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Preventing Photochemistry in Culture Media by Long-Pass Light Filters Alters Growth of Cultured Tissues

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ABSTRACT

Exposure of plant tissue culture media to light from fluorescent bulbs changed the growth regulating properties of the media. The light caused nutrient medium-dependent photosensitized degradation of the phytohormone indole-3-acetic acid and other media components. Photochemical changes in culture media were caused by light from 290 to 450 nanometers and were prevented with a yellow long-pass filter. The use of appropriately filtered light when culturing plant material can eliminate unnecessary variability by stabilizing the culture media composition.

The ability to grow and manipulate plant cells in culture has led to important applications in basic research and in agriculture. For example, plant tissue culture has been crucial to progress in genetic transformation of plants (17). Tissue culture techniques are also being used for commercial plant propagation and for variety development (20, 25). However, not all plants can be readily cultured, and many of those that can be cultured exhibit high levels of variation as a result of the culture process. Genetic change, or somaclonal variation, is one type of variability that occurs frequently in cultured cells and tissues (11, 20, 21, 23). This type of instability is undesirable where genetic fidelity is required, as is usually the case. Moreover, genetic and somatic variation may limit many tissue culture applications because it makes results difficult to reproduce.

Several factors have been implicated as origins of variability in plant tissue cultures. These include factors such as tissue source, media components, genotype, environment, and culture age (11, 21, 23). Factors which should be amenable to careful control, such as media components and environmental conditions, continue to be a sometimes unsuspected source of variability. Plant tissues and cells are usually grown on defined media in which the chemical nature and amounts of all components are known when the media are first prepared. However, once culture media are exposed to plant material, the medium composition changes as cells metabolize components, perhaps explaining tissue density-related differences in growth of cell cultures (3). Furthermore, abiotic factors such as light and heat can change the chemical composition of culture media (1, 6, 7, 9, 27).

Changes in growth regulator levels are of particular concern since excised plant tissues and cells are generally incapable of synthesizing their own growth regulators, which must therefore be provided in the culture medium. Auxins and cytokinins are probably the most critical regulatory substances involved in coordinating growth and development of cultured plant tissues. The natural auxin IAA is degraded rapidly in culture media and often is not very effective in supporting sustained growth of cultured plant tissues. This has led to the widespread use of more stable synthetic auxin-like growth regulators such as 2,4-D. However, although 2,4-D is very active in stimulating the growth of cultures, it has been associated with decreased regeneration and increased chromosomal abnormalities relative to those observed when IAA is used (5, 25, 26).

As an alternative to the use of synthetic auxin-like growth regulators we have been exploring ways to stabilize the levels of IAA supplied to cultures (15). As much as 90% of the IAA initially present in a standard Murashige and Skoog nutrient medium can be photochemically degraded within a few days in the absence of plant tissue (1, 7, 9). Since plant cultures are often grown in the light for weeks between subcultures, light-induced changes in growth regulator level could contribute significantly to variations in the growth and development of tissue cultured cells. This paper describes an efficient and simple method for eliminating nutrient media-catalyzed photochemistry which improves the stability of the media and results in improved plant tissue growth.

MATERIALS AND METHODS

Plant Material

Seeds of *Arabidopsis thaliana* Columbia wild type and tomato (*Lycopersicon esculentum* Mill. cv Marglobe) were surface-sterilized for 15 to 20 min in 30% (v/v) commercial bleach. After rinsing with sterile water, *Arabidopsis* seeds were germinated and grown on hormone-free media in polystyrene Petri dishes at 22 to 24°C with continuous illumination (65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD). Growth media contained Murashige and Skoog nutrients (22), 0.8% agar, 3% (w/v) sucrose for *Arabidopsis*, or 1% sucrose for tomato seed germination. The pH of all media was adjusted to 5.8 prior to autoclaving. Tomato seeds were germinated in darkness.

