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The Effects of Light Intensity and Spectral Quality on Growth and Shoot Initiation in Tobacco Callus

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ABSTRACT

The effects of eight different narrow band-emitting fluorescent lamps (371–750 nm) and four commercial broad band-emitting fluorescent sources upon growth and shoot initiation in tobacco callus (*Nicotiana tabacum* var. Wisconsin 38) have been characterized. Wavelength and intensity are equally important parameters in determining morphogenic changes. Near ultraviolet light (371 nm) was found to stimulate (0.024 mw/cm²) or inhibit (above 0.15 mw/cm²) callus growth and shoot initiation, depending on the light intensity. Stimulation of growth and shoot production occurs also in blue light region, but at higher intensity than in the near ultraviolet. Red and far red light (up to 1.7 mw/cm²) do not appear to affect callus growth or stimulate shoot initiation. The enhancement of callus growth and the stimulation of shoot initiation are controlled by the same near ultraviolet-absorbing photoreceptor system present in a small enough concentration that it cannot be recognized in the absorption spectrum of the intact tissue. Carotenoids, porphyrins, and phytochrome associated with the high irradiance response do not appear to qualify as the photoreceptor. Flavonoids are possible candidates. Radiation emitted by fluorescent lamps outside the near visible region was determined, and we concluded that energy levels were not sufficient to affect the reported results. The spectral output of several commercial lamps in the visible and near visible regions is such that there could be different effects on growth and development of tissue cultures.

Until about 20 years ago, biologists paid little attention to the role of light in the growth and development of plant tissue cultures. The normal practice at that time was to store sample tubes in the laboratory under prevailing light conditions which were sporadic exposures to an illuminance of about 100 ft-c. Fortuitously, this level was adequate for sustaining growth, although below the 350 ft-c optimal value found by de Capite (10) for callus growth in *Helianthus annuus*, *Parthenocissus tricuspidata*, and *Daucus carota*, de Capite did not identify his light source.

More recently, as interest in tissue culture increased, investigators have sought to determine whether different wavelengths have differential effects on growth and development of callus cultures. The results have been conflicting. Butenko *et al.* (7), Klein (18), Beauchesne and Poulain (3), Beauchesne (2), and Polevaya (27) have reported that near UV, blue, red, or near IR light inhibit callus growth in comparison with unlighted tissue. In contrast to Beauchesne's findings of inhibition of tobacco callus growth, Bergmann and Bälz (4) and Weis and Jaffe (35) claim that their tobacco callus growth was enhanced by blue light. Callus growth in other species (*Pelargonium*) has also been reported to be enhanced by blue light (34). Whereas Polevaya (27) reports that red light inhibits carrot callus growth, other investigators find either enhancement or null effects (7, 34, 35) of red light on callus in other species. Although there may be species differences, it is apparent that, in the case of tobacco, there are obvious discrepancies.

One of our objectives in the present study was to determine whether there were important differences between commonly used light sources with respect to the effects on the differentiation process in cultured tissue. Currently, little information exists about the effects of light quality on callus growth and the process of organ initiation in culture (35). Perhaps the conflicting results of previous investigators could be explained by considering the differences in light sources and intensities. Neither of these parameters are described well in the biological literature.

A second objective of this investigation was to measure the energy spectrum of conventional fluorescent light sources to determine the level of electromagnetic radiation present outside the visible region and establish whether the intensities were sufficient to elicit a biological effect. The present study utilizes both experimental narrow bandwidth (20–98 nm) sources emitting near UV, visible or near IR light as well as several broad band commercial lamps to study growth and shoot initiation in cultures of tobacco callus. Preliminary discussions on this work appear elsewhere (29, 30).

MATERIALS AND METHODS

Biological Samples. Callus stock material, isolated from the cambium of tobacco (*Nicotiana tabacum* var. Wisconsin 38), was continuously subcultured in the dark on a modified White's nutrient medium (23) except that Casamino Acids (No. 0288-01, Difco Laboratories, Detroit) were substituted for Edamin and the kinetin concentration was adjusted to 0.5 mg/l (24). Tissue used for experimental purposes was cut into uniform disks (0.5 cm diameter, 0.15 cm thick) by first using a spring-loaded plunger, tissue-ejecting borer shown on the left in Figure 1. The cylinders of callus produced were then cut with a slicer consisting of five parallel mounted razor blades, spaced 0.15 cm apart (shown at the right of Figure 1). The resulting wet weight of the disks was approximately 25 mg. For experimental purposes, these disks were cultured on a second medium which supports shoot initiation. This differentiating medium consisted of Murashige and Skoog salts (23) to which 2 mg/l kinetin, 2 mg/l IAA, 0.4 mg/l thiamine·HCl, 80 mg/l adenine sulfate dihydrate, 170 mg/l $NaH_2PO_4 \cdot H_2O$, 100 mg/l *i*-inositol, 30 g/l sucrose and 10 g/l Difco Bacto-agar were added. Before inclusion of agar, the pH of the medium was adjusted to 5.7 with NaOH. The tissue disks were placed on 25-ml slants of solidified medium in 25 × 125

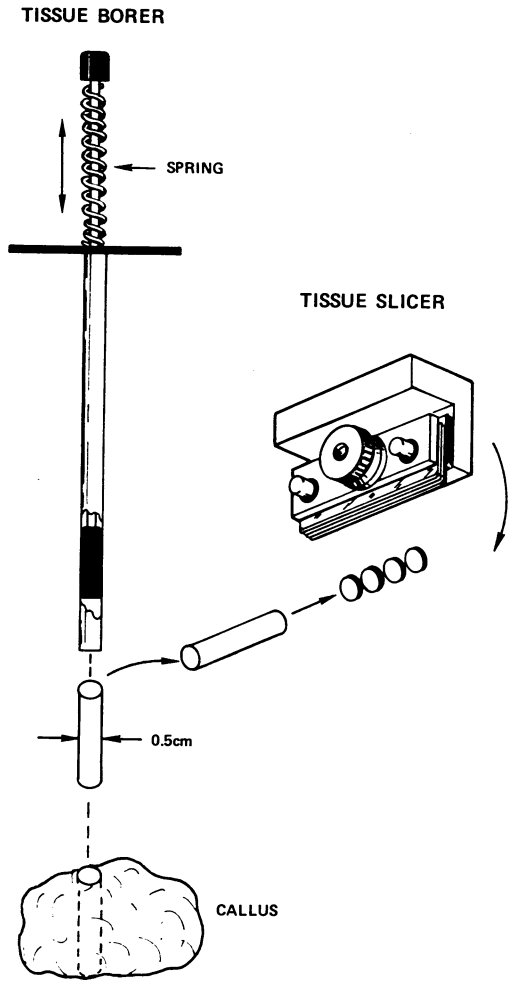

FIG. 1. Tissue borer (left) and tissue slicer (right) used to prepare uniform 0.5-cm diameter, 0.15-cm thick disks of callus for experimental purposes. The tissue borer cuts 0.5-cm diameter cylinders of callus while the spring-loaded plunger facilitates ejection of the tissue. Disks are cut from the cylinders using the tissue slicer, a device which holds five razor blades in a parallel arrangement. Spacers 0.15 cm wide, placed between the blades, determine the thickness of the disks.

mm Bellco culture tubes (which transmit 50% of the incident light at 303 nm and essentially all of the incident light at 360 nm) and capped with snug-fitting plastic Kap-uts (Bellco Glass Co., Vineland, N.J.).

