# Phytochrome-Mediated Bioelectric Potentials in Mung Bean Seedlings

Abstract. *Excised root tips from dark-grown mung bean seedlings* (Phaseolus aureus) *that adhere to a negatively charged glass surface when irradiated with red light and release when irradiated by far-red light develop a positive bioelectric potential (about 1.0 millivolt) at the tip in red light and a negative bioelectric potential in far-red light. The sign of the bioelectric potential was repeatedly photoreversible, and the adhesion and release kinetics were similar to those of the development of the bioelectric potentials. Photoconversion of the phytochrome holochrome perhaps changes permeability characteristics of the cell membrane, resulting in an induced localized electrochemical gradient manifested as a bioelectric potential. This supports the view that phytochrome* in situ *is membrane bound.*

Phytochrome reversibly potentiates responses of eukaryotic plants to low levels of red and far-red light manifested as morphological changes. Since its discovery (*1*) many such responses have been documented. Although some responses have been explored to the level of biochemical control (*2*) they are manifested hours after photoreception. Since only seconds elapse during phytochrome conversion by either red or far-red light, the great majority of phytochrome-mediated effects must be only distantly related to the initial photoconversion of the pigment. Recently phytochrome has been implicated in various tropic and nastic responses whose occurrence can be measured in minutes. Thus rapid leaf-sleep movement (nyctinasty) in both *Mimosa pudica* and *Albizzia julibrissin* is reversibly controlled by red and far-red light (*3*), and an apparent electrotropic response of barley and mung bean root tips is phytochrome mediated (*4*). This last phenomenon occurs when excised dark-grown root tips are placed in solution in a beaker whose glass has been negatively charged by washing with phosphate ions. When the preparation is irradiated with red light, the apices of the root tips adhere to the glass. Far-red light potentiates their release. Since only the apex adheres to the glass, a polarization of potential may be inferred.

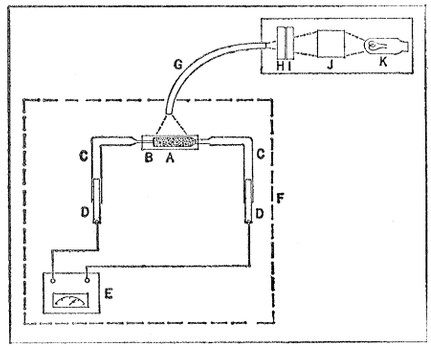


Fig. 1. Apparatus for the measurement of the bioelectric potential at the tip of excised dark-grown secondary root of mung bean. The preparation, including the electrode assembly, is shown greatly enlarged. *A*, excised root tip, about 3 mm long; *B*, capillary jacket, with an inner diameter of 750 $\mu$, containing the bathing solution and the root tip; *C*, glass electrodes, containing the bathing solution. The capillary meeting the apex had an inner diameter of about 500 $\mu$, and the electrode at the cut end of the root had one of about 200 $\mu$; *D*, silver–silver chloride electrodes; *E*, electrometer; *F*, Faraday cage made of copper screening; *G*, flexible glass-fiber optic "light wire," diameter 2 mm; *H*, Corning colored-glass filter; *I*, Bausch and Lomb second-order interference filter; *J*, a lens system which focused the light (filament image) from lamp, *K*, onto the base of the light wire. The entire assembly was used in a dark room maintained at 25°C under a green safelight.

Root tips (2 to 3 mm long) of dark-grown seedlings of *Phaseolus aureus* were excised and placed in Tanada's bathing solution (*4*) supplemented with 10 $\mu M$ $CaCl_2$. The solution was freshly made every 2nd day. For adhesion experiments, large boxes with either incandescent lamps (far-red) or fluorescent lamps (red) were used for irradiation with far-red or red plastic filters (*5*). During measurement of a bioelectric potential, the image of a 6.5-volt, 2.75-amp incandescent filament (after passing through a Corning-2408 glass filter and an appropriate Bausch and Lomb second-order interference filter) was projected onto one end of a flexible glass-fiber optic light wire. The insulated light wire passed through a copper-screen Faraday cage and irradiated the root-tip preparation in the electrode assembly (Fig. 1). This device ensured that the silver–silver chloride electrode would not be irradiated. The bathing solution, as well as the electrolyte solution in the glass electrodes, was the same as that used in the adhesion experiments. The electrodes were in circuit with a Keithley model 610B electrometer having a maximum sensitivity of 1 mv full scale, with accuracy of ± 1 percent of full scale. All experiments were performed at 25°C under a green safelight. The irradiances were obtained with an ISCO model SR spectroradiometer (Fig. 2).

Potentiation of, adhesion to, and release from the negatively charged glass surface was studied in relation to the development of bioelectric potentials. Figure 2 compares the kinetics of the Tanada effect with the kinetics of the development of bioelectric potentials. The adhesion and release curves indicate that the two phenomena have almost identical sigmoid kinetics with completion occurring after about 150 seconds and the inflection point at about 60 seconds. The bioelectric potential curves differ somewhat, each