For root growth assays 3-d-old *Arabidopsis* seedlings were transferred to Petri dishes containing growth media and the indicated growth regulators. The root tip positions were marked. The Petri dishes were oriented vertically so the roots grew on the agar surface. The increase in root lengths were measured after 2 d in darkness at 23°C (10).

Leaf explants were used as the tissue source for tissue culture experiments with *Arabidopsis*. Leaf tissue was excised above

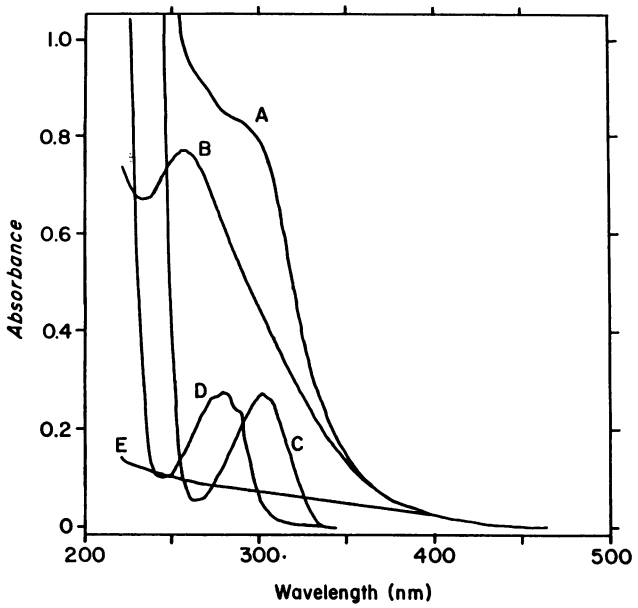

Figure 1. Light absorption properties of Murashige and Skoog nutrient media and components. Trace A, hormone-free liquid nutrient medium; trace B, 100 μM $FeSO_4 \cdot 7H_2O$ and 100 μM Na_2EDTA; trace C, 100 μM NH_4NO_3 and 100 μM KNO_3; trace D, 50 μM IAA; trace E, 100 μM $FeSO_4 \cdot 7H_2O$. All solutions were adjusted to pH 5.8 with HCl or NaOH.

the petiole from rosette leaves of 20-d-old aseptically grown plants. For tomato, hypocotyl explants (1 cm) were taken from 7-d-old aseptically grown etiolated seedlings (15). The explants from both plants were cultured at 22 to 24°C with continuous illumination in polystyrene Petri dishes on medium containing Murashige and Skoog nutrients (22), 3% (w/v) sucrose, 0.8% agar, 30 μM IAA, and 10 μM N^6-[2-isopentenyl]adenine.

Light Treatments

Light was supplied from cool-white fluorescent lamps (General Electric F96T12-CW). The PPFD at the surface of the Petri dishes was measured with a Li-185B quantum photometer (LiCor, Inc.). Light levels were varied by changing the distance from the source. The long-pass filters used are expressed here as the longest wavelength at which light transmission is below 0.1% of incident light as determined with a Hewlett Packard Diode Array Spectrophotometer. The 290 nm cutoff was provided by the polystyrene Petri dishes used for all experiments and corresponds to our standard unfiltered light conditions. Clear plexiglass (3.18-mm thick, Rohm and Hause) provided the 340 nm cutoff. Acrylic UF-3 and Yellow-2208 (3.18-mm thick, Polycast Technology Corp.) were the 388 and 454 nm filters, respectively. Absorbance spectra were measured for liquid nutrient solutions at pH 5.8.

IAA Degradation

IAA degradation was monitored two ways. One method measured the absorbance increase at 251 nm which is characteristic of the formation of 3-methyleneoxindole by oxidation of IAA (2, 24). Absorbance changes were calculated by subtracting spectra of dark controls from light-treated samples. IAA degradation was also monitored by assaying IAA-induced inhibition of *Arabidopsis* root growth (10).

