EFFECTS OF VISIBLE MONOCHROMATIC RADIATIONS ON GROWTH
OF PITH CALLUS TISSUE OF PELARGONIUM ZONALE

APPROVED:

[Signatures]

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EFFECTS OF VISIBLE MONOCHROMATIC RADIATIONS ON GROWTH
OF PITH CALLUS TISSUE OF PELARGONIUM ZONALE

THESIS

Presented to the Graduate Council of the
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For the Degree of

MASTER OF SCIENCE

By

H. Bailey Ward, B. S.
Denton, Texas
August, 1966
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CHAPTER I
INTRODUCTION

In the early 1920's, Robbins (27) and Robbins and Maneval (28) succeeded in growing excised plant roots for several weeks in a defined nutrient solution. However, it was not until 1934 that the field of plant tissue culture was opened up by two workers, White and Gautheret (6). White (43) had emphasized the usefulness of growing excised tomato roots in vitro for extended periods of time. These were the first successful cultures of plant organs. A few years later, White (44) published descriptions of the first successful cultivation of plant tissues. These early cultures represented the first true plant tissue cultures in the sense of cultures of unorganized plant materials.

Before long-continued cultivation of plant tissues grown in vitro was demonstrated, the development of tissue culture technique as a useful method in botanical research was outlined by White (41). Additional reviews concerning materials, techniques, and history have been presented by White (39, 40, 41, 42, 45), Gautheret (7, 9), Rappaport (25), Street (36), Riker and Hildebrandt (26), Butcher and Street (2), Heller (10), Narayanaswami and Norstog (21), Tulecke (37), and Hildebrandt, Riker, and Duggar (11).
A number of different media have been developed for the nutrition of plant tissue cultures. Many of these are entirely synthetic (9, 10, 23, 45) while others contain basic mineral elements combined with such ill-defined substances as yeast extract, coconut milk, or tomato juice. In attempts to analyze coconut milk, Pollard, Shantz and Steward (24) and Shantz and Steward (29) revealed growth-promoting substances which stimulated the growth of carrot phloem explants. Steward and Caplin (35) found synergistic effects between 2,4-dichlorophenoxyacetic acid and coconut milk after finding that a combination of the two was necessary for growth of potato-tuber tissue grown in vitro. Similar synergisms have been reported between indoleacetic acid and kinetin (6-furfuryl aminopurine) (32), and 2,4-dichlorophenoxyacetic acid and kinetin (1). Skoog and Miller (31) demonstrated the role of kinetin in the regulation of root, callus, and shoot growth in tobacco pith tissue.

The nutritional requirements of plant tissue cultures have thus been generally established. The search for better media, new growth-promoting substances, and defined culture conditions is at present in an active state.

The usefulness of plant tissue cultures in studies of disease, nutrition, and morphogenesis has been limited by the relative instability of the growth of plant tissues in in vitro cultures. Nutritional requirements (10), morphological and cytological form (3), and general organizational abilities
of plant tissue cultures often change with the passage of time. Although many studies have been made in an attempt to determine optimal nutritional requirements (10, 12, 33, 40) and temperature regimes (4, 5, 11), little is known about the influences of visible radiation on the growth and development of plant callus tissue. Much of the research being done with light-induced morphogenesis, or photomorphogenesis, has involved the use of intact plants or plant organs. It is at this level that plant tissue cultures may be effectively used. De Capite (4) studied the effects of different intensities of artificial light on callus tissue growth but failed to specify whether the source of radiation was incandescent or fluorescent. White (45) and White and Risser (46) simply stated that cultures should be incubated in the light or in darkness. Early in the history of plant tissue culture, La Rue (15) noted differences in root formation abilities of hypocotyls and cotyledons from various species when grown in the light and in darkness. Some workers have reported that respiration rates and differentiation were promoted by light (5, 8, 20). Nickell et al. (22) demonstrated that the growth of some tissues may be stimulated by light. However, Steinhardt et al. (34) reported that the growth of spruce tissue cultures was more pronounced in darkness than in light.

