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Chlorophyll is not the primary photoreceptor for the stimulation of P-type H^+ pump and growth in variegated leaves of *Coleus* × *hybridus*

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Abstract. There has been persisting controversy over the role of photosynthesis in the stimulation of the plasma membrane H^+-ATPase and growth of dicotyledonous leaves by light. To investigate this, we compared the effects of light on growth, H^+ net efflux and membrane potential (V_m) of strips which contained either only chlorophyll-free (white) mesophyll cells or chlorophyll-containing (green) cells cut from variegated *Coleus* leaves. White mesophyll cells responded to white, blue and red light with a hyperpolarization of V_m, an acidification of the apoplast and a promotion of growth, all of which began after a lag of 2–7 min. In contrast, green mesophyll cells showed a biphasic light response in which the hyperpolarization and the acidification were preceded by a rapid depolarization of V_m and an alkalinization of the apoplast. Nevertheless, green and white tissues showed comparable growth promotions in response to light. The light response of the leaf mesophyll is a composite of two separate photosystems. The initial depolarization and alkalinization are mediated by photosynthesis and blocked by 3-(3,4-dichlorophenyl)-1,1-dimethylurea. The slower hyperpolarization, acidification and growth response, on the other hand, are clearly in response to light absorption by pigments other than chlorophyll.

Key words: *Coleus* × *hybridus* – Electric photoresponse – Leaf growth – Photosynthesis – Photomorphogenesis – P-type H^+-ATPase

Introduction

Growth of etiolated leaves is promoted by low-fluence irradiation with blue or red light (Klein 1969). After de-etiolation, light stimulates the growth of dicot leaves by extensive cell enlargement with a high fluence rate optimum of >100 μmol photons cm^{-2} s^{-1} (Butler 1963; Sale and Vince 1963; Van Volkenburgh et al. 1990; Stahlberg and Van Volkenburgh 1999). In many cases light does so by causing cell wall acidification by stimulating a P-type H^+-ATPase (Van Volkenburgh and Cleland 1980; Brock and Cleland 1989; Keller and Cosgrove 1996; Keller and Van Volkenburgh 1999). The identity of the primary photoreceptor for the stimulation of this H^+-ATPase in leaf cells is a matter of some controversy. There is strong evidence that photosynthesis is required for the light-induced stimulation of the plasma membrane (PM) H^+-ATPase in leaf cells of *Elodea* (Jeschke 1970; Tazawa et al. 1986; Marrè et al. 1989; Okazaki et al. 1994; Bellando et al. 1995) and in several *Characean* algae (Fujii et al. 1978; Tazawa and Shimmen 1980). Similarities in the action spectra for light-stimulated leaf growth and photosynthesis (Vince-Prue and Tucker 1983; Van Volkenburgh et al. 1990) might suggest that chlorophyll is the photoreceptor in dicot leaves as well. This is also suggested by the fact that a reduced photosynthetic capacity often appears to limit cell expansion and cell size in dicot leaves (Kriedemann 1986; Lecoeur et al. 1995; Saradevi et al. 1996). On the other hand, persistence of light-induced growth in tentoxin-bleached bean leaves suggests that chlorophyll is not the primary photoreceptor (Van Volkenburgh and Cleland 1990). A role for phytochrome as an important photoreceptor for leaf expansion has long been suggested (Vince-Prue and Tucker 1983; Neff and Van Volkenburgh 1994; Van Volkenburgh 1999) and was recently supported by studies on the effect of overexpression of phytochrome C (Halliday et al. 1997; Qin et al. 1997).

Experiments with 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) – a photosynthetic inhibitor that interrupts linear electron transport from photosystem II to photosystem I (Vasil'ev and Venedictov 1993) had been expected to provide decisive evidence for the involvement of photosynthesis, but failed to do so. Although DCMU inhibits extracellular acidification by

Abbreviations: DCMU = 3-(3,4-dichlorophenyl)-1,1-dimethylurea; FR = far-red light; PM = plasma membrane; V_m = membrane potential

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illuminated discs or protoplast from dicot leaves (e.g. Kelly 1983; Petzold and Dahse 1988; Van Volkenburgh and Cleland 1990), its effect on leaf growth never reaches the level of complete inhibition (Van Volkenburgh and Cleland 1990; Blum et al. 1992). Even if chlorophyll is the primary photoreceptor, some light-stimulated leaf growth might still have occurred because DCMU was only partially effective in inhibiting photosynthesis. Also, DCMU does not affect cyclic photophosphorylation, which could supply ATP for the H^+ pump (Spanswick 1974). Alternatively, some pigment other than chlorophyll may be the primary photoreceptor for the stimulation of H^+ pump and leaf growth, while the process is enhanced by some photosynthetic product (Marrè et al. 1989; Takeshige et al. 1992), e.g. ATP (Mimura and Tazawa 1986; Blum et al. 1992).