Physical Conditions. Experiments were conducted in an environmental chamber (Controlled Environments, Ltd., Winnipeg, Canada) divided into five light-tight modules. Each module (painted flat black on the inside) was capable of holding five 20-w fluorescent lamps across the top. Light intensities were controlled by adjusting the distances between the lamps and the tissue. The lamps operated for 16 continuous hr out of every 24, a photoperiod found optimal for shoot initiation in tobacco callus (22). Temperature was maintained at 26 ± 1 C by forcing chamber air rapidly up through the modules, past the lamps, and out the top. This practice prevents thermal build-up in the modules from the fluorescent lamps.

Light Sources. Narrow bandwidth-emitting fluorescent lamps were used providing one of the following peak wavelengths (nm): 371, 419.5, 467, 504, 550, 590, 660, and 750. To eliminate near UV mercury line emission (in all but the 371 nm lamp), we covered the tube exterior with a 5 mil thickness of Weatherable polyester film (Martin Processing Co., Martinsville, Va.). Spectra of this material (obtained using a Cary 14 spectrophotometer) show that the transmission between 200 to 370 nm is 0%, the wavelength where transmission = 50% is 408 nm, and between 470 nm and 1000 nm transmission gradually increases from 80 to 85%. In addition, selected Cinemoid filters surrounding the UV prefilters served to absorb visible mercury lines not in the immediate spectral region of the narrow band phosphor emissions (19). The bandwidths and filters used for each source are given in Table I. Emission spectra of unfiltered broad band fluorescent sources A, B, C, and D are plotted in Figure 2 (5, 32). Source A is commonly used for commercial lighting. Sources B to D are special purpose fluorescent lamps.

Light irradiances for the narrow band-emitting lamps were measured at callus level using a Centralab No. EA7E-1 silicon solar cell (Centralab, Globe-Union, Inc., El Monte, Calif.). This cell was calibrated against an EG & G Model 580 radiome-

Table I. *Narrow Bandwidth Light Sources*

Wavelength Peak of Lamp	Half-Height Bandwidth of Lamp	Cinemoid Filter
nm		*No.*
371	19	6[1]
419.5	34	25
467	48	16
504	30	24
550	98	1
590	53	5
660	20	6 + 5 or 5a
750	90	6 + 5 or 5a

[1] The only lamp in this set in which UV light was not filtered with the polyester film described in the text.

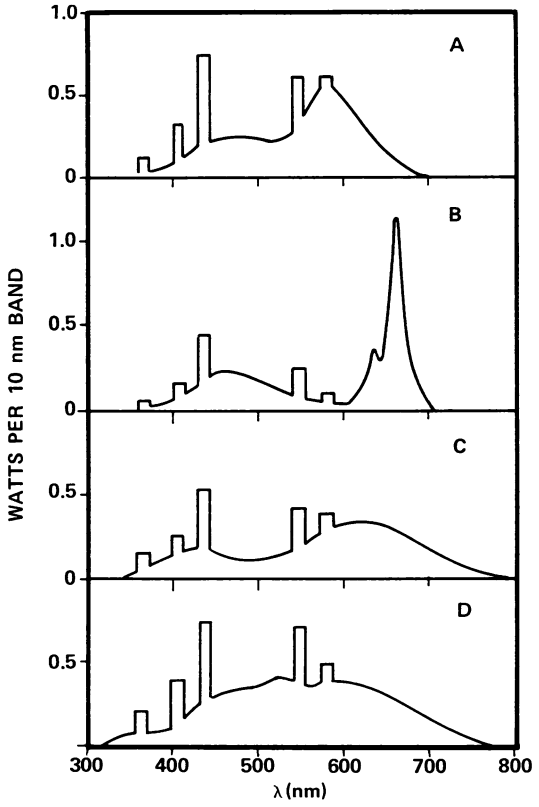

FIG. 2. Emission spectra of four commercial broad band-emitting fluorescent light sources.

ter (EG & G, Salem, Mass.). For the broad band sources a Tektronix J16 photometer/radiometer with a J6512 probe (Tektronix, Inc., Beaverton, Ore.) was used.

Absorption Spectra of Turbid Samples. Since plant tissue scatters light differentially as a function of wavelength, we used an integrating sphere (20, 26, 28) to obtain corrected absorption spectra. Our spectrometer consisted of a 6-v strip filament incandescent lamp powered by a stable voltage supply, a Spex ¾-m monochromator with a grating blazed at 500 nm, and a 30.5-cm diameter integrating sphere coated on the inside with No. 6080 white reflective paint (Eastman Kodak, Rochester, N.Y.). A 1.5-mm pathlength cuvette was positioned at the center of the sphere so that measuring light from the monochromator hit only the sample area. The detector, a photomultiplier tube with an S-20 response, was situated on the surface of the sphere, 90° off axis from the entering, measuring beam. A screen prevented light from the cuvette from directly entering the photomultiplier. The photomultiplier signal was fed into a micro-micro ammeter (No. 410, Keithley Instruments), and its output was recorded on chart paper.

Pieces of sample callus tissue were cut precisely using a modified Stadie-Riggs tissue slicer (A. H. Thomas, Philadelphia) to just fit the 1.5-mm pathlength cuvette. At a particular wavelength, the absorption of the sample was calculated using equation 1,

$$\text{Absorption} = (A - C)/(A - B) \quad (1)$$

where A is the signal with a nonabsorbing scatterer in the cuvette (200 g/l dry milk in our case), B is the signal with a totally absorbing sample (India ink), and C is the signal with the callus tissue.

