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Effects of Auxins and Cytokinins on Ethylene Evolution and Growth of Rose Callus Tissue in Sealed Vessels[1]

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Abstract. **Ethylene evolution from callus tissue of rose (*Rosa hybrida* L.) grown in air tight vessels was enhanced by the presence of auxin and cytokinin in the culture medium. Increased ethylene levels did not adversely affect the fresh weight gain of rose callus tissue.**

Tissue culture propagation and long term storage of plant tissue *in vitro* often necessitates the use of sealed vessels to prevent desiccation of the nutrient medium (7). However, such conditions also prevent or restrict gas exchange.

Significant amounts of ethylene are produced by plant tissue during *in vitro* culture (3), including rose cell suspension cultures (4). Ethylene is known to affect the growth and differentiation of many plant tissues (1), and auxins and cytokinins are capable of causing substantial increases in ethylene evolution from cultured tissues (2, 4). It seems reasonable, therefore, that tissues maintained for extended periods of time in static gaseous environments may be exposed to self-generated levels of ethylene which could have deleterious effects on the growth or survival of the tissue being cultured.

This study was initiated to determine to what extent auxins and cytokinins might effect the production of ethylene by 'White Masterpiece' rose stem tissue.

Stems of greenhouse grown roses were cut into 2 cm lengths and surface sterilized in a 0.5% (by volume) solution of sodium hypochlorite for 20 min. Stem segments were rinsed with sterile deionized water, and the bark was removed under aseptic conditions. Stem segments were split longitudinally, then 4–5 segments were placed cut surface down in 100 ml screw-top jars containing nutrient medium (5), with 2 mg/liter indolebutyric acid (IBA) and 2 mg/liter kinetin. The tissue was allowed to proliferate callus for 1 month under continuous Cool White fluorescent light (3 klx) at 20°C.

Callus tissue that proliferated on the basal medium was excised and subcultured in 30 ml test tubes containing 10 ml of the same nutrient medium, but with different types and concentrations of auxins and cytokinins. The design was a factorial with IBA or naphthaleneacetic acid (NAA) at 0, 1, 2, or 3 mg/liter and 6-benzylamino purine (BA), dimethylallyl amino purine (DMAA) or zeatin riboside at 0, 5, 10, and 15 mg/liter. Tubes were closed with plastic caps (Kaputs) and sealed with parafilm. The caps had been previously modified by drilling a hole in the center of the cap to which a small serum cap was attached with epoxy, allowing a syringe to be passed to withdraw gas samples.

The tubes plus medium were weighed immediately before and after the addition of callus in order to determine initial callus fresh weight. The initial callus fresh weight was subtracted from the callus weight at the end of the experiment for determination of fresh weight gain. Four replicates of each growth regulator treatment were grown in darkness at 20°C and 4 replicates were grown under continuous fluorescent lights (3 klx) at 20°C.

Two samples were selected from each treatment and 1 ml samples were withdrawn at 2-week intervals for ethylene determinations, using a Hewlett Packard 402 gas chromatograph equipped with a flame ionization detector and a Poropak Q column (183 × 0.32 cm, I.D.) at 70°C. The carrier gas flow rate (N_2) was 25 ml min^{-1}. Ethylene was quantified by comparing retention times and peak heights with those of ethylene standards. Each sample injection was repeated twice.

Data was subjected to analysis of variance, and for economy of presentation means were pooled when it was indicated that significant differences at the 5% level were not present. No significant effect from presence or absence of light was observed on ethylene evolution, so light and dark treatment means were pooled (Table 1). The mean gain in weights of plants in light or darkness was shown to be significantly different; however, no differences were indicated between different auxin concentrations. Weight gain means were pooled with regard to auxin concentration and are presented in Table 2 by "auxin type" only.

Some ethylene accumulated as a result of all treatments, including those receiving no growth regulators (Table 1). No significant increases in ethylene evolution resulted from treatment with a cytokinin alone. Treatment with 3 mg/liter NAA resulted in significant increases in ethylene evolution compared with calli receiving no growth regulator treatments. A synergistic enhancement in ethylene production resulted from treatment with 3 mg/liter auxin and either DMAA or zeatin riboside at 5 mg/liter. Little or no ehtylene evolution was associated with treatments where calli failed to grow.

The greatest weight gain was associated with the presence of auxin (Table 2). With auxin alone weight gain was as great as that occurring in the presence of auxin and cytokinin. No difference was observed between light and dark treatments when auxin alone was present; however, the addition of a cytokinin

Table 1. The effect of BA, DMAA, and zeatin riboside with IBA or NAA on ethylene evolution.

Auxin concn (mg/liter)	Ethylene evolution ($\mu l\ liter^{-1} mg^{-1}$)									
	BA				DMAA			Zeatin riboside		
	0(mg/liter)	5	10	15	5	10	15	5	10	15
NAA										
0	6a[z]	5a	18a	14a	12a	20a	12a	12a	8a	16a
1	44ab	66ab	116ab	15a	109bc	24ab	41a	93a	52a	17a
2	41ab	240c	60ab	4a	38ab	35ab	19a	59a	18a	31a
3	102b	128b	122b	0a	178c	78ab	40a	282b	13a	30a
IBA										
1	34a	18a	27a	13a	293d	107ab	18a	35a	33a	21a
2	24a	9a	28a	18a	58a	113b	25a	51a	63a	37a
3	95a	20a	102ab	14a	560e	8a	61a	64a	64a	38a

[z]Column means separated by HSD at P = 5%; means of 4 samples, 2 each of plants held in light and darkness.