Discrimination among these possibilities can be made using leaf cells completely lacking chlorophyll. Variegated young leaves of *Coleus* × *hybridus* grow at modest rates in the light. Leaf strips can be obtained with all mesophyll cells either containing (green) or completely lacking (white) chlorophyll at the same developmental age. By comparing the effects of light on membrane potential (V_m), apoplastic pH and growth, chlorophyll-mediated responses are separated from those driven by a different photoreceptor. It will be shown that in mesophyll cells of *Coleus* leaves chlorophyll is not the receptor for the light that stimulates growth and the PM H^+-ATPase.

Materials and methods

Plant material

Coleus plants were grown in a greenhouse at 22 °C day/18 °C night with a natural photoperiod. All experiments were carried out with young (30–40 mm midvein length which is about 50% final leaf size), variegated leaves of vegetatively propagated *Coleus* × *hybridus* plants. Handling and preincubation proceeded under dim green light with a fluence rate of less than 1 µmol m^{-2} s^{-1} (referred to as darkness).

Microscopic techniques

Fluorescence- and light-microscopic pictures were taken with an SLR camera body (N8000s; Nikon Corporation Tokyo, Japan) connected to an epifluorescence microscope (Labophot-2; Nikon Corporation Tokyo, Japan) at a magnification of 100× for cross-sections of *Coleus* leaves containing both green and chlorophyll-free tissue.

Absorption spectra

Green and white patches of *Coleus* leaves were excised and vacuum-infiltrated with a solution containing 10 mM KCl and 1 mM $CaCl_2$ to reduce light scattering and refraction. After mounting them inside the measuring path of a spectrophotometer (Beckman DU-40; Palo Alto, Calif., USA) we recorded the absorption in the range from 400 to 750 nm in 10-nm steps and against the background of the infiltrated solution.

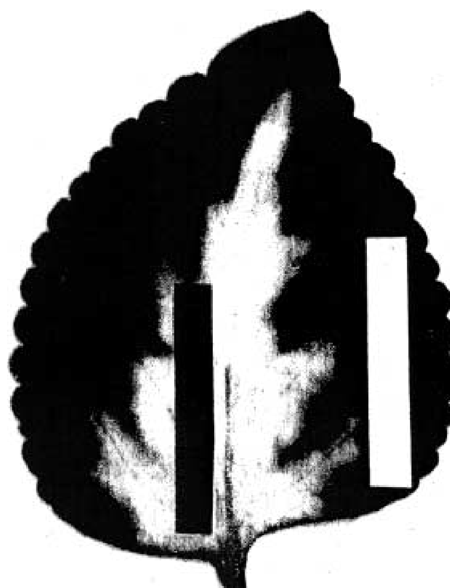

Fig. 1. Example showing the location of 20-mm-long, 4-mm-wide strips cut out of the white and green parts of a growing *Coleus* × *hybridus* leaf (50% final length)

Growth measurements

Leaf strips (20 mm × 4 mm) containing either chlorophyll-free or green mesophyll cells were cut parallel to the midvein of young, variegated *Coleus* leaves (Fig. 1) and then mounted between a fixed and a mobile clamp while floating on the incubation medium containing 10 mM KCl, 1 mM $CaCl_2$, and 1 mM Mes/BTP [1,3/bis-tris(hydroxymethyl)methylamino-propane] buffer at pH 6.0. The mobile clamp was connected to the axis of a rotary position transducer (RD 30; Schaevitz, Pennsauken, Pa., USA), which recorded the expansion of the strip under a load of 2 g to keep the strip in a stretched position. Upon mounting between the clamps under dim green light (<1 µmol m^{-2} s^{-1}) the leaf strips expanded rapidly for 5–20 min (not shown), after which a slow, steady rate was observed for at least 60 min before 200 µmol $m^{-2}s^{-1}$ white light, or 100 µmol m^{-2} s^{-1} red or blue light was applied from a projector (Techni-Quip Corp., Hollywood, Calif., USA) equipped with a 150-W bulb (Quartzline lamp; General Electric Co., Nela Park, Cleveland, Ohio, USA) and a fiberglass light guide. Blue light, 95–100 µmol m^{-2} s^{-1}, was obtained by inserting a blue Plexiglas cut-off filter (Rohm and Haas No. 2424, 472 nm peak) at the end of the light guide. The same fluence rate of red light was obtained by varying the number of layers of a red Plexiglas cut-off filter (650 nm peak). In order to confirm the efficacy of these filters, growth and V_m experiments were repeated with blue (Ealing No. 35–3417; 460 ± 6.8 nm) and red (Ealing No. 35–4001; 660 ± 11.6 nm) bandpass interference filters (Ealing Electro-Optics, Holliston, Mass., USA).