It has been amply demonstrated that specific wavelengths of visible radiations are factors in plant morphogenesis. The red portion of the visible spectrum has been implicated
in several morphological responses. The phytochrome system, reversibly responding to red (660 μm) and far-red (730 μm), has perhaps been given more attention than any other system. Liverman and Bonner (16) reported on the reversibility of the phytochrome system and Lockhart (17) has studied the system in relation to physical growth factors. Lane et al. (14) have partially purified the phytochrome pigment.

Visible radiation with selected wavelengths also has an effect on internode elongation of intact plants. Some workers have suggested that the absence of short wavelength light produces an increase in stem length (38). Meijer (18) reported that Calendula and Petunia plants exhibited a pronounced elongation of internodes in blue light or in darkness. However, other plants such as Mirabilis, Rivina, and Mentha showed a pronounced internode elongation in red or green light and had short internodes in blue light.

Using relatively low intensity radiations (about 500 ergs/cm² per second), Klein (13) subjected tissue cultures of Parthenocissus crown-gall tissue to monochromatic light in the visible blue, green, orange, red, and far-red regions of the spectrum. In Klein's study, green radiation repressed growth, as measured by fresh weight, while all other wavelengths used produced growth of about the same degree above the dark control. At somewhat higher intensities (5,000 ergs/cm² per second), green repressed growth even more. The growth of the Parthenocissus crown-gall tissue also was inhibited by full-spectrum radiation at about 1340 lux.
In 1957, it was demonstrated that phytochrome was not the only photoreactive system in photomorphogenesis (30). The experimental data collected at that time indicated that a different photoreactive system was involved. This reaction was demonstrated only if the material was irradiated with relatively high intensities of radiation (over 2000 ergs/cm² per second) and has been termed the "high-energy reaction of photomorphogenesis." The high-energy reaction seems to be more important than the phytochrome system under natural conditions of high irradiance. According to Mohr (19), the system is not photoreversible and the action spectrum of the reaction shows peaks in the blue and far-red with slight activity in the red. It was suggested that a metal-flavoprotein enzyme might be the light-activated species involved in the system.

In view of the lack of data concerning the responses of plant tissue cultures to high intensity monochromatic radiations, and with the conviction that in vitro cultures may provide a useful tool for study of the photoreactive systems involved, the present investigation was undertaken. It was the purpose of the investigation to determine the effects of selected high intensity monochromatic radiations on the growth of pith callus tissue of Pelargonium zonale, variety Enchantress Fiat. In addition, the extent of cell differentiation was to be determined for tissues grown under each experimental treatment.
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CHAPTER II

MATERIALS AND METHODS

Initial callus cultures were obtained from pith tissue of greenhouse-grown Pelargonium zonale variety Enchantress Fiat. The plants were grown from cuttings and were about ten months old at the time of harvest. Stock plants were obtained from Jack Cannon Florist, Arlington, Texas.

Procedures similar to those presented by Chen and Galston (1) were used to initiate pith callus growth. Stem segments, three to four centimeters long, were taken ten centimeters below the apex of the stem. After removing all leaves and buds, each segment was washed with tap water, sterilized in a beaker containing 15 per cent commercial Clorox for thirty minutes, and rinsed three times with sterile, distilled, demineralized water. The pith was cut into small, uniform disks by first boring cylinders of tissue with a sharpened glass tube and then slicing disks of uniform thickness with a glass knife. The resulting disks were 6.0 millimeters in diameter, 1.0 millimeter thick, and weighed about 20 milligrams, fresh weight. The disks were then inoculated onto a solid medium contained in twenty-five by one hundred millimeter culture tubes, two disks per tube.
The tubes were capped with loose-fitting aluminum caps that permitted aeration and prevented contamination from the atmosphere.

The medium used was White's basic inorganic (4), supplemented with organic constituents (1), and solidified with one per cent Difco Iod-Agar. Table I lists both the inorganic and organic constituents of the modified medium used.

