[y]± SE

Table 2. The effects of NAA and the BA concentration of the previous medium on the percent of bulblets of 'Anna Marie' and 'Delft Blue' which could be transplanted to soil.

NAA (mg/liter)	Previous growth medium concn of BA (mg/liter)	Anna Marie		Delft Blue	
		No. of bulblets	Transplantable bulblets (%)	No. of bulblets	Transplantable bulblets (%)
0	0	20	15	15	40
	0.1	38	3	18	56
	0.3	43	7	15	45
	1.0	46	9	18	61
	3.0	42	10	24	46
	10.0	32	16	16	69
0.1	0	15	93	13	100
	0.1	40	95	18	100
	0.3	33	100	18	100
	1.0	40	100	17	100
	3.0	39	100	20	100
	10.0	30	100	17	100

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[3]Plant material for this research was donated by Michigan Bulb Company, Grand Rapids, Michigan.
[4]This paper is dedicated to the memory of our colleague and friend, Yil-Jong Kim.

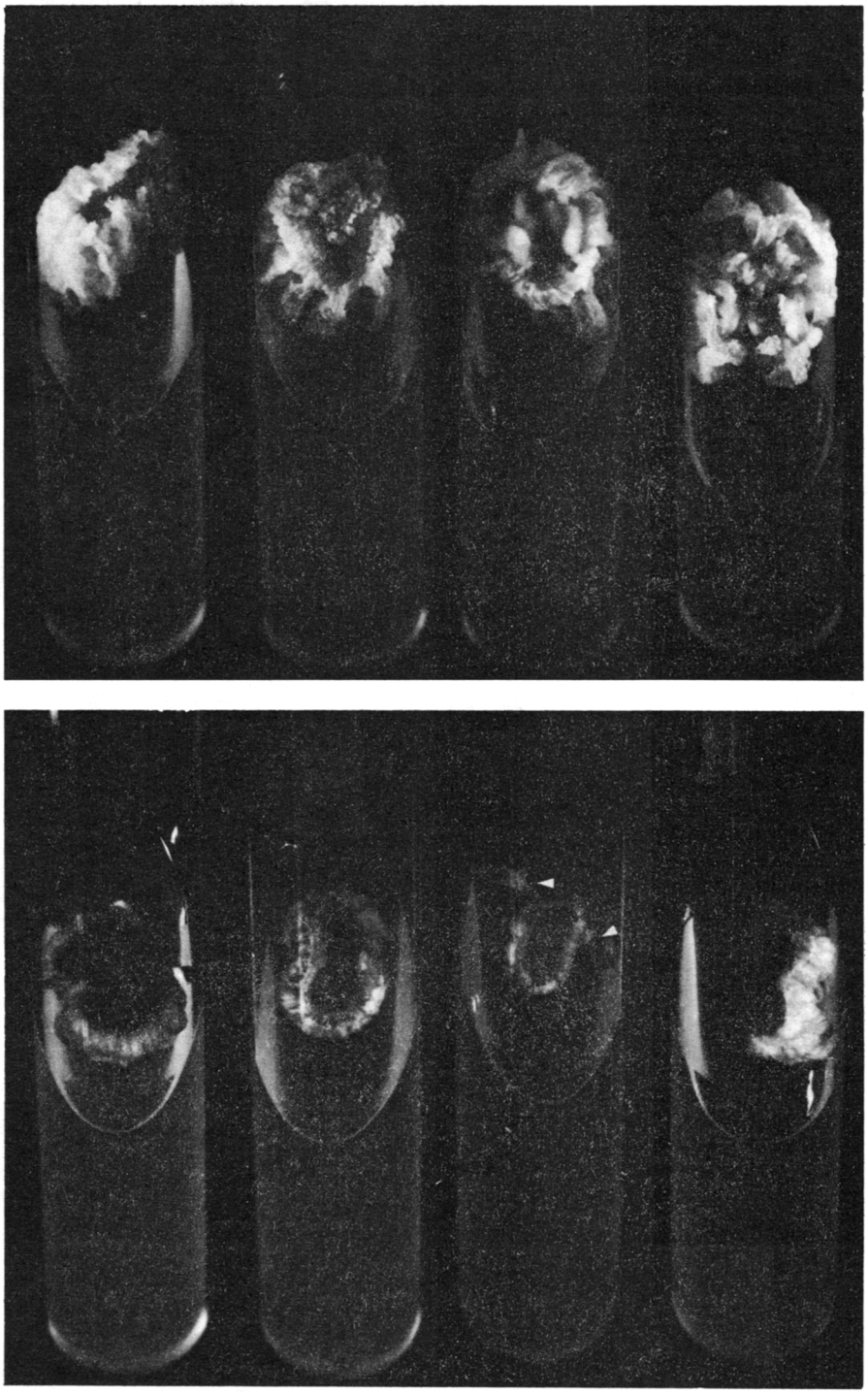

Fig. 2. Callus and bulblet initiation (arrows) from cultured scape of 'Anna Marie' as influenced by BA, NAA, light, and darkness. Left to right, in mg/liter: BA 0.1, NAA 1.0; BA 0.1, NAA 3.0; BA 0.3, NAA 1.0; BA 0.3, NAA 3.0. The top row of cultures were in darkness and the bottom row in the light.

was superior in darkness (Table 1).