Measurements of membrane potential of mesophyll cells

Individual leaf strips (20 mm long, 4 mm wide) were cut parallel to the midvein of young leaves and floated upon a solution containing 10 mM KCl, 1 mM $CaCl_2$, and 1 mM Mes/BTP (pH 6.0). Strips contained either pure white or green mesophyll tissue. After an incubation period of 3–5 h (in which the leaf cells recovered from excision and regained a sizable V_m) the strips were secured against a Plexiglas stage with strands of the elastic taping mass Terostat (Teroson Werke, Chemische Fabrik Heidelberg,

Germany) and mounted into a perfusion chamber on a microscope stage that contained the reference electrode. The mounted strip was continuously perfused with incubation solution (see above) and a microelectrode was inserted under microscopic observation (×100, using perpendicular green light of < 1 μmol m^{-2} s^{-1}) and micromanipulator control (Narishige, Tokyo, Japan). The microelectrodes were pulled from borosilicate glass capillaries (Kwik-Fil; World Precision Instruments, Sarasota, Fla., USA), backfilled with 300 mM KCl, had 0.1-μm tips with resistances ranging between 10 and 30 MΩ and tip potentials of less than 10 mV. Continuous recordings of V_m were made of the second or third cell encountered during the impalement process and assumed to be mesophyll cells. After impalement mesophyll cells were required to show a steady V_m value for at least 30 min before 200 μmol m^{-2} s^{-1} of white light or 100 μmol m^{-2} s^{-1} red or blue light was applied from a projector lamp through a fiberglass light guide

Measurements of the extracellular pH of mesophyll cells

Using fine carborundum (Rottenstone particles, Empire White Products, Newark, N.J., USA) we abraded about 50 mm^2 of the cuticle from the adaxial epidermis of a *Coleus* leaf segment. The segment was excised from one half of the leaf and contained either purely white tissue or green tissue. The leaf segment was placed abaxial surface down on ten layers of water-soaked filter paper stacked in a small plastic petri dish (55 mm diameter). About 20 mm^2 of the abraded area was covered with a 50-μl drop of an incubating solution containing 10 mM KCl, 1 mM $CaCl_2$, and 0.1 mM Mes/BTP at pH 6.0. Through a hole in the cover of the dish, the tip of a small-volume (gel type) combination pH electrode (Model MI-410; Microelectrodes Inc., Bedford, N.H., USA) was lowered onto the leaf. Surface tension led to a covering of the electrode tip with an amount of solution that remained constant over time. After the electrode read a constant pH for about 1 h, white light of 200 μmol m^{-2} s^{-1} was applied. Incubation of cross-sections of the leaf segment after the experiment in a dilution (1:100 standard solution, v/v) of a fluorescein diacetate-saturated ethanol solution showed yellow fluorescence in all the mesophyll but very few cells of the abraded epidermis (not shown). We conclude that the recorded pH is a measurement of the mesophyll apoplast.

Results

The goal of this study was to compare photoresponses in young leaf cells of comparable developmental age which either contain or lack functioning chloroplasts. To verify that white tissue of variegated *Coleus* leaves does not harbor functioning chloroplasts, white and green tissue were tested for the red fluorescence which is characteristic of excited chlorophyll molecules (Lichtenthaler and Miehe 1997). Our observations showed that the emission of red light occurred only from those leaf cells that appear green and contain chloroplasts (Fig. 2). With the exception of guard cells in the abaxial epidermis, there was no red emission from UV-irradiated white areas of *Coleus* leaves. A closer investigation of the abaxial epidermis in white leaf and epidermal strips confirmed that red emission was confined to guard cells. While the chlorophyll in guard cells is important for the photocontrol of the stomates, Elzenga et al. (1997) have shown that the guard cells do not contribute to the light-induced acidification of the leaf epidermis.

The results from the fluorescence microscopy were confirmed by the absorption spectra from green and white leaf segments (Fig. 3). While green segments showed the typical absorption of chlorophyll, with peaks in both the red and blue part of the spectrum, white tissues showed an increased absorption only in the blue and violet, which reflects the presence of blue-absorbing carotenoids and flavonoids.