Characterization of Fluorescent Lamps. Because of the questions raised concerning the spectral quality of the light sources used to illuminate tissue culture materials, we sought to quantitate nonvisible, electromagnetic emissions from fluorescent lamps (including the very low frequency radio to the x-ray region) which might possibly affect our conclusions. Radio signals were probed using a Hewlett-Packard No. 8556A spectrum analyzer with a Singer Instrument Co. No. 90114-3 antenna (22 Hz to 200 kHz), a Hewlett-Packard No. 8553B spectrum analyzer with a Singer VA-105A vertical remote antenna connected to a 1-m brass wire (150 kHz to 30 MHz), and a Hewlett Packard No. 8554B spectrum analyzer with a Singer bi-conical antenna Model No. 94455-1 (20 MHz–200 MHz). Emissions were measured from the center of the fluorescent lamp perpendicular to the axis of the tube. Radio and microwave frequencies (20 MHz to 10 GHz) were measured using a Hewlett-Packard 8551-B spectrum analyzer with a No. 852-A display section and either a Singer No. 93490-1 conical log spiral antenna or a wire probe. The UV to IR (200–1800 nm) was scanned using a ½ meter Jarrel-Ash monochromator in combination with a lead sulfide cell or a photomultiplier tube (S-5, S-20, or S-1 response). The x-ray region (>6–7 kev) was probed with a Victoreen 440 RF meter. Measurements were made with the detector held less than 1 cm from the tubes. In the case of lamp A, x-ray energies greater than 5 kev (2.5 Å, where 1 mil of aluminum foil transmits more than 50% [17]) were also measured using three different types of x-ray film: Kodak type AA, Kodak No. NS54T, and Ilford type G. Pieces of film were wrapped with aluminum foil of 1 mil thickness. The foil-film package was then covered with three layers of black paper to make a light-tight container. The films were placed 6 cm away from the tubes to eliminate heat effects. Film packets were exposed to lamp A for a total of 75 hr. During this time the lamp was started six times.

RESULTS

Lamp Characterization. Table II represents a summary of results determined for four commercial fluorescent lamps and the 419.5 nm emitting lamp. Besides the visible (and near visible) regions, the only area where the five lamps display spectral differences in emission characteristics (perhaps due to differences in inductance due to cathode geometry) centers in the 0.15 MHz to 10 MHz range. Since the amplitudes involved are about the same as those detected for private and commercial broadcast signals, it is not likely that any differential noise signals in this region would cause appreciable differences in tissue culture growth and differentiation. Similarly, variation among the same types of lamps is not apt to have a significant effect as long as the lamps are operating normally.

The differences between the commercially used lamps in the visible portion of the spectrum are presented in Figure 2. Salient differences are that B has a more pronounced peak in the red than any of the others while C and D lamps have significantly more near UV than any of the others. These differences could be important in influencing plant growth and development. To test this, these processes were first followed under a defined light regime over a period of time. Then time was held constant and light sources were compared.

Growth Curves. Figure 3 plots callus weight and shoot production as a function of time under saturating 419.5 nm light conditions (see the 419.5 nm curves in Figs. 7 and 8). The fact that both curves are parallel suggests that shoot production is related to callus growth. The next few sections will expand upon this observation in an effort to identify the nature of the relationship.

Effects of Narrow Band Wavelengths. Light of different wavelengths influences the fresh weight of plant callus cultures in comparison with dark controls. Discrepancies in the results of others were also noted. Figures 4 to 6 represent the photomorphogenic state of tobacco callus cultures after 35 days of exposure to different amounts of 371, 467, and 660 nm light, respectively. Salient features of these irradiance profiles demonstrate that callus growth and differentiation depend not only on the wavelength of illumination but also on the intensity. Figure 4 (371 nm) is the best example of this. Compared with dark-grown callus (lower left-hand corner), light-induced callus weight enhancement and shoot development peak at approximately 0.024 mw/cm². Moreover, higher levels of irradiance inhibit this phenomenon. At 467 nm (Fig. 5) enhancement of callus weight continues through higher irradiances (0.57 mw/cm²) as does shoot initiation. Whether even higher intensities at this wavelength would inhibit both parameters was not studied. Figure 6 shows that 660 nm levels to 1.7 mw/cm² have little effect on callus growth; also, few shoots developed over the same range of irradiance.

Quantification of Callus Growth Results. Data similar to those in Figures 4 to 6 are quantified in Figure 7 which plots the wet weight of tobacco callus after 35 or 36 days of exposure to various quantum levels of the indicated wavelengths. Weights of calluses which formed shoots were obtained by weighing the callus after all shoots had been removed. The saturation curve at 371 nm demonstrates the fact that light can stimulate or inhibit callus growth depending on the quantum level of radiation. The curve at 419.5 nm might also extend below the dark control at higher intensities but this was not verified. With the maximum available light level at 467 nm, it appears that the saturation value for callus weight increase was just reached. Stimulation of callus growth in comparison with dark controls also resulted using 504 and 550 nm light. No observable increase or decrease in callus weight appeared even at the highest intensities tested if the incident wavelength was 590 nm or longer.

Quantification of Shoot Initiation Results. Figure 8 presents data similar to that of Figure 7, except that in this case shoot initiation was monitored. Note that some shoots formed in the dark. This growth occurs occasionally in tobacco callus grown on shoot differentiating medium (see "Materials and Methods") in the dark for an extended period of time. However, dark-

Table II. *Comparison of Emissions of Several Fluorescent Lamps Throughout Electromagnetic Spectrum*

Spectral Region	Source A	Source B	Source C	Source D	419.5 mm
22 Hz–200 kHz[1] (ELF, UF, VLF, and LF bands)	60 Hz harmonics below 40 kHz,[2] nothing else detected up to 200 kHz (sensitivity of the instrument was 840 μv/m above 40 kHz).				
150 kHz–30 MHz[3, 4] (LF, MF, and HF bands)	Broad band noise detected between 150 kHz and 2.7 MHz. Small bands observed around 4 MHz and from 8 MHz to 10 MHz	Broad band noise detected between 150 kHz and 1.5 MHz. Small bands observed around 4 MHz and from 8 MHz to 10 MHz.	Broad band noise detected between 150 kHz and 1.5 MHz. Small bands observed from 8 MHz to 10 MHz.	Broad band noise detected between 150 kHz and 3.0 MHz. Small bands observed around 4 MHz and from 8 MHz to 10 MHz.	Small bands detected between 150 kHz and 180 MHz. Quietest lamp in this spectral range.
20 MHz–200 MHz[3] (HF, VHF, and UHF bands)	Nothing detected besides commercial broadcasting stations. Field strength sensitivity was between 8 and 30 μv/m.				
200 MHz–12.4 GHz[1, 3] (VHF, UHF, and SHF bands)	Nothing detected besides broadcasting stations. Sensitivity of the probe was 5 μv/m up to 1 GHz.[5]				
Upper end of the SHF band, the EHE band, and the far IR	Not observed.				
7000 Å–18000 Å (near IR)	Hg lines noted were at 10140, 11287, 13570, 13673, and 15295 Å. All lamps displayed broad band emissions above 10000 Å emanating from both cathodes. Several small emission lines appearing from 7000 to 10000 Å and from 13000 Å to 14000 Å were also localized at the cathodes.				
3000 Å–7000 Å (near UV and visible)	See Fig. 2 and description in the section on light sources.				
2000 Å–3000 Å (far UV)	Nothing detected in this region including the 2537 Å Hg line which would be the strongest emission. All emissions were absorbed by the fluorescent tube glass.				
100 Å–2000 Å (vacuum UV)	Measurements in this region were not attempted since fluorescent tube glass, O_2, and N would absorb any radiation emitted.				
2.5 Å–100 Å (soft x-ray)	Fluorescent tube glass is opaque. The per cent transmission for wavelengths above 1.8 Å was $<10^{-5}$ (calculated from data in ref. 15).				
<2.5 Å (x-ray and γ-ray)	Nothing detected above background (0.03 mroentgen/hr) with either the RF meter or using any of the three films.				