**Table I**

**Composition of White's Modified Medium**

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Milligrams Per Liter</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inorganic:</strong></td>
<td></td>
</tr>
<tr>
<td>KCL</td>
<td>65.0</td>
</tr>
<tr>
<td>KNO₃</td>
<td>80.0</td>
</tr>
<tr>
<td>Ca(NO₃)₂.4H₂O</td>
<td>300.0</td>
</tr>
<tr>
<td>MgSO₄.7H₂O</td>
<td>720.0</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>200.0</td>
</tr>
<tr>
<td>NaH₂PO₄.2H₂O</td>
<td>16.5</td>
</tr>
<tr>
<td>Fe₂(SO₄)₃</td>
<td>2.5</td>
</tr>
<tr>
<td>MnSO₄.4H₂O</td>
<td>7.0</td>
</tr>
<tr>
<td>ZnSO₄.7H₂O</td>
<td>3.0</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>1.5</td>
</tr>
<tr>
<td>KI</td>
<td>0.75</td>
</tr>
<tr>
<td>CuSO₄.5H₂O</td>
<td>0.001</td>
</tr>
<tr>
<td>MoO₃</td>
<td>0.0001</td>
</tr>
<tr>
<td><strong>Organic:</strong></td>
<td></td>
</tr>
<tr>
<td>Thiamin</td>
<td>0.1</td>
</tr>
<tr>
<td>Pyridoxine</td>
<td>0.5</td>
</tr>
<tr>
<td>Nicotinic Acid</td>
<td>0.5</td>
</tr>
<tr>
<td>Glycine</td>
<td>2.0</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>100.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>30000.0</td>
</tr>
<tr>
<td>Kinetin (6-furfuryl aminopurine)</td>
<td>0.1</td>
</tr>
<tr>
<td>2,4-dichlorophenoxyacetic acid</td>
<td>0.1</td>
</tr>
</tbody>
</table>

All solutions were made in distilled, demineralized water. The pH of the medium was adjusted to 5.3 with hydrochloric acid or potassium hydroxide before addition of the
agar. After heating and addition of the agar, the medium was dispensed to the culture tubes, twenty milliliters per tube, and autoclaved at 1.2 kilograms per square centimeter pressure for fifteen minutes at 121 degrees Celsius (C).

The preparation of pith explants and all subsequent operations involving the tissues were performed in a stainless steel sterile transfer chamber, the inside of which was washed with 10 percent commercial Clorox and then irradiated with ultraviolet light for at least four hours prior to use.

The pith explants were incubated in a dark chamber at 27±2°C. After three weeks of culture, approximately 60 percent of the explants had developed callus tissue ranging from one- to three-fold over the initial volume. Callus pieces from these initial cultures were used as the inoculum for subsequent procedures (see Figure 3, B-C).

Pieces of pith callus from the initial cultures were excised, weighed individually to the nearest milligram on a Mettler Model H16 analytical balance, and placed in culture tubes. Weights were made by placing the tissue in a sterile petri dish, weighing, and taring the dish after the tissue had been removed. A sterile filter paper inside the petri dish served to remove excess water from the tissue. All weights were made inside the sterile chamber. Three pieces of tissue, ranging in weight from eighteen to thirty milligrams, were placed in each tube. All manipulations were made in diffuse, incandescent light. The use of colored "safe"
light was avoided since experimental treatments consisted of monochromatic light within the same wavelength range. The extreme friability of the callus tissue made it possible to use glass loops fashioned from glass rods for excising and transferring the tissue.

Three tubes, each containing three callus pieces selected from three different stock tissues, were placed in each of five culture chambers to receive the respective light or dark treatment. Figure 1 illustrates the apparatus used to culture and irradiate the callus tissue.