The light responses of chlorophyll-free leaf cells

Among the many responses of leaves to light are the activation of the PM H^+-ATPase and cell enlargement (Dale 1988). An activation of this ATPase is usually manifested as a hyperpolarization of V_m and an acidification of the apoplast (Spanswick 1981). Indeed,

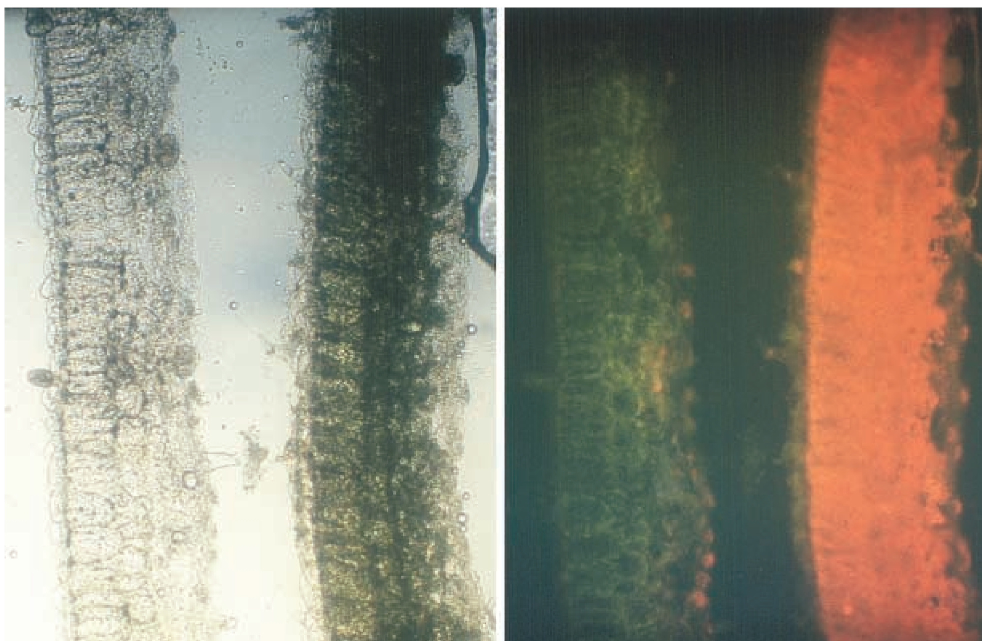

Fig. 2. Comparison of cross-sections through green and white areas of a variegated *Coleus* × *hybridus* leaf. The left pair of images was photographed under bright-field illumination (100× magnification) and the right pair are images of the same sections illuminated with ultraviolet light. Only chlorophyll-bearing mesophyll cells show the emission of red-fluorescing light upon excitation with ultraviolet light. With the noted exception of guard cells in the abaxial epidermis, there is no red emission from UV-irradiated white areas of *Coleus* leaves

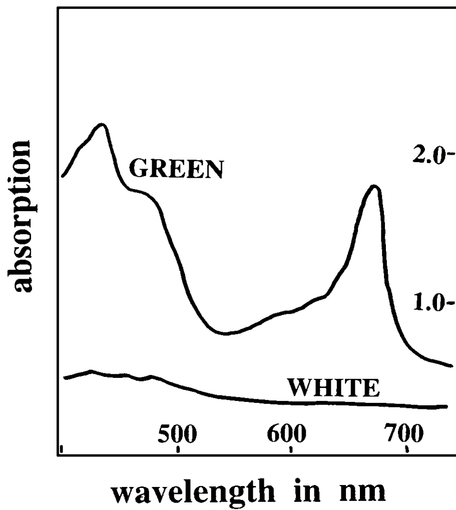

Fig. 3. Absorption spectra for an isolated green and white segment from variegated *Coleus* leaves. The white segment lacks increased absorption in the red, an indication of the absence of chlorophyll

when white *Coleus* cells were illuminated, there was a slow hyperpolarization of V_m (Fig. 4A), starting after a lag of 1–8 min (average 4 ± 2 min, $n = 8$). The hyperpolarization had an amplitude averaging 10 ± 4 mV ($n = 8$) and was blocked by vanadate (Fig. 4B), but not by DCMU (Fig. 4C). It occurred simultaneously with an acidification of the mesophyll apoplast (Fig. 5B). These data indicate that light activates the PM H^+-ATPase of *Coleus* mesophyll cells in the absence of chlorophyll.