[1] Electromagnetic radiation detected was circularly polarized.

[2] Low frequency pick-up was localized at one or both of the cathodes (this was variable but independent of the phosphor). The amplitude of the harmonics between 22 and 40 kHz was no greater than the amplitude of 60 Hz room noise when the lamps were not operating.

[3] Electromagnetic radiation detected was linearly polarized.

[4] Radio noise emission from fluorescent lamps (6) and ballasts (33) is a well known phenomenon.

[5] Guentzler (13) has described microwave noise emissions from fluorescent lamps.

initiated shoots should not affect our results since they represent less than 10% of the shoots present under saturating light conditions. The results at 3 neinsteins cm^{-2} sec^{-1} are consistent with those reported by Weis and Jaffe (35) at similar intensities, *i.e.* blue light initiates shoot production and red light has no effect. Qualitatively, the data in Figures 7 and 8 are similar, suggesting a common mechanism for light effects on growth and shoot production.

Figure 9 shows the spectral dependence of shoot production in tobacco callus at saturation. In spite of an apparent peak in the mean values, it is important to emphasize that this figure is not an action spectrum (which will be discussed in the next section).[1] A straight line drawn through the data can be justified since the error bars represent plus and minus one standard error of the arithmetic mean. Physically, then, Figure 9 is consistent with the idea that shoot initiation involves one photoreceptor because the number of organs initiated at saturating light levels is independent of wavelength. This idea would not preclude the possibility of photosensitized reactions or multiple photoreceptors having different absorption spectra but which cause the same effect at saturation.

Action Spectra. An important criterion which must be satisfied before an action spectrum for a photobiological reaction can be compared with the absorbance spectrum of a suspected photoreceptor chromophore is that reciprocity must hold (31), *i.e.* the effect is independent of how the dose (defined as the product, irradiance × time) is delivered (16). The fact that a lag period of about 2 weeks exists before shoots appear (35) makes it difficult to check the reciprocity relationship for organ initiation by conventional means. Consequently, isolated callus disks were placed in duplicate modules under 467 nm light for 16 hr/day. The irradiance (0.155 μw/cm^2) was kept constant in the control module. However, in the other module the irradiance was adjusted to one-half that of the control value for the first 8 hr and one and one-half times that of the control for the second 8 hr. Thus, over every daily 16-hr light period the total incident dose for each of the duplicate sets of callus was identical. If simple reciprocity holds for this intensity range (at which the 467 nm points in the action spectra of Fig. 11 were obtained), it would be expected that the amount of callus and the number of shoots produced at any specified time would be the same under both sets of conditions. Table III verifies this expectation. Since no significant differences appear in the weight of the callus or the

[1] The 550 nm point in Figure 9 probably represents a low estimate for the saturation value since the 504 nm curve in Figure 8 does not appear to reach saturation at the highest light level available.

number of organs produced under the two light regimes, reciprocity appears to hold at 467 nm. The same is probably true at the other wavelengths used in this investigation.

A second aspect of action spectroscopy which an investigator must examine concerns the interaction of light and nonphotoreceptor substances in the tissue. Unless absorption and scattering within the tissue by inactive materials is negligible or constant as a function of wavelength, corrections accounting for these parameters must be made to action spectra obtained using incident light quantum levels. Since our callus samples grew to a thickness of about 1 cm, the potential for differential absorption was considerable. Indeed, reflectance spectra of 0.15-cm thick pieces of dark-grown tobacco callus obtained using a Cary 14 spectrophotometer suggest more light absorption in the blue region than in the red. A comparison of absorption spectra of freshly cut dark- and light- (467 nm) grown tissue slices, calculated from integrating sphere measurements using equation 1, is seen in Figure 10. The peaks around 430 nm and at 675 nm in the light-grown tissue are due to Chl. The broad series of peaks between 400 nm and 500 nm in the dark-grown tissue are attributed to other known pigments. In Figure 11, the uncorrected action spectra for both light-induced callus weight enhancement and shoot production are plotted as the reciprocal of the incident quantum light level (proportional to the dose) required to cause 50% of the difference between the saturation and dark values (1.75 g of callus or 28 shoots, see Figs. 7 and 8) at each wavelength of investigation. The 750 nm point for shoot initiation, although recorded, is based on the extrapolation of the two highest intensity points on the 750 nm curve of Figure 8 whose differences from the dark control values are of questionable significance. Correction for absorption by a 0.15-cm pathlength piece of dark-grown or light-grown tissue appears to raise the action spectrum for shoot initiation between 400 nm and 500 nm only slightly. Similar results are obtained for the corrected action spectra for callus growth. We chose the value of 0.15 cm since that was the thickness of the original experimental callus explants. However, in developing callus cultures, cell division is not induced throughout the tissue but is restricted to the peripheral areas (36). Presumably, any effect of light must also occur in these same outer layers, and the dashed curves of Figure 11 represent limits to the amount of correction due to absorption.

Corrections due to scattering (which increases the effective pathlength of light) were not attempted. However, such modifications would raise the values at the blue end of the action spectra as well as those to the long wavelength side of any tissue absorption peak (12). Nevertheless, scattering should not significantly alter the action spectra of Figure 11 since again we are observing surface effects. The final assumption made in plotting the action