Light treatments consisted of monochromatic radiation obtained by filtering incandescent light from General Electric Reflector Flood lamps through layered, plexiglass filters (2). A relatively full spectrum treatment ("white") also was administered with a General Electric Lumiline incandescent tube by passing the light through transparent plexiglass. Three one hundred-watt lamps were used for the red and green treatments, and a one hundred fifty-watt lamp used for the blue treatment. Variations in intensities were obtained by adjusting the height of the lamps over the filters. A Powerstat variable transformer was used to adjust the radiant output of the light sources to 15,000 ergs/cm² per second as measured with a YSI-Kettering Model 65 Radiometer. The radiant energy measurements were made at the level of the culture tubes.

Three monochromatic treatments were used: one in the visible blue, the filter system transmitting wavelengths from 410
millimicrons to 460 millimicrons and peaking at 450 millimicrons; one in the green, the filter system transmitting from 500 millimicrons to 590 millimicrons and peaking at 545 millimicrons; and one in the red, the filter system transmitting from 600 millimicrons to 690 millimicrons and peaking at 650 millimicrons. The transmission data were obtained from published spectral transmission curves for the filters used (2). All light treatments were continuous. The dark treatment was accomplished by use of a light-tight chamber heated with a thermostatically controlled hot plate (see Figure 1).

Temperatures were maintained at 27±2° C. by an oxygen-tent cooling unit. Figure 1 illustrates the manner in which the unit was attached to the treatment chambers.

After twenty-two days of culture, each tissue piece was individually weighed (fresh weight). Microscopic examinations were made to determine the extent of differentiation. Since lignification is often an indication of differentiation, at least in vessel elements (3), phloroglucinol-HCl stain was used to aid in determining the nature and degree of differentiation of the callus tissue. Direct observation of cells was made by preparing fresh mounts of different parts of representative tissues. No microtome sections were prepared because the callus tissues were extremely friable and were easily broken into fractions suitable for wet mount preparations. Photographs of the callus tissues were made with a
Polaroid Land camera equipped with a close-up lens. Photomicrographs of fresh mounts of callus tissue cells were made with an AO Spencer Series 10 microscope equipped with a 35 millimeter camera.

This investigation was designed to permit a statistical analysis of the data, using a randomized block design and the studentized range test. All experiments were repeated three times. For each test, new media were prepared and precautions were taken to obtain identical conditions. The first test was begun on March 23, 1966, and the last one ended on June 12, 1966.
Figure 1—Diagram of the tissue culture chamber.
CHAPTER BIBLIOGRAPHY


CHAPTER III

RESULTS

After twenty-two days of culture, all tissue units were pale yellow in color and were quite friable. Maximum growth resulted in fresh weight increases representing a twelve-fold increase over the initial weight. Minimum growth was represented by tissue units which did not show a fresh weight increase over the initial weight. The initial and final fresh weights of each piece of callus tissue used in this experiment are presented in Table II. That the growth of the tissue is quite variable with respect to fresh weight increases is apparent in the variability of final weights. The mean weight increases shown in Table II reveal that greatest growth occurred in the blue and "white" light treatments and that less growth occurred in the dark treatment than in any other treatment.

All three trials yielded similar results with respect to treatments producing maximum and minimum growth. However, the mean increases for the red and green treatments, respectively, in trial one were reversed in trial two and three. The mean for each treatment of the combined trials is presented in Figure 2.
### Table II

**Initial and Final Weights of Callus Tissue of Pelargonium Zonale Variety Enchantress Flat Grown in Five Different Light Treatments**