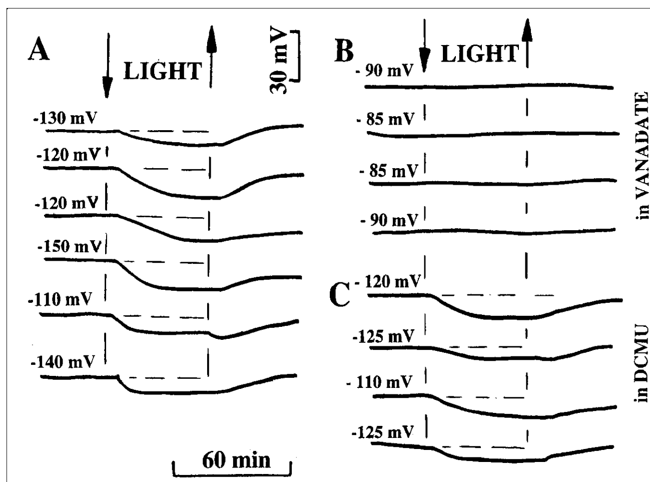

Fig. 4. The action of white light (200 µmol m^{-2} s^{-1}) upon the V_m of mesophyll cells in exclusively chlorophyll-free leaf strips that were not preincubated (**A**) or were preincubated in 5×10^{-4} M sodium orthovanadate (**B**), or 5×10^{-5} M DCMU (**C**). The leaf strips were submersed and perfused in a flow-through chamber and a conventional microelectrode was inserted into the first or second layer of mesophyll. After reading a steady value in a mesophyll cell for more than 15 min in the dark, the leaf strip was illuminated (*downward arrow*; *upward arrow* indicates darkness). The only V_m response of white mesophyll cells to light is a hyperpolarization which starts 2–4 min after the onset of illumination and is completely prevented by vanadate, but is unaffected by DCMU

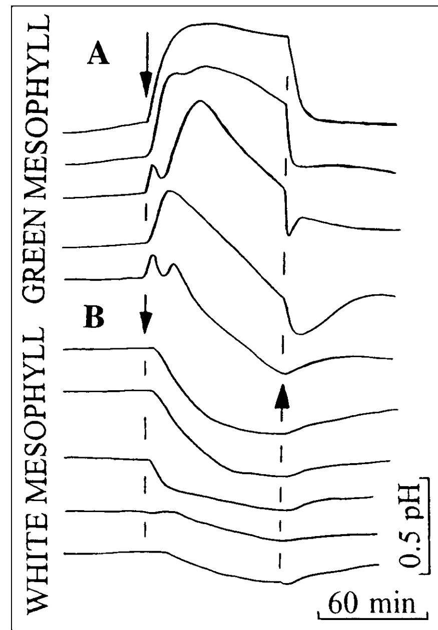

Fig. 5. The effect of white illumination on the extracellular pH of mesophyll cells that were either part of a green leaf strip that contained chloroplasts (**A**) or of a white strip that lacked photosynthesizing cells (**B**). After reading a steady value for more than 30 min in the dark, the measured leaf segment was illuminated with 200 µmol m^{-2} s^{-1} white light (*first arrow*; *second arrow* shows moment when illumination ceased). The pH values before the time of illumination were between 6.4 and 6.8 for both isolated green and white tissue

Similarly, the growth rate of strips cut from the white region of *Coleus* leaves increased nearly 4-fold upon illumination (Fig. 6, Table 1) after a lag of 1–6 min

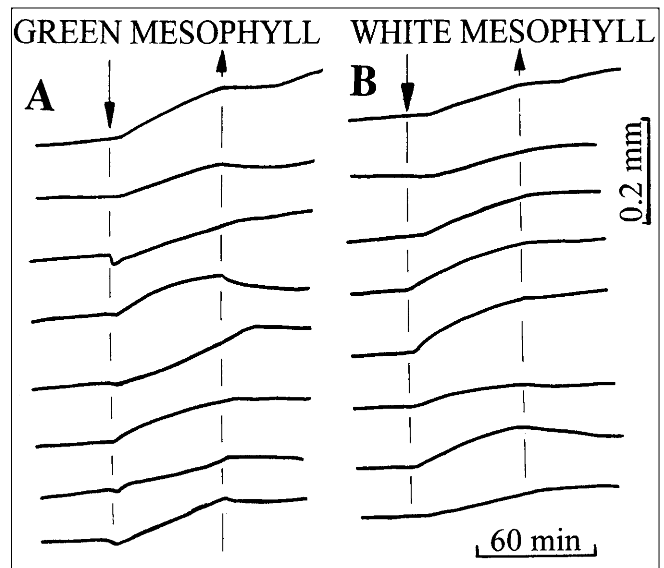

Fig. 6. The effect of illumination with white light (200 µmol m^{-2} s^{-1}) on the growth of green (**A**) or white strips (**B**) of variegated *Coleus* leaves. The white and green strips were parallel cuts from the same leaves, which had been kept in the dark for 3 h before the experiment (see Fig. 1). Other conditions were as in Fig. 4. *Downward arrows* indicate start of illumination; *upward arrows* show its termination. Eight separate runs are illustrated

Table 1. The effect of functional chloroplasts and photosynthesis on the growth rate of *Coleus* leaf strips in darkness and light. Growth rates were estimated during a 60-min-long interval before and during illumination (200 μmol m^{-2} s^{-1} white light) for white and green strips from the same variegated leaf (cf. Fig. 6) and values are given in μm h^{-1} ± SE ($n \geq 7$). Leaf strips were incubated for 5 h in standard solution with or without 5×10^{-5} M DCMU. Light caused a nearly 4-fold increase in the growth rate of both green and white leaf strips; DCMU reduced this response by 20% only in the green strips

	Darkness	Light	Light ± DCMU
Green strips	15 ± 1.6	58 ± 2.1	47 ± 4.0
White strips	14 ± 1.6	54 ± 2.9	56 ± 1.6

[4 ± 2 min (SE), $n = 2$]. The growth effect was sometimes only transient, but more often it was sustained. Treatment with DCMU had no effect on this growth response (Table 1).