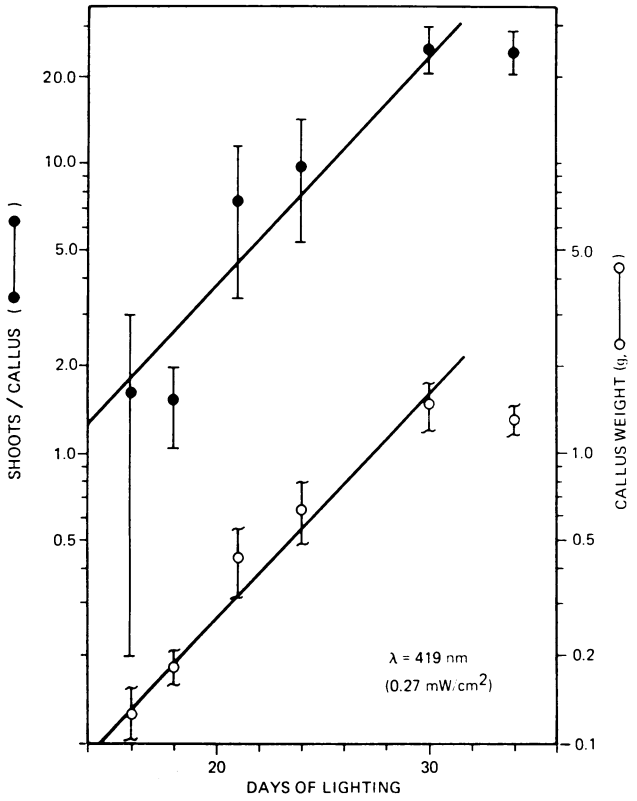

FIG. 3. Growth curves for tobacco callus exposed to 0.27 mw/cm² of 419.5 nm light for 16 hr each day. The number of shoots produced, defined as number of stem-like projections having at least one leaf (●) and the callus weight, as determined after all shoots are removed (○) are plotted as a function of time. Error bars represent ±1 SEM.

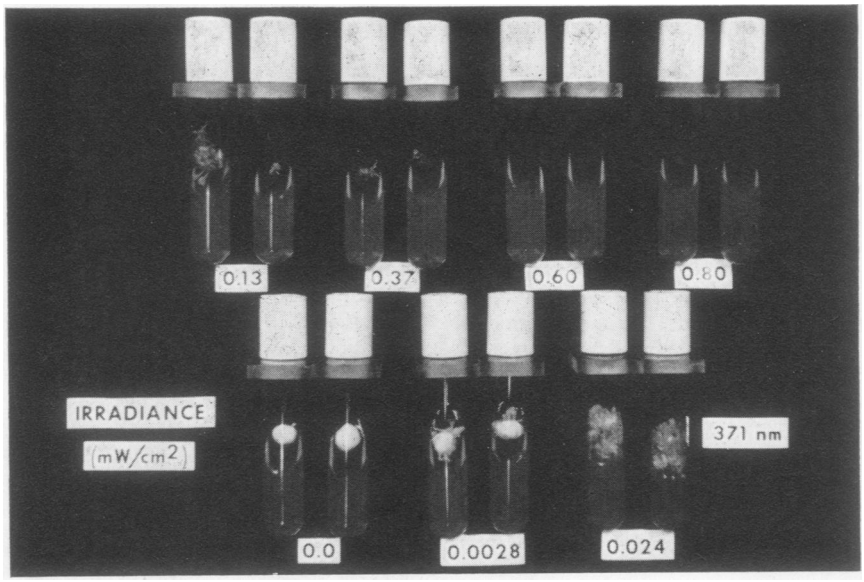

FIG. 4. Representative tobacco callus cultures exposed to the indicated incident irradiance levels of 371 nm light, 16 hr/day for 35 days.

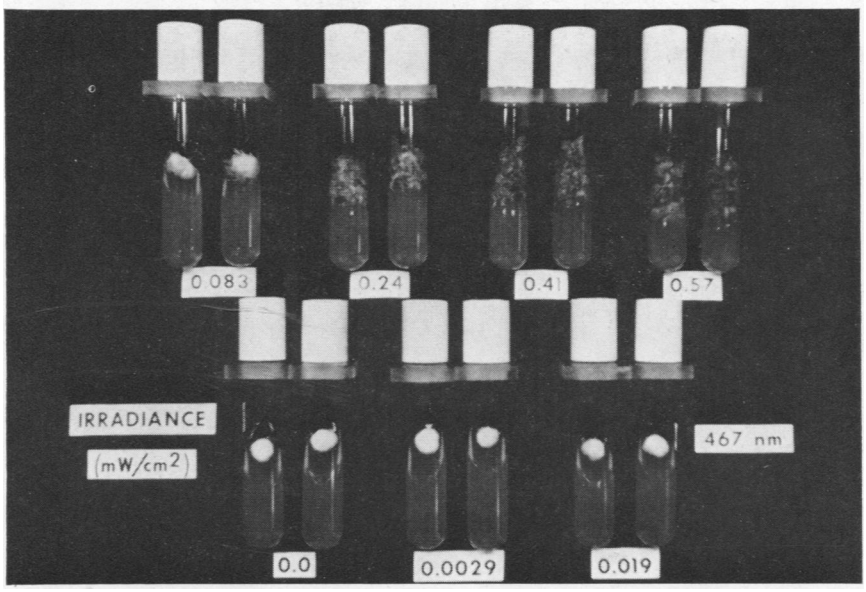

FIG. 5. Same as Fig. 4, except that the wavelength was 467 nm.

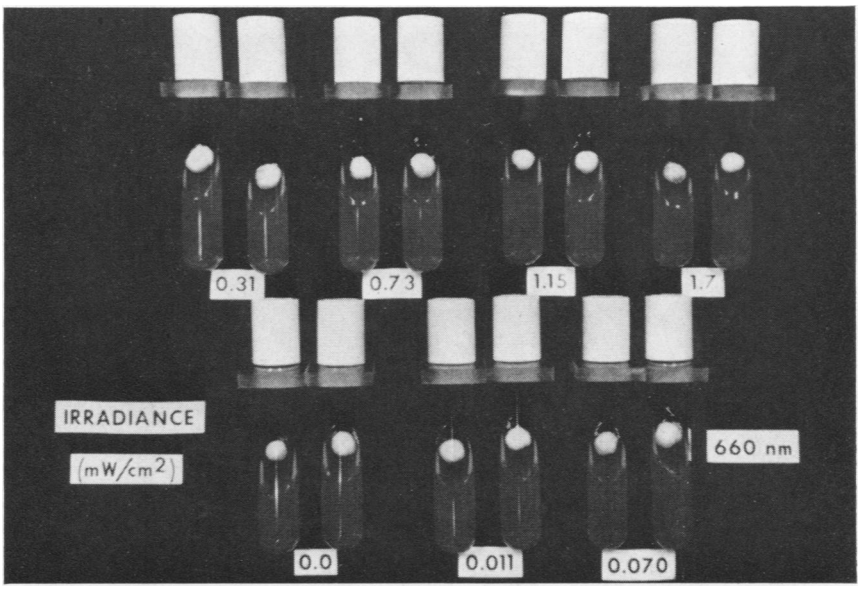

FIG. 6. Same as Figs. 4 and 5, except that the wavelength was 660 nm.

spectra of Figure 11 is that the quantum yield of the photoreceptor remains constant as a function of wavelength.