After 4 weeks in culture, all scape explants of 'Anna Marie' and 'Delft Blue' produced highly friable callus in darkness and 33 to 40% of scape explants in light produced callus on medium with 0.1 or 0.3 mg/liter BA and 1 or 3 mg/liter NAA. No bulblet formation was observed at this point. However, bulblets were initiated from scape explants grown on media containing 1 mg/liter NAA and 0.1 or 0.3 mg/liter BA 8 weeks after implantation (Fig. 2). Callus formation was greater in darkness, but low intensity illumination increased the formation of bulblets (Fig. 2). Bulblet initiation from scape tissue was considerably lower than from flower bud tissue.

The initiation and growth of callus in bulb-forming plants is affected by the type and quantity of the auxin, and the response to auxin varies with the cultivar (5). Callus initiated from freesia corm tissue will form shoots or roots depending on the concentration of NAA in the medium on which the callus was initiated (1). This may explain our inability to stimulate bulblet formation from callus derived either from scape or floral tissue of hyacinth although, regeneration of plantlets from callus of 'Ostara' and 'Princess Irene' hyacinths has been reported (4). However, regeneration from callus may not be desirable because of the frequency of aberrent plants which often arises from regenerants from callus (3).

Bulblet multiplication of hyacinth from scales has been reported to be stimulated by indoleacetic acid, indolebutyric acid, and NAA, but could not be influenced by cytokinin (11). In our experiments we observed that a reduction in the concentration of either BA or NAA in the nutrient medium resulted in a significant decrease in bulblet formation (Table 1) suggesting that both auxin and cytokinin work synergistically to influence bulblet regeneration. Such an interaction has been suggested previously (13, 14).

Growth of bulblets. Bulblets initiated in culture were recultured onto a medium containing the basal components and BA (0, 0.1, 0.3, 3, and 10 mg/liter) or GA (0, 0.3, and 3 mg/liter) or were maintained on basal medium at 8° C for 4 weeks. Bulblets initiated *in vitro* did not grow when cultured on medium without BA. Growth of bulblets was maximal at 3 mg/liter BA for 'Anna Marie' and at 1.0 and 3.0 mg/liter BA for 'Delft Blue'. At 10 mg/liter BA the bulblets of both cultivars had elongated leaves and total growth was reduced.

GA_3 at concentrations of 0.3 and 3 mg/liter and chilling (4 weeks at 8° C) had no effect on bulblet growth. The reported promotive effect of GA (16) or chilling (6) on bulblet growth was not observed.

Rooting of bulblets and transfer of plants to soil. Bulblets initiated *in vitro* were transferred to medium containing 0 or 0.1 mg/liter NAA to stimulate rooting. The individual plants were then transplanted into a soil mixture (2 spagnum: 2 perlite: 1 soil; v/v/v). The highest degree root formation was achieved

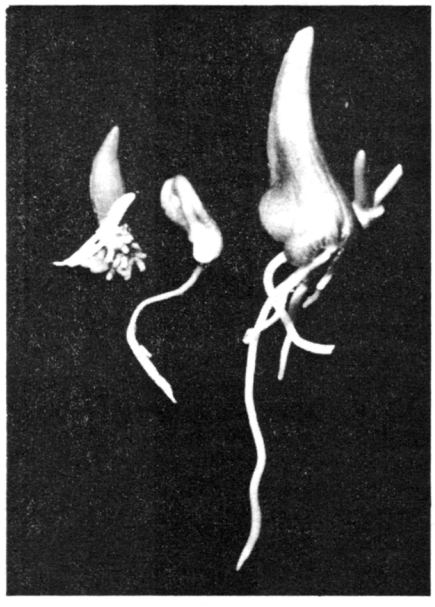

Fig. 3. Root induction on medium containing 0.1 mg/liter NAA. The plantlets are ready for transfer to soil.

when individual bulblets were separated and transferred to media containing the basal constituents with 0.1 mg/liter NAA (Fig. 3). Bulblets of 'Delft Blue' had a higher percentage of root initiation than did those of 'Anna Marie'.