The growth of green leaves is stimulated by either red or blue light (Vince-Prue and Tucker 1983; Van Volkenburgh et al. 1990; Blum et al. 1992). To see if this holds true for leaf tissues lacking chlorophyll, the growth of white leaf strips was recorded during a sequence of light treatments with 200 μmol m^{-2} s^{-1} white light, 100 μmol m^{-2} s^{-1} red light and 100 μmol m^{-2} s^{-1} blue light, interspersed with dark periods (Fig. 7). Blue or red light caused similar increases in growth rate. The response to 100 μmol m^{-2} s^{-1} blue or red was slightly smaller (amplitude on average reaching 70%) than to 200 μmol m^{-2} s^{-1} white light. An addition of 100 μmol m^{-2} s^{-1} of blue to a leaf strip already illuminated by red light (or of 100 μmol m^{-2} s^{-1} of red to a leaf strip already illuminated by blue light) gave a new growth response which was always smaller than the first response to either blue or red light (data not shown).

Since both red and blue light were active in stimulating growth of white (Fig. 7) and green *Coleus* leaf tissues (data not shown), their effects on the V_m of white tissues were determined as well (Fig. 8). The results show that both red and blue light have the ability to induce a hyperpolarization in chlorophyll-free mesophyll cells. The hyperpolarizations of V_m to red and blue showed no apparent or statistically significant differences in either lag or magnitude.

The light responses of green leaf cells

The light-induced changes in V_m and apoplastic acidification of green *Coleus* cells were more complex than those of white tissues. There was a biphasic response of both V_m and pH, similar to that found earlier with green pea leaf tissues (Stahlberg and Van Volkenburgh 1999). Upon illumination, there was a rapid depolarization of the membrane potential, followed by a rapid recovery (Fig. 9A). This response was inhibited by DCMU, but not by vanadate. After the initial depolarization/repolarization there was (as in white tissue) a slow hyperpolarization of V_m, which was blocked by vanadate (Fig. 9B) but not by DCMU (Fig. 9C). The differences between white and green cells show that the complex V_m response of green cells is the composite of two different photoresponses and systems; a rapid depolarization/re-polarization mediated by chlorophyll and a slow hyperpolarization mediated by non-photosynthetic pigments.

The apoplastic pH of green tissues, upon illumination, showed a rapid rise, followed by a slower recovery (Fig. 5). The recovery continued until the net effect was an acidification of the apoplast. This slow acidification was similar to the acidification that was seen in the white mesophyll. The changes in V_m and apoplastic pH in green *Coleus* leaf tissues, then, are identical to those found in other green leaf tissues (Brock and Cleland

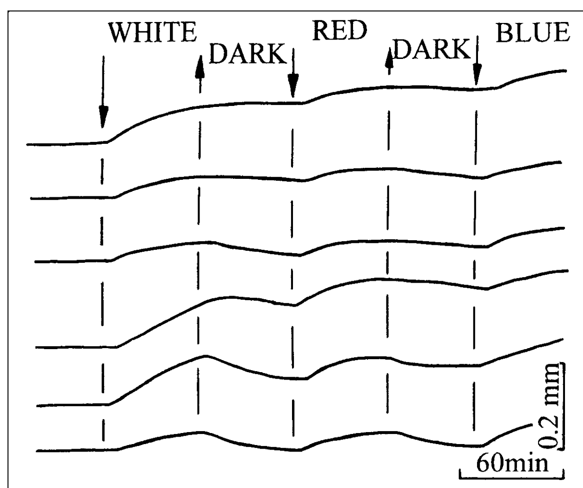

Fig. 7. The effect of white, red and blue light on the growth of strips cut from exclusively white parts of young *Coleus* leaves. After reading a steady growth rate for more than 60 min in the dark the leaf strips were illuminated with 200 μmol m^{-2} s^{-1} white light, and, after 30-min dark periods, with 100 μmol m^{-2} s^{-1} red and blue light