Broad Band Light Sources. Figure 12 presents callus growth and shoot initiation saturation curves for each of four broad band fluorescent light sources. The general features of the curves are quite similar to those of the 371 nm and 419.5 nm curves in Figures 7 and 8. Namely, the effect which a given light source will exert depends upon both the intensity and spectral properties at the tissue level. Figure 12 shows that there is an optimal irradiance for callus weight enhancement and shoot production with the broad band sources which was the case for the narrow band short wavelength sources. Deleterious effects appear only at light levels higher than in the case of the narrow band sources. The light intensity scale in Figure 12 is not directly convertible to the scales in Figures 7 and 8 because the sources cannot be characterized by a single wavelength. However, a rough comparison can be made by observing that for the 467 nm source, saturation was achieved at about 2.2 neinsteins cm^{-2} sec^{-1} which corresponds to an irradiance of 0.57 mw/cm^2. Although it is difficult to assess the differences in the saturation curves attributable to differences in the spectral emission of the four lamps, we noted that the number of shoots produced at saturation is greater in the case of B. In Figure 2, the most salient difference between B and all other sources is the presence of a pronounced red component in B's spectrum. The results from source D did not appear significantly different from those of the A or C. Apparently the additional UV component in both C and D has no important consequences for this plant system.

DISCUSSION

Explanation of Past Ambiguities. The pioneering work of many investigators has clearly established the important role light plays in the development of cultured plant tissue. The object of

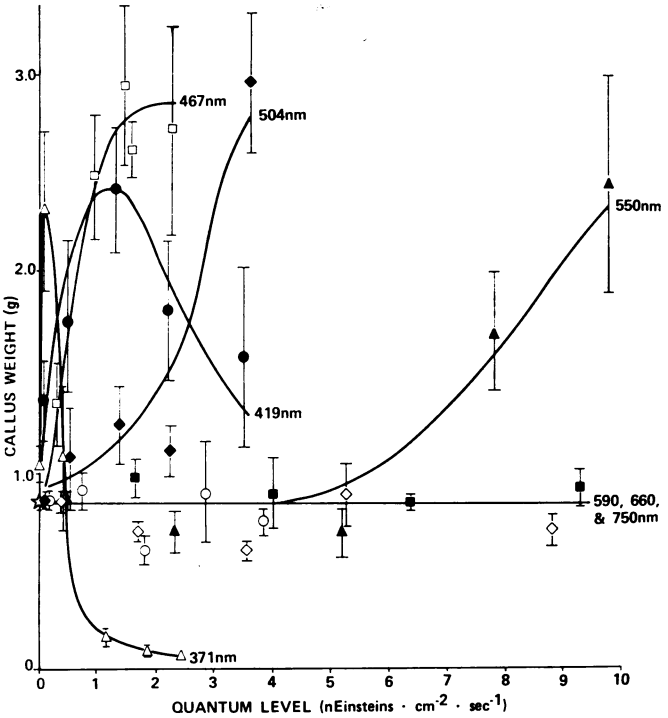

FIG. 7. Saturation curves for light-induced callus weight increase in tobacco. Each point represents the mean of the wet weight (weight after the removal of all shoots) for 10 callus samples. Callus weights are plotted as a function of the quantum level of incident light at each wavelength. The quantum level (Q) in neinsteins cm^{-2} sec^{-1} is related to the irradiance (I) in mw/cm^2 by the equation $Q = I \cdot \lambda/119.6$ where λ is the wavelength of the light source in nanometers. The exposure period was 16 hr/day for 35 or 36 days. Error bars represent ±1 SEM. Dark control (☆), 371 nm (△), 419.5 nm (●), 467 nm (□); 504 nm (◆); 550 nm (▲); 590 nm (○); 660 nm (◇); 750 nm (◇).

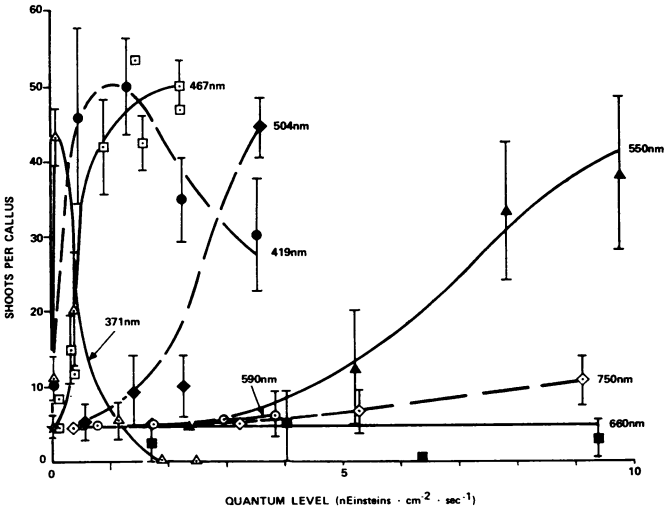

FIG. 8. Saturation curves for light-induced shoot production in tobacco callus. Experimental details are the same as in Fig. 7.

the present study has been to carefully characterize this role by demonstrating that both wavelength and intensity are important parameters for understanding photomorphogenesis in plant tissue culture. As a consequence, it is possible to clear up some of the inconsistencies mentioned in the introduction. Tobacco callus growth can be either stimulated or inhibited (Fig. 7) in comparison with dark controls, depending upon the level of near UV or blue light irradiance.

Our experiments revealed that light at 371 nm causes maximal stimulation at 0.024 mw/cm^2 but inhibits at levels above 0.15 mw/cm^2. Light at 419.5 nm and 467 nm induces maximal stimulation at higher levels of irradiance (0.3 mw/cm^2 and 0.57 mw/cm^2, respectively). Bergmann and Bälz (4) claim that 435 nm light stimulates callus growth, whereas Beauchesne and Poulain (2, 3) report that it is inhibitive. The former results were obtained at an irradiance of 0.24 mw/cm^2, whereas the latter were at 1.07 mw/cm^2. Thus, the lower irradiance stimulates callus growth while the higher value inhibits it, as one might expect from the curves in Figure 7. The inhibition of growth by high intensity light in the near UV and blue might involve low IAA oxidase activity (noted under 1.6 mw/cm^2 of blue light in *Parthenocissus tricuspidata* callus which grew much less than dark controls (7),

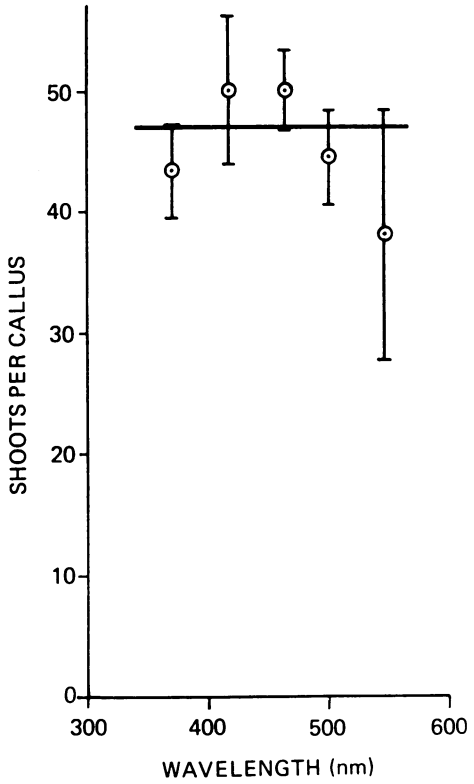

FIG. 9. Spectral dependence of shoot production at saturating light levels. Data were obtained from Fig. 8. (The point at 550 nm may not be quite at saturation level.)