RESULTS AND DISCUSSION

To investigate the photochemistry involved in light-induced IAA degradation, we analyzed the light absorption properties of materials used for plant tissue culture. The absorption spectra of Murashige and Skoog medium and the light-absorbing components of such media are shown in Figure 1. Hormone-free medium absorbs light from 220 to about 450 nm. No light absorption was detected between 450 and 700 nm. Analysis of individual medium components showed that light absorption by the nutrient media is mostly due to Fe-EDTA and nitrate salts. Unchelated iron also absorbs light but to a lesser degree. None of the other component salts absorb light significantly between 220 and 700 nm. Growth regulators such as auxins contribute to UV absorption as indicated by the absorption spectrum of IAA.

Transmission spectra of several common plant culture vessels are shown in Figure 2. With the exception of containers made from polypropylene the culture vessel materials screen out most of the UV light below 300 nm. Because light absorption by IAA occurs mostly below 300 nm, IAA degradation by direct absorption of light is largely prevented by typical culture vessels. All of the containers examined transmit

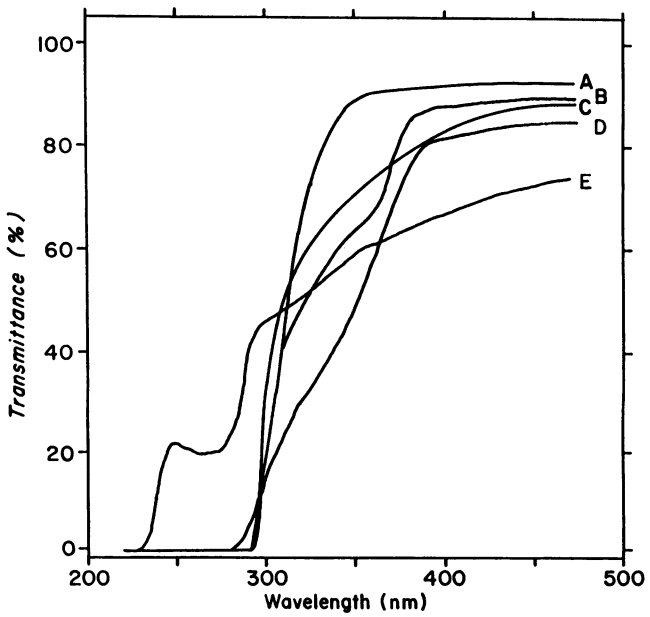

Figure 2. Transmission spectra of different types of plant tissue culture containers. Trace A, glass Petri dish; trace B and D, polystyrene Petri dishes from two sources; trace C, polycarbonate Magenta vessel; trace E, polypropylene Magenta vessel cover.

Table I. *Protection of IAA from Photodegradation by Long-Pass Light Filters*

The absorbance increase at 251 nm is characteristic of the formation of 3-methyleneoxindole by oxidation of IAA (2, 24). Liquid Murashige and Skoog medium containing 100 μM IAA was incubated in polystyrene Petri dishes for 6 d at 23°C in continuous light at 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at the surface of the Petri dishes. The 290 nm cutoff is from the polystyrene Petri dishes.

Filter Cutoff	ΔA_{251}
nm	
290	0.590
340	0.480
388	0.320
454	0.030

light longer than about 300 nm. The different materials showed some variation in the extent of transmittance to UV-A (320–400), but transmittance to photosynthetically active radiation (400–700 nm) was usually between 80 and 95% of the incident radiation.

Because typical culture container materials do not block light between 300 and 450 nm, a region of the spectrum where nutrient media absorb light, photochemistry in the media is possible when using such vessels. The light-absorption properties of plant culture materials shown here are consistent with the earlier demonstration that iron and nitrate, the major light-absorbing components of Murashige and Skoog-based culture medium (Fig. 1), can catalyze light-induced IAA degradation (7).