<table>
<thead>
<tr>
<th>Trial and Tissue Number</th>
<th>Fresh Weight in Milligrams</th>
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<tr>
<td></td>
<td>Blue (450 μm)</td>
<td>Green (545 μm)</td>
</tr>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>A. 1</td>
<td>21</td>
<td>127</td>
</tr>
<tr>
<td>2</td>
<td>18</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>27</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
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<td>9</td>
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<td>134</td>
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<tr>
<td>Mean</td>
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<tr>
<td>S.D.</td>
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<tr>
<td>B. 1</td>
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<td>136</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>165</td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td>105</td>
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<td>7</td>
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<td>8</td>
<td>30</td>
<td>75</td>
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<tr>
<td>S.D.</td>
<td>4.2</td>
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<tr>
<td>C. 1</td>
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<tr>
<td>Mean</td>
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<td>64</td>
</tr>
<tr>
<td>S.D.</td>
<td>4.2</td>
<td>64.6</td>
</tr>
</tbody>
</table>
Figure 2—Fresh weight increases over initial weights of *Pelargonium zonale* callus tissue grown in vitro under different light treatments. Each value is the mean of 42-45 pieces of callus tissue.
Photographs of the final tissues from each treatment in trial two are presented in Figure 3, A (1-5). Although volume differences are not so apparent in the two-dimensional photographs, the fresh weight differences given in Table II reveal the growth differences of individual tissue units in each treatment. The smallest piece of tissue shown in Figure 3, A (1), represents the relative size of the initial tissue units and the largest piece in the same photograph represents the maximum size attained by any single tissue unit.

Photomicrographs of typical cells from representative callus tissues are shown in Figure 3, D-H. The characteristically large, highly vacuolated cells found in all tissues are shown in Figure 3, D. These cells ranged in shape from nearly spherical to greatly elongated cells. The spherical cells were 0.05 to 0.1 millimeter in diameter whereas the elongated cells were about 0.1 millimeter in diameter and up to 1.0 millimeter in length. The cells shown in Figure 3, D were magnified 100 times. The individual cell shown in Figure 3, E was magnified 450 times and was included to show the cellular inclusions of a typical callus cell.

All tissues yielded callus growth under each treatment and no shoot or root formation was observed. However, the tissue did develop aggregates of differentiated material in the form of vessel elements. Figure 3, F is a photomicrograph of a typical aggregate of vessel element cells found.
Figure 3—Photomicrographs of Callus Tissue of *Pelargonium zonale*, variety Enchantress Fiat. A, Sets of tissue from each light treatment in trial two. Numbers 1-5 represent blue (450 μm), green (545 μm), red (650 μm), "white," and dark treatments, respectively. Two-thirds actual sizes are shown. B, Disk of *Pelargonium* pith tissue before cultivation, X2. C, Pith tissue from initial explant after three weeks of culture, X2. D, Typical callus tissue cells, X100. E, Single callus tissue cell, X450. F, Aggregate of differentiated cells, X100. G, Aggregate broken apart to show individual elements, X100. H, Individual vessel element, X450.
scattered throughout all tissues. A typical aggregate, spread apart to reveal individual elements, is shown in Figure 3, C. A higher magnification (X450) of a typical vessel element common in tissues from all treatments is depicted in Figure 3, H. The secondary thickening of the cell wall is distinguishable. It was not possible to determine a significant difference in the degree of cell differentiation between treatments. Phloroglucinol-HCl staining revealed a high degree of lignin deposition in the aggregates and in individual vessel elements.

A statistical analysis using mean fresh weight increases for each treatment with a randomized block design revealed significant differences at the one per cent level of significance due to treatments (Table III).
### TABLE III

**VARIANCE TABLE FOR DIFFERENT LIGHT TREATMENTS ON MEAN FRESH WEIGHT INCREASES OF CULTURES OF *PELARGONIUM ZONALE* GROWN IN VITRO AS DETERMINED BY USING A RANDOMIZED BLOCK DESIGN FOR ANALYSIS OF VARIANCE**

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>SS</th>
<th>DF</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatments</td>
<td>2975.0</td>
<td>4</td>
<td>744.0</td>
<td>14.66**</td>
</tr>
<tr>
<td>Blocks</td>
<td>463.6</td>
<td>2</td>
<td>231.8</td>
<td>4.60*</td>
</tr>
<tr>
<td>Error</td>
<td>402.4</td>
<td>8</td>
<td>50.3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3740.8</td>
<td>14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*0.05 level of significance.
**0.01 level of significance.