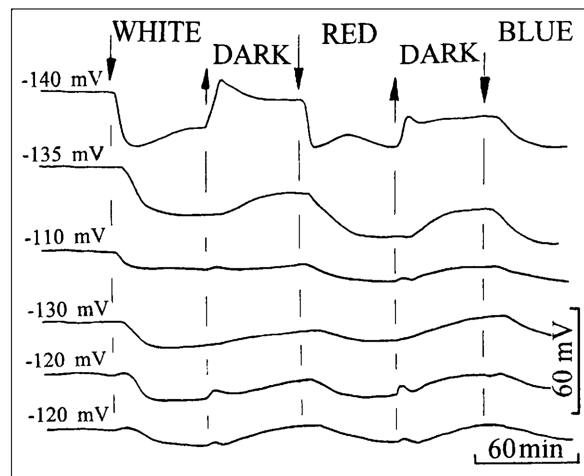

Fig. 8. The effect of white, red and blue light action upon the membrane potential (V_m) of chlorophyll-free mesophyll cells. After reading a steady V_m for more than 15 min in the dark the leaf strips were illuminated sequentially with 200 μmol m^{-2} s^{-1} white light, 100 μmol m^{-2} s^{-1} red light and 100 μmol m^{-2} s^{-1} blue light, with 1-hour dark periods between each illumination

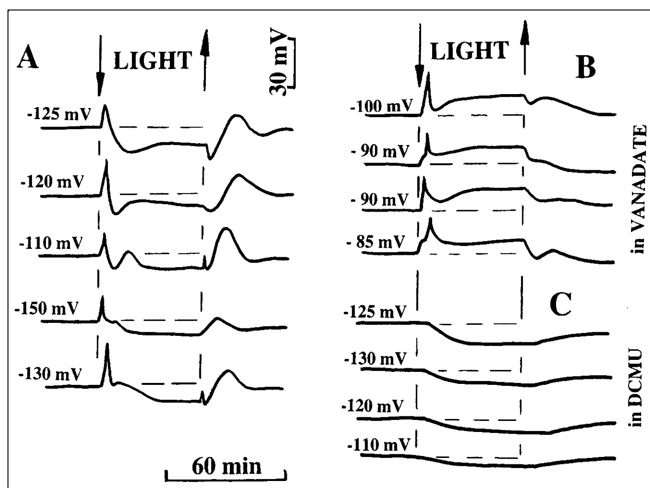

Fig. 9. The effect of illumination (200 μmol m^{-2} s^{-1} white light) on the membrane potential (V_m) of green (chlorophyll-containing) mesophyll cells which were not preincubated (**A**) or were preincubated in 5×10^{-4} M sodium ortho-vanadate (**B**), or in 5×10^{-5} M DCMU (**C**). Green leaf cells show a biphasic V_m response to light. Conditions were identical to those in Fig. 4 showing the response of white mesophyll cells

1989; Shabala and Newman 1999; Stahlberg and Van Volkenburgh 1999).

The growth response of green *Coleus* leaf strips to light was comparable to that of white strips (Fig. 6, Table 1). In both cases there was a short lag [averaging 4 ± 2 min (SE), $n = 8$] before a nearly 4-fold increase in growth rate. These results show that after their separation from each other, white and green tissues of a variegated *Coleus* leaf still grow equally in response to light. There were small differences between the growth responses of green and white strips, however. Some green leaf strips (but no white strips) underwent a small initial shrinking upon illumination. While white strips were unaffected by DCMU, the growth of green strips declined by about 20% in the presence of DCMU (Table 1). This was a much smaller effect than occurs in the faster growing leaf strips from pea (Stahlberg and Van Volkenburgh 1999).

Discussion

The effects of light on growth rate, membrane potential and apoplastic pH are interrelated (Stahlberg and Van Volkenburgh 1999). It has been proposed that light stimulates a PM H^+-ATPase, which hyperpolarizes V_m, acidifies the apoplast, and leads to acid-induced wall loosening and growth (reviewed by Palmgren 1998). An unresolved question is whether the light is perceived by chlorophyll or by some other photoreceptor(s).

Chlorophyll is a possible candidate for the primary photoreceptor. Photosynthesis affects a multitude of processes in leaf cells including the light activation of enzymes such as kinases and carboxylases (Jiao and Chollet 1992). Moreover, photosynthesis is required to activate the PM H^+-ATPase in green leaf cells of *Elodea* (Jeschke 1970; Tazawa et al. 1986; Marrè et al. 1989) and several algae (see *Introduction*). Since DCMU causes at least a partial inhibition of light-stimulated growth and H^+ net efflux in leaves of dicot land plants as well (Van Volkenburgh and Cleland 1980; Petzold and Dahse 1988; Blum et al. 1992), it could be that land plants also share this mechanism. However, the results of this study reject such a generalization.

Mesophyll cells, from young *Coleus* leaves that lack chlorophyll, still respond to light. The response consists of a slow (average lag 4 min) hyperpolarization and apoplastic acidification, and, simultaneously, a growth stimulation. Inhibition of these responses by vanadate implies a strong dependence on the activity of a light-stimulated P-type H^+-ATPase. As expected because of the lack of chlorophyll, these slow responses are unaffected by DCMU. The stimulation of the P-type H^+-ATPase must be mediated by photoreceptor(s) other than chlorophyll.

