The Effect of Different Light Quality on the Change of Membrane PD of the Guard Cell in *Tradescantia virginiana* L.

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Abstract - The effects of different light quality on the change of membrane potential difference (PD) of the guard cell in the intact leaf have been investigated. The membrane PD was about −5.5 mV by white light of 600 μmoles m^{-2} s^{-1}. The mean PD of change caused by red light was about −5.2 mV at the light intensity of 80 μmoles m^{-2} s^{-1}. Membrane PD of guard cells in response to blue light was saturated at low light intensity. However, red and green light enhanced the change of membrane PD of guard cells with increasing light intensity. In green light the biggest change of memrane PD was around −4 mV, whereas, with blue light the change of of memrane PD was around −2 mV. Accordingly, the membrane PD of guard cell showed the different degree of hyperpolarization by each wavelength.

Key words : membrane PD, light quality, guard cells

INTRODUCTION

Changes in membrane potential in response to light are a known feature of many cells (Spanswick 1973; Murdoch and Sinclair 1976), and their demonstrations in stomatal guard cells have a special significance as a plausible electrical correlation of an electrogenic mechanism driving the large ion fluxes associated with stomatal movements (Hsiao 1976). The increase in turgor of guard cells is the result of ions accumulated by the generation of a primary electrochemical potential gradient across the plasmalemma. Since the most observed time courses of stomatal opening and closing range between several minutes and a few hours (Meidner and Mansfields 1968), in this study, the leaf was first exposed to dark-light cycles. In this system, white, red, blue and green light were used to trace the changes of membrane PD when the leaf was exposed to the onset of each light.

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One method for the determining the identity of a photoactive pigment is to compare the wavelength dependence of the light induced action with the absorption spectrum of a pigment suspected to be involved.

However, no attempts to investigate how the different light quality can contribute to guard cell membrane hyperpolarization has been made. Therefore, this study was carried out to investigate the influence of wavelength on the guard cell membrane hyperpolarization.

MATERIALS AND METHODS

1. Plant material

Tradescantia virginiana L. was potted in John Innes No. 2 compost supplemented with Phostrogen and watered every morning. They were grown in a glass house (minimum temperature of 20°C during the day and 15°C at night) under a light regime of 18 hours day and 6 hours night (natural daylight supplemented by high pressure sodium lighting (150 μmoles m^{-2} s^{-1}).

Table 1. Irradiation source of each light used for measuring the changes of guard cell membrane PD.

Light	Source	Filter	Maximum peak	Light intensity (μmoles m^{-2} s^{-1})
White	Lux-150 4-port Projector	Glass	–	900
Red	Lux-150 4-port Projector	Glass (transmitting above 625 nm)	650 nm	300
Green	Lux-150 4-port Projector	Plastic (transmitting 475 ~ 575 nm)	513 nm	100
Blue	Lux-150 4-port Projector	Plastic (transmitting 412 ~ 512 nm)	450 nm	60

2. Vacuolar PD measurements

The measurements were conducted on the abaxial surface of the leaves. Four or five weeks old fully expanded leaves were excised early in the morning and floated on distilled water in the dark for 1 h. to ensure that the stomata were tightly closed. After 1 h, the leaf was put, abaxial side uppermost, on a side which was placed in a chamber with two holes provided for the air stream. The slide was inclined at 30° to facilitate micro-electrode implantation. The cut end of the leaf was dipped in KCl (10 mM). The chamber was installed on the stage of stereomicroscope and the leaf surface was viewed at a magnification of × 100 with the microscope lamp giving 100 μmoles m^{-2} s^{-1}. Light intensity used in in the measurements was 550 μmoles m^{-2} s^{-1}, provided by a Lux 150 4-port Projector. Irradiation source of each is shown in in Table 1.

When a steady reading of PD had been obtained, light was given to the leaf.