We tested a series of long-pass light filters for their ability to prevent photochemical degradation of IAA in Murashige and Skoog medium (Table I). The greatest protection occurred when all wavelengths less than 450 nm were blocked. Although there was relatively little absorption by the media between 388 and 450 nm (Fig. 1), blocking light below 388 nm decreased IAA degradation by approximately 50%. Since root growth is very sensitive to IAA exposure, we measured *Arabidopsis* root growth on IAA-containing nutrient media that had been exposed to different light conditions. When Murashige and Skoog medium containing IAA was incubated in unfiltered light for 10 d IAA activity was lost, but when it was incubated under light filtered through yellow acrylic (which blocks wavelengths below 454 nm) IAA activity was unaffected (Fig. 3). The apparent I_{50} for IAA-induced inhibition of root growth shifted from 0.06 μM on media incubated in darkness and under yellow-filtered light to 7.4 μM on media incubated under unfiltered light. Other experiments with the root growth assay and UV absorbance measurements showed that the yellow light filter prevented IAA degradation in the absence of tissues for at least 25 d (data not shown).

Slow release formulations of IAA have been suggested as a possible means of controlling IAA levels (7, 14, 15). In previous work we showed that conjugates of IAA could be used as slow release sources of IAA (14, 15). One of the attractive features of IAA conjugates is that many of them are protected from peroxidative attack (2, 24). Unfortunately, UV spectra of light-treated Murashige and Skoog media containing IAA-alanine, IAA-glycine, IAA-aspartate, or IAA-phenylalanine showed these compounds to degrade to the same extent as did free IAA (data not shown). Thus, conjugation does not protect the IAA moiety from the nonspecific photosensitized degradation that occurs in culture medium incubated in unfiltered fluorescent light.

We also observed that the photochemical activity of plant nutrient culture media led to growth inhibition when no phytohormones were used (Fig. 4). Prior incubation of hormone-free Murashige and Skoog medium for 21 d in unfiltered light at 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$ resulted in 53% inhibition of *Arabidopsis* root growth compared with roots grown on media previously incubated in darkness. Yellow-filtered light did not cause the medium to become inhibitory to root growth. However, light-induced inhibition in hormone-free controls was not significant at the lower light level and shorter exposure used in the experiment in Figure 3.

In experiments with cultured *Arabidopsis* leaf and tomato hypocotyl tissue we observed growth differences when the tissues were cultured under yellow-filtered and unfiltered light (Fig. 5). Growth of the tissue explants was three to four times greater under yellow-filtered light than under unfiltered light. Growth under yellow-filtered light was similar to the growth observed in unfiltered light when IAA levels were maintained by frequently transferring the tissues to fresh medium (Fig. 5). Under yellow-filtered light, frequent renewal of the media also improved growth compared with the untransferred tissues but to a lesser degree than the same treatments under unfiltered light. The differences between transferred and untransferred tissue in the yellow light was probably due to metabo-

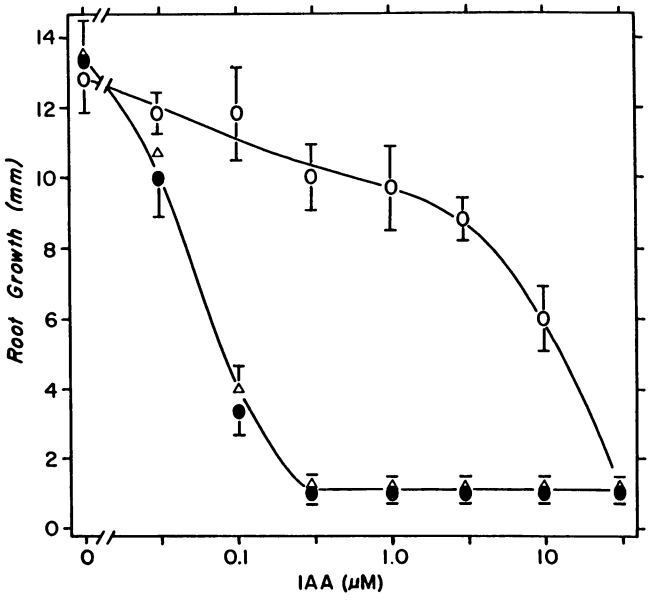

Figure 3. Inhibition of *Arabidopsis* root growth on IAA-containing nutrient medium previously exposed to different light conditions. Unfiltered light (○), yellow-filtered light (△), darkness (●). Each point represents the mean of eight measurements ± SE. PPFD at the surface of the culture dishes was 25 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the light treatments. Yellow acrylic was used as the light filter.