Ninety to 100% of the bulblets of both cultivars grown on medium with 0.1 mg/liter NAA had developed sufficiently to be transplanted to a soil mixture (Table 2). All of the transplanted bulblets had produced new growth after 3 weeks.

Using this technique, it is possible that more than 1,000 plantlets can be obtained from a single inflorescence in a period of 4 to 6 months. Further multiplication could be obtained by inducing bulblet formation from the *in vitro* generated bulblets.

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Sensitivity of Ginseng to Ozone and Sulfur Dioxide[1]

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Abstract. **American ginseng (*Panax quinquefolius* L.), was injured by exposure to 20 pphm ozone and/or 50 pphm (v/v) sulfur dioxide for 6 hr daily for 4 days. Ozone induced upper surface leaflet stippling along the veins and interveinally, and sulfur dioxide induced mild chlorosis to irregular necrotic areas. Ginseng was less sensitive to ozone and as sensitive to sulfur dioxide as 'Cherry Belle' radish (Raphanus *sativus* L.) and 'Bel W-3' tobacco (*Nicotiana tabacum* L.).**

Ginseng (*Panax* sp.) grows wild and is cultivated in countries known to have air pollution problems. For instance, ginseng production areas of southern Ontario, Canada (8) frequently experience phytotoxic levels of air pollutants, specially ozone (Air monitoring data, Air Resources Branch, Ontario Ministry of the Environment, Toronto). Injury to ginseng by ambient air pollutants in this area has not been documented (7) and to our knowledge has not been reported for any other ginseng growing area (6). The purpose of this study was to establish, under controlled conditions, the sensitivity of American ginseng to 2 common air pollutants, ozone and sulfur dioxide, singly and in combination.

Three-year-old field grown roots of American ginseng were transplanted, 1 root per pot, to 15 cm diameter plastic pots containing 1 sphagnum peat:1 perlite (v/v) on November 1, 1979. Pots were placed in storage at 7° ± 2°C and 50 ± 5% relative humidity to satisfy the dormancy requirements of the roots. On January 8, 1980, pots were moved to the greenhouse (23° ± 3° day, 18° ± 2° night) and placed under a nylon mesh shade cloth which permitted 30% of full sunlight to reach the top of the pots. By February 11 shoot development had started and by March 5 the plants had reached maximum height and the leaves were fully developed. The plants were then moved to controlled environment chambers (Controlled Environment Model EY8M) maintained at day/night temperature of 20°/15° ± 2°, photosynthetically active irradiance at the top of the plants of 155 ± $\mu Em^{-2}s^{-1}$ provided by 73% input wattage Cool White fluorescent and 27% incandescent lamps, a photoperiod of 12 hr, and relative humidity of 45 ± 5%. The plants were irrigated alternately with deionized water and half-strength complete nutrient solution (3). Pots were sequentially removed at monthly intervals from cold storage to the greenhouse and then to the controlled environment chambers to establish 3 replications. Also periodically 'Cherry Belle' radish and 'Bel W-3' tobacco were seeded in 5 cm diameter plastic pots and grown in the controlled environment chambers with the ginseng. These plants, which have known sensitivity to O_3 and SO_2, were included for comparison with ginseng.

Ginseng, radish, and tobacco plants were moved to the exposure chambers one day before beginning the pollutant treatments. Exposure conditons were 350–400 $\mu EM^{-2}s^{-1}$ high pressure sodium light, 12 hr photoperiod, 22 ± 2°C temperature and 50 ± 5% relative humidity. The plexiglass exposure chambers were 70 cm cubes and the exposure system included a Grade Model LG-2-L2 ozone generator with a Dasibi Model 1003AH ozone monitor, sulfur dioxide from a gas cylinder and a Beckman Model 953 sulfur dioxide analyzer. Six-hour daily exposures in the middle of the photoperiod for 4 days were given. Treatments were (a) control; (b) 15 pphm ozone; (c) 50 pphm sulfur dioxide, and (d) a mixture of (b) and (c). There were 5 plants per treatment, the experiment was repeated 3 times and the combined data are presented.

Pollutant injury severity as percent of leaf area injured was determined on the 4th day after the end of exposure. The rating was based on the Horsefall and Barratt (4) scheme.

Tobacco was more severely injured by ozone than either radish or ginseng (Table 1). Characteristic upper surface stippling (2) was obvious by day 2 of the exposure, and by day 4 a high proportion of the leaf had bifacial necrosis. The ozone sensitive 'Cherry Belle' radish (9) had the expected necrotic fleck and bifacial necrosis on leaves and cotyledons. Ginseng leaflets had upper surface stippling along the veins and interveinally, which was more pronounced near the base of the leaflets (Fig. 1A). Injury was similar on all 5 leaflets.

Ginseng was less sensitive to ozone as the

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