Micro-electrodes for PD measurements were pulled from glass capillaries (2.0 mm outside diameter, 1.6 mm inside diameter with internal filament, Clark Electromedical Instruments, Pangbourne, Berks, U.K.) filled with 3 M KCl and connected by way of a Ag/AgCl half cell to a votage follower and chart recorder. The circuit was completed by a Ag/AgCl referenece electrode dipping into the bathing solution. Microelectrodes were inserted into the cells using a micromanipulator (Reaserch Instruments TVC 300). The PD was measured under an Olympus CK2 inverted microscope (Olympus Optical Co. Ltd., Tokyo, Japan) at a magnification of × 200.

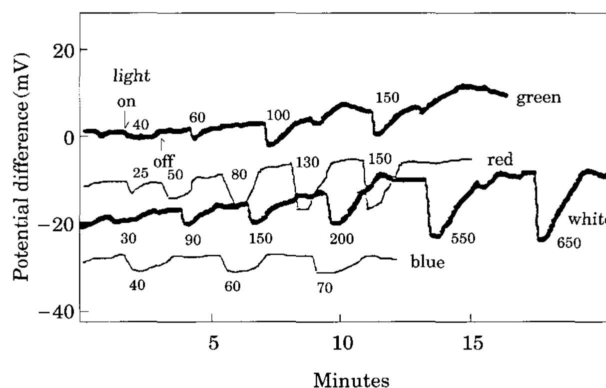

Fig. 1. The effect of light quality on the change of membrane PD of the guard cell of a closed stoma in the intact leaf of *T. virginiana*. Each number indicates light intensity (μmoles m^{-2} s^{-1}).

RESULTS AND DISCUSSION

The saturation point of the membrane PD was around 500 μmoles m^{-2} s^{-1} in response to white light, but respectively in response to red, green and blue light, they were 80, 100 and 40 μmoles m^{-2} s^{-1} (Fig. 1). Even though the saturation points betwen white and red light were different, the magnitude of white and red light induced hyperpolarization was almost same, indicating red light was the most effective in changing membrane PD of guard cells. Membrane PD of guard cell in response to blue light was saturated at low light intensity however, red and green light enhanced the change of membrane PD of guard cell with increasing light intensity (Fig. 1). In green light the biggest change of membrane PD was around −4 mV, whereas, with blue light the change of membrane PD of guard cells was around −2 mV.

How can green light cause the membrane hyperpolarization of gaurd cells? Absorption spectra of chloroplasts show that green light can be absorbed by chloroplasts, even if the action spectrum of gree light is lower than that of red light. Therefore, it can be hypothesized that green light effect might be the result of energy consumption from ATP or $NADPH_2$ already stored in the cell. To test this hypothesis, green light was repeatedly

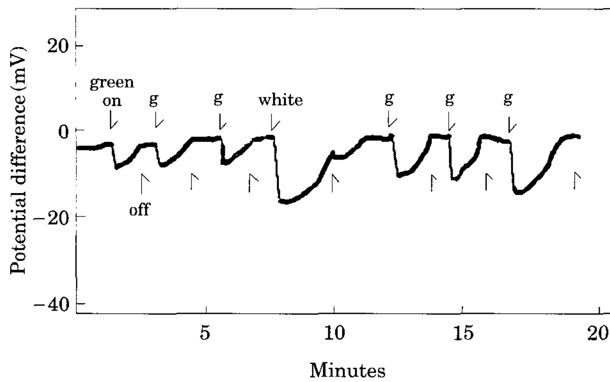

Fig. 2. The effect of green light on the change of membrane PD of the guard cell of a closed stoma in the intact leaf of *T. virginiana*.