Table III. *Effect of Different Distributions of Same Daily Dose on Callus Weight and Shoot Initiation after 42 Days of Illumination*

Distribution of Daily Dose	Callus Weight	No. of Shoots
	g	
One-half daily dose both 8-hr periods[1]	1.525 ± 0.105[2] (20)[3]	9.05 ± 2.10[4] (20)
One-quarter the daily dose the first 8 hr, three-quarters the daily dose the second 8 hr	1.580 ± 0.108[2] (20)	11.24 ± 2.25[4] (20)

[1] Irradiance of the 467 nm light was 0.155 μw/cm^2.

[2] No significant difference at the 0.05 level as determined by the *t* test.

[3] Values in parentheses are the number of samples; errors are SEM.

[4] No significant difference at the 0.05 level.

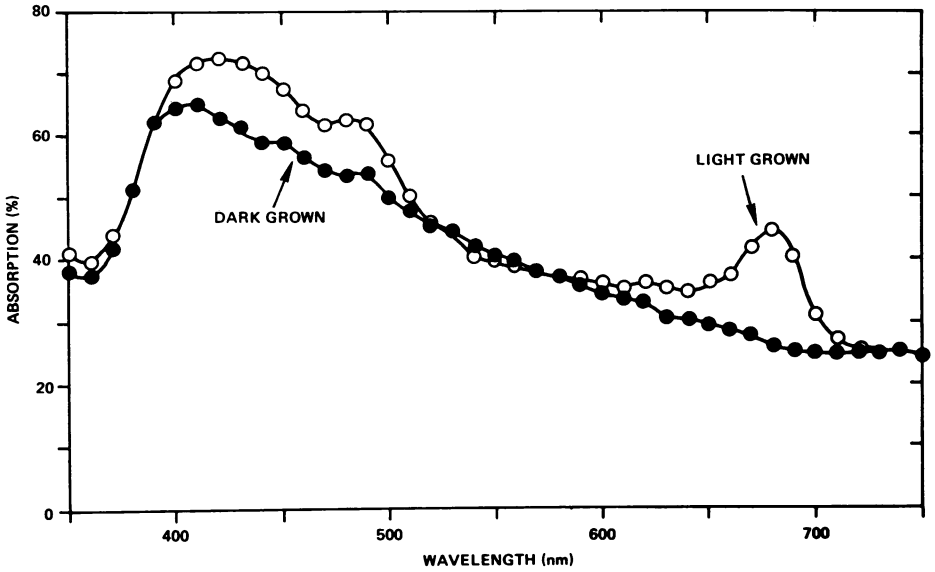

FIG. 10. Absorption spectra of freshly cut 1.5-nm thick pieces of dark- and light- (467 nm) grown tobacco callus samples obtained using an ntegrating sphere.

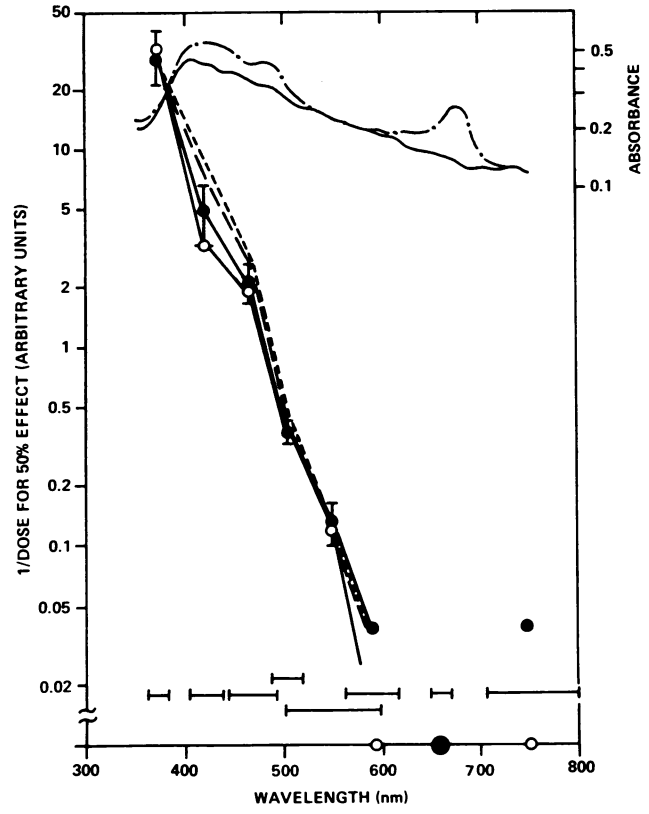

FIG. 11. Superposition of action spectra for callus weight enhancement (○) and shoot production (●) with the absorbance spectra of 1.5-mm thick pieces of dark- (——) and light-grown (-·-) tobacco callus tissue. The dashed curves represent corrections to the action spectrum for shoot initiation due to the absorption of a 1.5-mm thick piece of dark-grown (long dashes) or light-grown (short dashes) tissue. The absorbance data was obtained from the per cent absorption data in Fig. 10. The two sets of spectra were plotted on equivalent logarithmic scales to facilitate comparison of action and absorbance spectra (see "Discussion"). Vertical error bars represent ±1 SEM. The horizontal bars at the bottom represent the half-height bandwidth of the light sources used to obtain the action spectra data (Table I).