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resulted in a decrease in growth of dark grown calli. This is especially apparent in treatments receiving cytokinin alone, but can be seen in a comparison of most weight gains in the light to the same treatment in the dark (Table 2).

Our results indicate that substantial levels of ethylene may be accumulated in sealed vessels where tissue is kept for an extended period of time, and that the type and concentration of growth regulating compounds employed may strongly influence ethylene synthesis. The synergistic effect on ethylene production is not unique to rose callus tissue and has been observed by other workers (6). Different auxins and cytokinins, although resulting in equally good growth, elicit various degrees of ethylene evolution, but growth of rose callus tissue was not adversely affected by increased ethylene levels.

Although at higher cytokinin concentrations fresh weight gain of calli was greater in light than in darkness, no statistical difference between light and dark treatments with regard to ethylene evolution was observed. This suggests that the reduced growth in dark treatments was not related to increased ethylene accumulations.

Table 2. The effect of BA, DMAA and zeatin riboside with IBA or NAA on callus weight gain maintained in light (L) or darkness (D).

		Callus weight gain (mg)									
			BA			DMAA			Zeatin riboside (mg/liter)		
Auxin	Light	0(mg/liter)	5	10	15	5	10	15	5	10	15
0	L	17a[z]	207b	292d	0a	217b	150b	197b	170b	130b	129bc
0	D	32a	18a	0a	16a	46a	0a	10a	4a	30a	0a
IBA	L	271b	269bc	336d	5a	340c	371d	271c	287c	316c	327d
IBA	D	280b	52a	89b	8a	290bc	188bc	207bc	178b	270c	158c
NAA	L	393c	318c	185c	0a	452d	410d	424d	423d	262cb	305d
NAA	D	313b	230b	132bc	0a	348c	229c	243bc	172b	191b	76b

[z]Column means separated by HSD at P = 5%; means of 12 samples, 4 weight gain replications at 3 auxin concentrations.

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In Vitro Propagation of *Hosta sieboldiana*[1]

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Abstract. **Plants were initiated from florets of *Hosta sieboldiana* (Lodd.) Engler cultivars cultured on a modified Murashige-Skoog medium containing 0.5 – 2.5 mg/liter naphthaleneacetic acid (NAA) and 0.5 – 2.5 mg/liter 6-benzylamino purine (BA) and grown in the light. These plants could be separated and proliferated on a similar medium to give large numbers of plants. Plant propagated from 'Frances Williams' had a chimeral separation into green and gold sports as well as retaining the chimeral organization.**

The hosta or plantain lily is a perennial plant popular for established gardens or other shady areas in the landscape. Plants are usually propagated by crown division, thus, introduction of a new cultivar can take several years. An underground bud or "eye" can be used to propagate Hosta by tissue culture (1), however this excision reduces the growth potential of scarce new cultivars and using underground structures increases the contamination rate of explants.

Young emerging inflorescences have been a good explant source for *in vitro* propagation of other herbaceous perennials (3, 4), but young inflorescences were tried unsuccessfully with hosta (2). The florets of the inflorescence proved a satisfactory explant source for *Hosta sieboldiana,* if the flower scapes were allowed to develop until the bottom florets were separate and 0.5 – 1 cm in length. Other workers (1, 6, 8) have reported that shoot tips, florets and scape slices have worked for other hosta species.

Rapid multiplication of 3 cultivars of the large-leaved *Hosta sieboldiana,* 'Helen Doreo', 'Frances Williams', and 'Frances Williams Gold Sport', and the separation of the chimeral 'Frances Williams' was achieved with the following techniques.

Flower scapes were excised as the bottom florets became separated and 0.5 – 1 cm in length but were still tightly closed. Larger florets on more mature scapes will not proliferate plants readily. The florets still attached to the scape were disinfected in a solution of 0.5% sodium hypochlorite (10% Clorox) and 0.1% polyoxyethylene sorbitan (Tween 20) for 15 min, rinsed with sterile water, transferred to a sterile surface, and removed from the scape by cutting just below the ovary. The florets were transferred to 2.54 cm tubes, pressed half way into an agar medium, and placed under Cool White fluorscent light ca. 3 klx for 18 hr a day. Earlier studies with other herbaceous perennials (3, 4) indicated greatest plant proliferation when the flower tissue was placed in the dark with an appropriate medium for callus production. The tissue was transferred to a new medium in the light for plant proliferation. This technique reported earlier for *H. sieboldiana* cultivars caused only 10-15% of the florets to produce plants (2). Starting the florets *in vitro* in the light resulted in 50-75% of the florets producing plantlets. Papachatzi et al. (6) obtained callus from *H. plantaginea* 'Grandiflora' in the dark in a high NAA medium and formed shoots by transferring the callus to a high BA medium in the dark. These shoots then had to be transferred to a medium with no growth

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