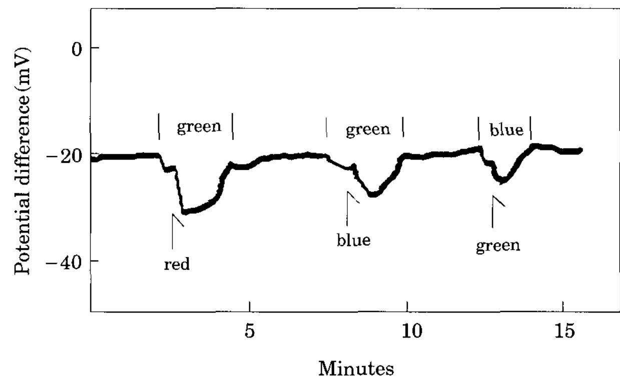

Fig. 3. The effect of superimposed light quality on the change of membrane PD of the guard cell of a closed stoma in the intact leaf of *T. virginiana*.

given with green on-off cycles (Fig. 2). The result was that the magnitude of membrane PD of the guard cells was not decreased when the leaf was repeatedly exposed to green light. However, white light onset enhanced the green light effect. This result seems to indicate that green light induces the same mechanism (electrical component) as white light in stimulating the changes of membrane PD, and the green light is likely to consume the energy which was already produced by white light. Because exposure of the leaf to white light could generate energy and the energy used by green light induced membrane hyperpolarization could be restored by exposure of the leaf to white light. That could be why there was a higher PD change in response to green light if the leaf was previously exposed to white light however, this light effect needs to be investigated further.

Sometimes, green light was given first and then supplemented with red or blue (Figs. 3, 4). Red and blue light enhanced another hyperpolarization of the guard cell membrane over membrane hyperpolarization produced by the green light. However, if red light was given first and then blue or green was given second, there was no secondary hyperpolarization over the initial hyperpolarization. This important result was that the megnitude of red induced hyperpolarization was almost same as red plus green induced hyperpolarization. Red was superior in changing PD to blue and green light, supporting the theory that chloroplasts are important to membrane hyperpolarization in response to red-light. However, in blue light the sensor might be different.

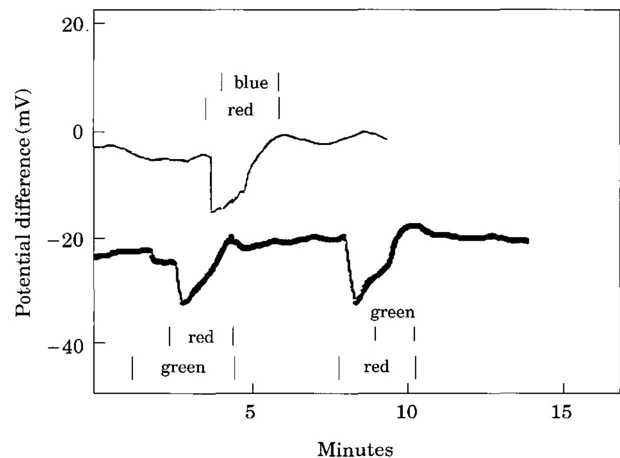

Fig. 4. The effect of superimposed light quality on the change of membrane PD of the guard cell of a closed stoma in the intact leaf of *T. virginiana*.

Blue light induced hyperpolarization was always lower than that caused by green light. Nevertheless, blue light produced another hyperpolarization over green light induced hyperpolarization (Fig. 3). This finding clearly indicates the pigment which can absorb each light was different. The idenity of the blue light photoreceptor or photoreceptors in plant cells remains to be established (Scmidt 1984). Munoz and Butler (1975) have proposed that flavin could absorb blue light and reduce a cytochrome in a membrane-bound electron transport system at the onset of sensory transduction. Spectroscope data of several blue light responses, including that in stomata (Karlsson 1986; Ogawa *et al.* 1978; Sharkey and Raschke 1981; Taiz and Zeiger 2002), give evidence of the involve-

ment of flavins in blue light absorption. Accordingly, possibly a flavin or flavin protein situated in or on the guard cell plasmalemma or tonoplast, may be a sensor of blue light.

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