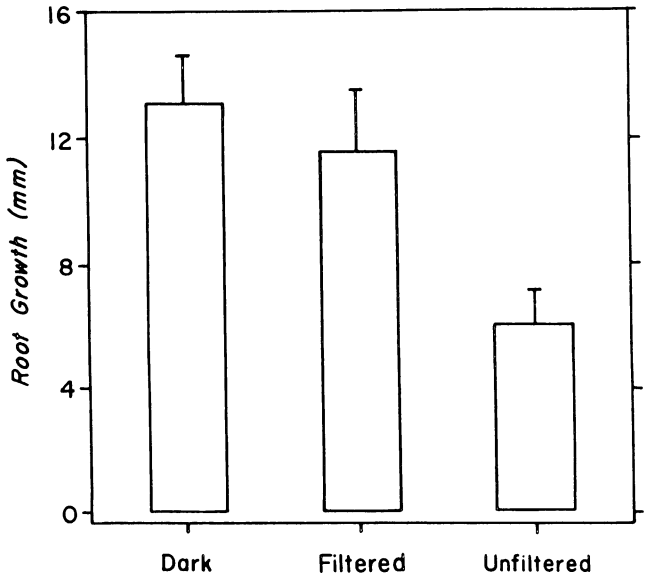

Figure 4. Inhibition of *Arabidopsis* root growth by prior exposure of hormone-free nutrient medium to light. The experiment was carried out as in Figure 3, except that the medium lacking hormones was incubated for 21 d prior to placing the seedlings on it, and the light was increased to 65 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Data represent the mean of 10 measurements ± SE. Unfiltered light rendered the media inhibitory to *Arabidopsis* root growth whereas yellow-filtered light was almost without effect.

lism of media components by the untransferred tissues. However, the larger difference between the transferred and untransferred tissue under unfiltered light, compared with the difference under filtered light, indicates that the media was altered more by light than by the tissue itself. Furthermore, the slightly different responses of tomato and *Arabidopsis* might also be due to differences in their rate of metabolism of IAA. From these observations and the rapid degradation of IAA by unfiltered light (Table I), it would appear that the increased growth under yellow-filtered light was related to improved stability of IAA. However, it must be realized that the levels of all media components were renewed and that metabolic products were also removed with each transfer.

It remains possible that some aspects of growth under the filtered light are due to removal of the growth-inhibiting effects UV and blue light have on plant tissues (12, 18). Action spectra for various blue light responses are known to have two maxima, one in the near UV between 370 and 380 nm and another in the blue region between 400 and 500 nm (16). It is unlikely that blocking light below 450 nm would completely prevent blue light responses because action spectra for blue light responses extend to 500 nm. On the other hand, responses regulated by UV light, such as phenylpropanoid metabolism (13), will almost certainly be affected by yellow-filtered light. The induction of phenylpropanoid synthesis is thought to provide protection from various stresses, including UV damage (13). Indeed, we have observed that cultured tomato hypocotyl tissues synthesize red pigments when grown under unfiltered fluorescent light but not under yellow-filtered light, consistent with the idea that commonly used fluorescent light sources are stressful to cultured plant tissues (18).