The slow light responses also occur in green *Coleus* mesophyll cells, but here they are preceded by a rapid depolarization/re-polarization of V_m and a rapid alkalinization of the apoplastic pH, just as they are in green *Pisum* leaf cells (Stahlberg and Van Volkenburgh 1999). Since these rapid responses are completely inhibited by DCMU in both *Coleus* and *Pisum*, they must be mediated by photosynthesis. The light response of green mesophyll cells, then, is a composite of a rapid photosynthetic response, which does not lead to growth, and a slower non-photosynthetic component, which is responsible for the leaf cell expansion.

While growing leaf tissues show this composite response, non-growing cells may lack the slow component. White mesophyll cells from mature *Coleus* leaves hyperpolarized very little in response to light, while mature green cells showed only the rapid depolarization/recovery and alkalinization responses (data not shown). It might be because mature cells were used that white mesophyll cells from *Oenothera* leaves failed to show a light response (Brinckmann and Lüttge 1974), that *Arabidopsis* mesophyll cells showed only the DCMU-inhibited transient depolarization (Spalding and Goldsmith 1993) and that *Avena* leaf protoplasts and *Vicia* mesophyll cells showed only the alkalinization in response to light (Kelly 1983; Mühling et al. 1995).

There are clear differences between *Coleus* and pea and the aquatic *Elodea* in regards to the light-stimulation of the PM H^+-ATPase. Whereas mesophyll cells of pea and *Coleus* maintain V_m in the presence of DCMU (Elzenga et al. 1995; Stahlberg and Van Volkenburgh 1999), illuminated *Elodea* cells respond to DCMU with a steep depolarization (Marrè et al. 1989). In *Elodea*, but not in *Coleus* or pea leaf cells, light-induced hyperpolarization and apoplastic acidification is inhibited by DCMU. It must be concluded that *Elodea* uses a different primary photoreceptor to stimulate the PM H^+-ATPase than is used in growing pea and *Coleus* leaf tissues.

Even though chlorophyll is not the primary photoreceptor for leaf growth, over longer periods photosynthesis must play some role in the growth process. This is indicated by the fact that DCMU causes some inhibition

of light-stimulated leaf growth. In the slow-growing green *Coleus* leaf tissue this inhibition is only 20% (Table 1) while in the fast-growing *Pisum* leaves it is up to 70% (Stahlberg and Van Volkenburgh 1999). The contribution of photosynthesis to growth may be to provide sugars as the basis for osmotic solutes and ATP. This is suggested by the fact that added sucrose enhances the growth of bean leaf discs, in particular when treated with DCMU (Van Volkenburgh and Cleland 1990; Blum et al. 1992). This might also be a reason for the close correlation found in some cases between the photosynthetic capacity and the final leaf size (e.g. Kriedemann 1986; Lecoeur et al. 1995; Saradevi et al. 1996).

The growth of chlorophyll-free *Coleus* leaf strips and the hyperpolarization in chlorophyll-free mesophyll cells are equally stimulated by blue and red light. This raises the possibility that blue and red light act as redundant signals in the same transduction process or that two different photosystems are involved. This would not be unique. Proton efflux from epidermal and guard cells is stimulated by both blue and red light acting through different photoreceptors (Assmann et al. 1985; Serrano et al. 1988; Elzenga et al. 1997). Likewise, different photoreceptors are involved in the inhibition of hypocotyl growth by red and blue light (Chory 1997). However, the nature of the photoreceptor(s) in the white *Coleus* mesophyll is yet to be determined. The likelihood that one photoreceptor is phytochrome is raised by the findings that phytochrome B is required for cotyledon expansion in *Arabidopsis* (Neff and Van Volkenburgh 1994), that overexpression of phytochrome C in *Arabidopsis* and *Nicotiana* increased final leaf size (Halliday et al. 1997; Qin et al. 1997) and that in *Phaseolus* primary leaves light stimulates expansion in a red/far red reversible manner (Van Volkenburgh et al. 1990).

The fact that isolated white and green leaf strips have indistinguishable growth responses is clear evidence that light-stimulated cell expansion in *Coleus* leaves does not directly involve photosynthesis. This is consistent with the situation in intact, variegated *Coleus* leaves that give no morphological indication of differences in blade expansion between green and white areas or entirely green and largely white leaves. Other variegated dicot species as well, such as ivy where white regions occupy from 0 to nearly 100% of the leaf area, show no apparent difference in final leaf size. It appears that a non-photosynthetic mechanism of leaf expansion is widespread among dicots.

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