Significant differences between individual treatments were determined with the studentized range test. Results of the test, presented in Table IV, showed significant differences at the five per cent level between blue and red, blue and green, blue and dark, "white" and red, "white" and green, "white" and dark, and red and dark.
TABLE IV

SIGNIFICANT DIFFERENCES OF MEAN FRESH WEIGHT INCREASES OF CALLUS TISSUE OF *Pelargonium zonale* BETWEEN INDIVIDUAL LIGHT TREATMENTS AS DETERMINED BY THE STUDENTIZED RANGE TEST

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dark</th>
<th>Green (545 mu)</th>
<th>Red (650 mu)</th>
<th>&quot;White&quot;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue (450 mu)</td>
<td>38*</td>
<td>25*</td>
<td>20*</td>
<td>4</td>
</tr>
<tr>
<td>&quot;White&quot;</td>
<td>34*</td>
<td>21*</td>
<td>16*</td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td>16*</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>13</td>
<td></td>
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<td></td>
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</tbody>
</table>

*0.05 level of significance.*
The growth of the Pelargonium pith callus tissue was significantly greater in the blue and full spectrum ("white") treatments than in the red, green, or dark treatments. In the blue and full spectrum treatments, the differences in weight increases were not significantly different. Thus the action spectrum of the Pelargonium callus tissue growth is comparable to the high-energy action spectra reported for several plant systems, including synthesis of anthocyanin (8, 9), synthesis of flavonoids (9), control of hypocotyl lengthening (8), and internode elongation (2).

The action spectrum of the high-energy reaction in the range of the visible spectrum has been determined for growth phenomena of several plant species. It shows peak effectiveness in the far-red and in the blue portion of the visible spectrum (7, 8, 9).

Wassink et al. (10), using radiant intensities of 10,000 ergs/cm² per second, reported that stem elongation in Hyoscyamus niger was more pronounced under blue and far-red irradiation than under green, yellow, or red. The addition of red to blue inhibited the promotive effects of blue. The addition of red to far-red treatments produced some inhibition.
but less than in the blue light. It was proposed by the authors (Wassink et al.) that far-red and blue have similar effects on stem elongation of *Hyoscyamus* and that red light suppresses elongation. Green light was shown to have no positive effects on stem elongation. Similar results were obtained by Fletcher et al. (2), who irradiated bean seedlings with relatively high intensities of monochromatic blue, red, and far-red and observed greater elongation under the blue and far-red treatments along with an apparent suppressive effect of red light. The same workers also showed that root formation of the bean plants was controlled by the blue/far-red system in that both wavelengths inhibited formation to the same degree.

Since the full spectrum source ("white") used in the present study to irradiate the callus tissue of *Pelargonium* was an incandescent lamp, and since this source emitted far-red radiation, it is possible that the growth effects observed under this treatment were due to the far-red light. However, it should be remembered that the full spectrum treatment also contained blue, red, and green wavelengths and that no data are available concerning the effects of light combinations on callus tissue growth. The full spectrum treatment effects were consistent with the reported actions of blue and far-red irradiation under relatively high intensities of light and would well explain the low degree of growth response that occurred in the dark treatment relative to the full spectrum treatment.
In their work with dodder seedlings, Lane and Kasperbauer (6) separated two photoresponses associated with hypocotyl hook opening and stem twining. Hook opening was dependent on the low-energy red/far-red system while twining action was controlled by high-energy blue and far-red irradiation. The nature of the results prompted these authors to propose two different photoreceptors involved in the high-energy reaction, one sensitive to blue and the other, perhaps phytochrome itself, sensitive to far-red. According to Mohr (7) and Vince (9), the high-energy system is not photoreversible and may operate simultaneously with the low-energy phytochrome system, either synergistically or independently. In either case the high-energy reaction will override the low-energy system. It is, of course, possible that phytochrome was present in the Pelargonium callus tissue and that a low-energy reaction was operating along with the proposed high-energy reaction. However, there have been no reports on the presence of phytochrome in plant callus tissue and the only action spectrum reported for the growth of such tissue at low energy levels was not the action spectrum of the phytochrome system (5).