We have not yet determined what photochemical changes are responsible for the inhibition of root growth observed in hormone-free medium (Fig. 4) nor the mechanisms involved in the photochemistry of plant culture media. However, UV spectra of light-induced IAA degradation products showed a marked increase in absorption at 251 nm indicative perhaps of 3-methyleneoxindole, the major product formed by peroxidase-catalyzed oxidation of IAA (2, 24; Table 1). Furthermore, many photosensitizers react with molecular oxygen to form reactive species such as superoxide and singlet oxygen (4, 8, 19). Thus it seems probable that photosensitized oxidations catalyzed by nutrient media lead to degradation of IAA and possibly to alterations in other media components. Action spectra and mechanisms of the media-catalyzed photochemistry are being investigated.

Plant tissue cultures have become an indispensable tool for both commercial and research applications. Unfortunately, current practices of controlling plant tissue growth, such as by adjusting the auxin to cytokinin ratio with unstable hormone sources, is such an empirical procedure that it approaches an art. When it works it can work well, but failures leave little recourse. Any steps that remove sources of variation and stress are certainly desirable. Preventing photochemical changes in culture medium, such as those leading to IAA degradation, eliminates an otherwise uncontrolled source of

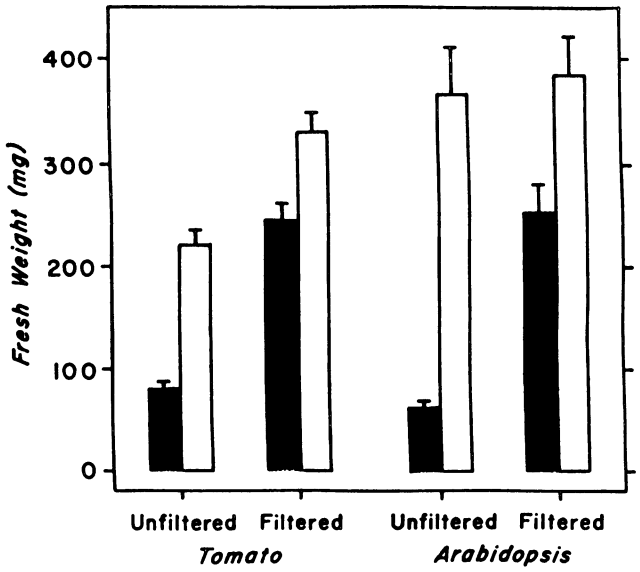

Figure 5. Prevention of white light-induced growth inhibition of tomato hypocotyl and *Arabidopsis* leaf tissues by yellow light and media renewal. Filled bars, untransferred tissue; open bars, tissues transferred every 3 d. Hormone levels were renewed every 3 d by transferring the tissues to fresh media as described in "Materials and Methods." Data represent the means of the callus fresh weight for 24 to 30 measurements ± SEM. Tomato and *Arabidopsis* tissues grew larger under yellow-filtered light compared with unfiltered light. Frequent transfer to fresh culture medium caused the tissue to grow as well (tomato) or better (*Arabidopsis*) in unfiltered light as did the untransferred tissue in yellow light.

variation and potential stress. It is conceivable that genetic stability may be improved by preventing potentially mutagenic radical species from forming. The observed growth inhibition with light-modified, hormone-free medium suggests that precautions should be taken even when using light-stable, auxin-like compounds or when no hormones are used. Moreover, many of the reported effects of light intensity and quality on tissue cultures may be due to indirect effects caused by photochemical alterations of the media.