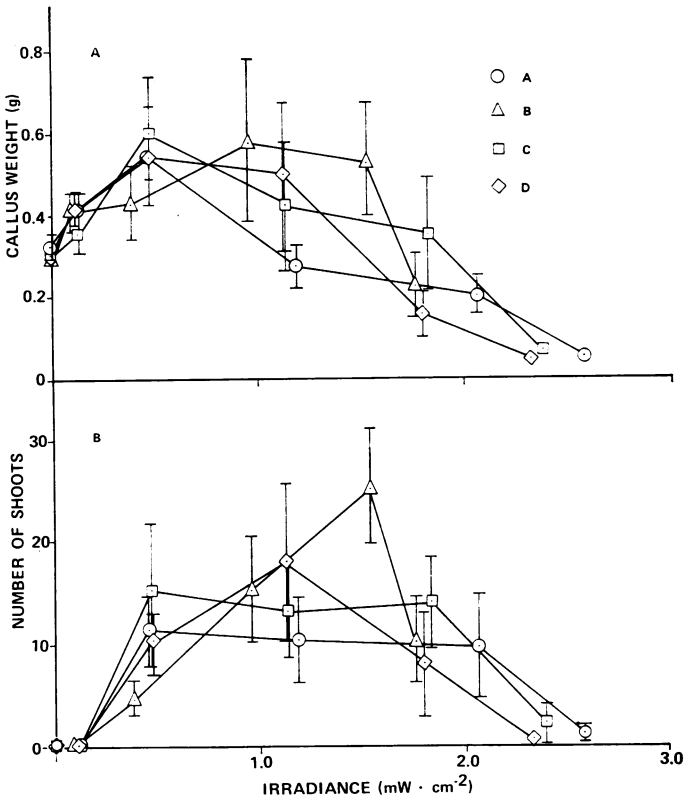

FIG. 12. Irradiance profiles for growth and shoot production in tobacco callus using four broad band commercial fluorescent light sources. No filters were used on these lamps. Irradiance levels are the incident values at tissue level. Error bars represent ±1 SEM.

destruction of Cyt oxidase (11), increases in the amount of phenolic compounds in the tissue (1), or vitamin B_{12} destruction (18).

Small amounts of contaminating short wavelength light, on the other hand, would suffice to explain why some investigators report enhanced callus growth at red light (660 nm) levels on the order of 10 neinsteins cm^{-2} sec^{-1}. We, in fact, did observe in-

creased callus growth and a great deal of shoot initiation in tobacco under our 660 nm light source if the near UV and blue mercury lines were not properly filtered (Seibert, unpublished results). Ohlenroth and Mohr (25) noted a similar type phenomenon in developing moss gametophytes.

Another source of confusion can arise when comparing the effects of several wavelengths at one quantum flux. As an example, if *a priori* in Figure 8, the effects of various wavelengths of light at 3.5 neinsteins cm^{-2} sec^{-1}, were arbitrarily chosen to compare, it might be concluded that shoot initiation is a green light (around 505 nm) effect instead of a near UV (around 370 nm) light phenomenon. This sort of comparison (one intensity for each wavelength) is common in the tissue culture literature.

Relationship between Growth of Tobacco Callus and Shoot Initiation. As seen from the parallel curves of Figure 3, callus tissue weight increase and shoot initiation are directly related, at least when illuminated with 419.5 nm light. The same general shape and relative position of the corresponding wavelengths in the saturation data of Figures 7 and 8 suggest a similar effect at all wavelengths investigated in this study. Figure 9, showing an essentially flat response for maximal shoot formation *versus* wavelength, suggests that a single type of photoreceptor controls the shoot initiation response. The coincidence of the two action spectra in Figure 11, furthermore, shows that the active photoreceptor which increases callus growth of tobacco compared with the dark controls also induces shoot initiation. Thus, we concluded that the same photoreceptor system which regulates callus growth also controls shoot production.

Photoreceptor. The photoreceptor absorbs in the near UV to blue region. It is also present in a small enough concentration that it's absorption (a sharply rising band peaking at 371 nm or lower) is not apparent in the absorption spectrum of either dark- or light-grown (green) tissue. (See Fig. 11 in which the action spectrum of the photoreceptor is plotted on the same scale as the absorbance spectrum of the tissue.)

Blue-absorbing photoreceptors in lower plants have been identified as either carotenoids or flavoproteins. According to Mohr (21) flavoproteins are the favored candidates. The action spectra of Figure 11 seem to eliminate the former because of their dissimilarity to the absorption spectra of carotenoids (21). In addition, preliminary experiments were performed using 2-(4-chlorophenylthio)triethylaminehydrochloride, a substance which interferes with carotenoid synthesis in higher plants by causing lycopene accumulation (9). The results are consistent with the conclusion that carotenoids are not the active pigment, since the inhibitor (up to 1 mM) has no effect on callus grown in the light. Various flavoprotein spectra show absorption peaks in a band between 400 and 500 nm, but do not look like the action spectra of Figure 11. On the other hand, the flavoprotein inhibitors, quinacrinehydrochloride and lumichrome (8), substantially inhibit growth of callus in the light (as low as 1 μM) while having no effect (up to 0.1–1 mM) on callus grown in the dark (Seibert, unpublished results). Perhaps some other type of flavonoid might be involved, since flavone glycosides, flavonal, flavonal glycosides, and aurones all have sharp absorption peaks (in organic solvents) in the near UV with little absorption in the visible (14). Porphyrins (other than Chl) might be another possible candidate for the photoreceptor, except that most of them peak at 400 nm or longer and have secondary absorption maxima in the 500 to 700 nm regions. For example, cytochromes in the reduced, but not the oxidized state, absorb in the 550 to 570 nm region. Ward and Vance (34) have proposed the "high irradiance response" mechanism (21) to explain photomorphogenesis in *Pelargonium* callus. Since there is no effect of light above 590 nm (in our intensity range) on the growth of tobacco callus and little effect on shoot initiation, we tentatively rule out high irradiance response effects.[2] An extension of this work further into the UV and a more detailed investigation of the pigments actually present in tobacco callus should aid in identifying the photoreceptor.

The broad bandwidth results of Figure 12 are of interest in relationship to the narrow bandwidth data. In spite of the considerable difference in the relative amounts of near UV and blue light components of four commerical lamps, the callus growth and shoot initiation curves appear quite similar. Thus, it would appear that sources C or D and increased UV in particular, offer no advantage for these types of studies. The plant lamp B, on the other hand, did give distinctively higher saturation values for shoot initiation (1.7 μw cm^2). It is distinguished from the others by emitting the greatest amount of 600 to 700 nm light. This fact provides additional evidence that a red light effect is obtained only in the presence of near UV or blue light and would mean that broad band-emitting lamps with different emission spectra may well have different effects on development in tissue culture. Examination of the nonvisible spectra indicated that other electromagnetic radiation from fluorescent tubes are not likely to have any effect on growth of these plant tissues.

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[2] The ratio of the 718 nm to 419 nm points of the high irradiance response action spectrum for hypocotyl lengthening in lettuce seedlings is 2.4 (21); that for shoot initiation is <0.04 from Figure 11, assuming no shoot initiation at 750 nm, maximal shoot initiation at the 719 nm high irradiance response peak, and a 90 nm half-height bandwidth of our 750 nm lamp.

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