The theoretical background of the high-energy reaction has not yet been firmly established. Mohr (6) proposed the involvement of metal-flavoproteins, such as butyryl-coenzyme A dehydrogenase, which have absorption spectra similar to the action spectra of the high-energy reaction.
It is possible that the effects of the blue and full spectrum light treatments on the growth of the *Pelargonium* callus tissue were brought about by the induction of changes in the levels of certain enzymes, flavonoids, and/or related phenolic compounds. Indoleacetic acid oxidase may be regulated by flavonoids in isolated systems. Some flavonoids inhibit indoleacetic acid oxidation while others enhance it (6). The action spectrum for flavonoid biosynthesis (8) is similar to the one presented in this paper for the growth of *Pelargonium* callus tissue.

In addition to enzyme effects, total protein synthesis has been shown to be dependent on light. Zucker (11) reported that protein synthesis was dependent on light in isolated disks of potato tuber tissue. The action spectrum for the response showed a broad maximum in blue light.

That the *Pelargonium* callus tissue did not develop differentiated tissue beyond simple vessel elements is consistent with results reported by other workers. Chen and Galston (1), the only workers who have reported the use of *Pelargonium* tissue cultures, stated that only tracheids were formed in the pith callus tissue. There might be some question as to the sole presence of tracheids in angiosperm tissue. It is more probable that the structures observed by Chen and Galston were vessel elements rather than tracheids.

The finding that no differences occurred in the degree of differentiation under the different light and dark treatments
appears to be consistent with the findings of Gautheret (3) that wavelength is not important in differentiation when high intensity light is used.

Although the growth factors, kinetin and 2,4-dichlorophenoxyacetic acid, used in the Pelargonium culture media have been reported to initiate bud and root formation, respectively, it has been reported that balanced amounts of kinetin and indoleacetic acid produce neither bud nor root formation (4). Since the action of 2,4-dichlorophenoxyacetic acid is similar to that of indoleacetic acid, it is proposed that the 0.1 milligram per liter concentrations of both kinetin and 2,4-dichlorophenoxyacetic acid used in the Pelargonium culture media contributed to the lack of organ formation in all treatments.

The findings of this investigation indicate that the high-energy reaction typical of intact plants is also in operation in pith callus tissue of Pelargonium zonale, variety Enchantress Fiat, grown in vitro under high intensity monochromatic radiations. It can be inferred from these findings that the tissue contains the photoreceptor(s) involved in the high-energy reaction and that the factors necessary for the response were synthesized in the in vitro cultures or were transferred from the initial explants. Further work with the rate of tissue growth and with the degree of response with age of tissue would help to elucidate the nature of the photoreceptor(s) involved.
In addition, these results, and the findings of others cited in this paper, indicate that the influences of light on plant tissue culture growth must be taken into account when such cultures are used for experimental purposes. If plant tissue used for experimental purposes responds to either the low- or high-energy reactions, and most plant tissues have been shown to be sensitive to one or both, then the cultures should be grown in either the dark or under specifically defined light regimes, preferably those for which growth responses have been determined. For *Pelargonium zonale*, the use of full spectrum, high intensity illumination for routine or experimental cultivation approximating natural conditions is indicated.

Keeping in mind the variability that usually is observed in callus tissue growth, and the even more variable data available concerning the effects of light on plant growth, it is obvious that a great deal of work should be done in both areas. Improved culture techniques must be developed that will yield rapid, consistent, and uniform growth of *in vitro* plant tissues before these cultures can be used with a high degree of confidence in experimental procedures.

At present the field of photomorphogenesis is in an active state indeed. As more work is reported and more data accumulated, the rather elusive photoreactive systems involved in the biological responses of plants to light will perhaps be elucidated.
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