LITERATURE CITED

1. **Bender L, Neumann K-H** (1978) Investigations on the indole-3-acetic acid metabolism of carrot tissue cultures (*Daucus carota* L.). Z Pflanzenphysiol **88:** 209–217
2. **Cohen JD, Bandurski RS** (1978) The bound auxins: protection of indole-3-acetic acid from peroxidase-catalyzed oxidation. Planta **139:** 203–208
3. **Cousson A, Toubart P, Tran Thanh Van K** (1989) Control of morphogenic pathways in thin cell layers of tobacco by pH. Can J Bot **67:** 650–654
4. **Daub ME** (1982) Cercosporin, a photosensitizing toxin from *Cercospora* species. Phytopathology **72:** 370–374
5. **Deambrogio E, Dale PI** (1980) Effect of 2,4-D on the frequency of regenerated plants in barley (*Hordeum vulgare*) cultivar "Akka" and on genetic variability between them. Cereal Res Commun **8:** 417–424
6. **Dunlap JR, Kresovich S, McGee RE** (1986) The effect of salt concentration on auxin stability in culture media. Plant Physiol **81:** 934–936
7. **Dunlap JR, Robacker KM** (1988) Nutrient salts promote light-induced degradation of indole-3-acetic acid in tissue culture. Plant Physiol **88:** 379–382
8. **Elstner EF** (1982) Oxygen activation and oxygen toxicity. Annu Rev Plant Physiol **33:** 73–96
9. **Epstein E, Lavee S** (1975) Uptake and fate of IAA in apple callus tissue using IAA-1-^{14}C. Plant Cell Physiol **16:** 553–561
10. **Estelle MA, Somerville C** (1987) Auxin resistant mutants of *Arabidopsis thaliana* with altered morphology. Mol Gen Genet **206:** 200–206
11. **Evans DA, Sharp WR, Medina-Filho HP** (1984) Somaclonal and gametoclonal variation. Am J Bot **71:** 759–774
12. **Fridborg G, Eriksson T** (1975) Partial reversal by cytokinin and (2-chloroethyl)-trimethylammonium chloride of near-ultraviolet inhibited growth and morphogenesis in callus cultures. Physiol Plant **34:** 162–166
13. **Hahlbrock K, Scheel D** (1989) Physiology and molecular biology of phenylpropenoid metabolism. Annu Rev Plant Physiol Plant Mol Biol **40:** 347–369
14. **Hangarter RP, Good NE** (1981) Evidence that IAA conjugates are slow-release sources of free IAA in plant tissues. Plant Physiol **68:** 1424–1427
15. **Hangarter RP, Peterson MD, Good NE** (1980) Biological activities of indoleacetic acids and their use as auxins in tissue culture. Plant Physiol **65:** 761–767
16. **Hart JW** (1988) Light and Plant Growth. *In* M Black, J Chapman, eds, Topics in Plant Physiology. Unwin Hyman Ltd., London, pp 71–75
17. **Klee H, Horsch R, Rogers S** (1987) *Agrobacterium*-mediated plant transformation and its further applications to plant biology. Annu Rev Plant Physiol **38:** 467–486
18. **Klein RM, Edsall PC** (1967) Interference by near ultraviolet and green light with growth of animal and plant cell cultures. Photochem Photobiol **6:** 841–850
19. **Knox JP, Dodge AD** (1985) Singlet oxygen and plants. Phytochemistry **24:** 889–896
20. **Larkin PJ, Skowcroft WR** (1981) Somaclonal variation: a novel source of genetic variability from cell cultures for crop improvement. Theoret Appl Genet **60:** 197–214
21. **Lee M, Philips RL** (1988) The chromosomal basis for somaclonal variation. Annu Rev Plant Physiol Plant Mol Biol **39:** 413–437
22. **Murashige T, Skoog F** (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15:** 473–497
23. **Orton TJ** (1983) Experimental approaches to the study of somaclonal variation. Plant Mol Biol Rep **1:** 67–76
24. **Park RD, Park CK** (1988) Oxidation of indole-3-acetic acid amino acid conjugates by horseradish peroxidase. Plant Physiol **84:** 826–829
25. **Shepard JF** (1981) Protoplasts as sources of disease resistance in plants. Annu Rev Phytopathol **19:** 145–155
26. **Singh BD, Harvey BL** (1975) Does 2,4-D induce mitotic irregulatiries in plant tissue culture. Experienta **32:** 785–787
27. **Weaterhead MA, Burdon J, Henshaw GG** (1978) Some effects of activated charcoal as an additive to plant tissue culture media. Z Pflanzenphysiol **89:** 141–147