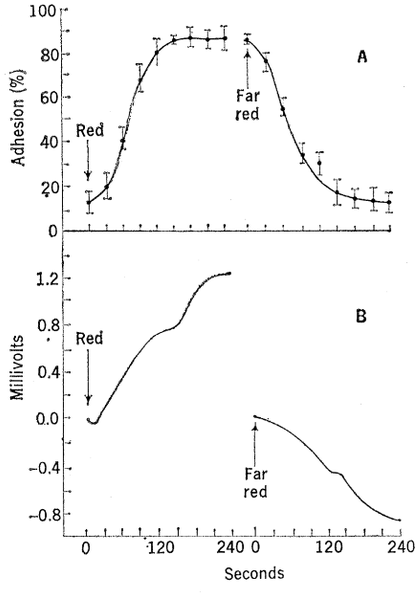


Fig. 2. Kinetics of root adhesion (A), and development of the bioelectric potential (B). Irradiation of root tips in beakers for adhesion was performed in large irradiation chambers consisting of incandescent lamps with a plastic far-red filter interposed (irradiance 730 nm, 5.95 $\mu$w/cm$^2$, or cool white fluorescent lamps with a red plastic filter interposed (irradiance 660 nm, 0.11 $\mu$w/cm$^2$). Irradiation of root tips during development of the bioelectric potential was as shown in Fig. 1. The irradiances were 730 nm, 0.31 $\mu$w/cm$^2$; 660 nm, 0.18 $\mu$w/cm$^2$. Typical bioelectric potential curves are shown representing the change in potential from the beginning of the irradiations. Zero- and 240-second measurements of the electrode assembly without the root tip were 0.00 and +0.01 mv in red light, and 0.00 and 0.00 mv in far-red light.

Table 1. Repeated photoreversibility of root adhesion and bioelectric potential. Roots were irradiated with red (R), far-red (FR), and then red light for 4 minutes each, and the development of a bioelectric potential as well as the percentage of the roots adhering to glass were measured.

| Treatment | Change in bioelectric potential* (mv) | Adhesion† (%) |
|---|---|---|
| R | + 1.20, + 0.28, + 2.00 | 100, 90, 90, 90 |
| R–FR | − 0.88, − 0.25, − 0.70 | 10, 0, 30, 20 |
| R–FR–R | + 0.35, + 0.23, + 0.15 | 90, 90, 80, 90 |

* Results of three experiments. † Results of four experiments.

reaching a plateau after about 150 seconds, and then resuming the previous rate of change. It is not known what the plateau represents, but it occurred quite regularly at the time of completion of the Tanada effect. It may be a manifestation of the termination of photoconversion of one form of phytochrome and the beginning of that of another form (*6*). The bioelectric potential slopes and time courses were, however, generally similar to those of the Tanada effect. Thus, red light (which produced adhesion to a negatively charged surface) produced a positive bioelectric potential, and both effects were reversed by far-red light. The repeated photoreversibility of both phenomena are shown in Table 1. The general decrease in amplitude of bioelectric potential change with time probably shows that the root tip was closely confined in a small volume of solution and may have used up the available oxygen as time elapsed. Nonetheless, the sign of the generated bioelectric potential was repeatedly associated with quality of light.

Because the development of a bioelectric potential, distinctive as to sign, is correlated so well with a phytochrome-mediated electrotropic response, it is concluded that this is one of the first events, if not the first, following photoreception and photoconversion of the phytochrome holochrome, and possibly is itself another manifestation of the same event. This last could be true if the holochrome were structurally part of the plasmalemma and, as light altered its own configuration, it in turn altered the configuration of the membrane, changing permeability characteristics and inducing a localized electrochemical gradient, manifested as a bioelectric potential. Such a chain of events might explain the sudden increase in electrolyte efflux from pinnae of *Albizzia julibrissin* exposed to red light (*7*).

Since the induction of a bioelectric potential must occur across a cell membrane due to electrochemical gradients across that membrane (*8*), these data support the view (*7*, *9*) that the primary phytochrome events are membrane bound.

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**References and Notes**

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## Anaerobic Brain Function: Effects of Stagnant and Anoxic Anoxia on Persistence of Breathing in Reptiles

Abstract. *Turtles tolerate anoxic anoxia about 14 times longer than stagnant anoxia. In snakes and crocodiles this difference is much less marked. Apparently, the remarkable anaerobic viability of turtles is dependent on blood circulation. Analyses of plasma indicate that loss of brain function in anoxic crocodiles is not caused by systemic acidosis or hypoglycemia. It is suggested that the ability of the central nervous system of the turtle to function without oxygen is due to a comparatively high rate of anaerobic uptake or metabolism of glucose.*

Lack of oxygen may result from stopping the circulation (stagnant anoxia) or from breathing gas mixtures which contain no $O_2$ (anoxic anoxia). Turtles as a group are much more resistant to anoxic anoxia than are the squamata and the crocodilia (*1*). In pure $N_2$, most turtles continue to breathe spontaneously at least ten times longer than other reptiles. Other behavioral indicators of central nervous system (CNS) function, such as corneal reflex, are

Table 1. Time (minutes, mean ± standard deviation) from first to last breath under conditions of acute anoxia. Numbers in parentheses are numbers of animals used.

| Species | Stagnant anoxia | Anoxic anoxia |
|---|---|---|
| *Caiman crocodilus* | 26.4 ± 2.2 (8) | 32.0 ± 3.1 (8) |
| *Natrix fasciata* | 39.3 ± 3.5 (20) | 62.2 ± 11.1 (20) |
| *Chrysemys picta* | 72.4 ± 8.0 (8) | 1104 ± 170 (8) |
| *Pseudemys concinna* | 65.2 ± 6.4 (20) | 900 ± 77 (20) |

Table 2. Analyses of heparinized blood and plasma from $N_2$-breathing crocodiles and turtles; mean values. Two animals were used under each condition; duplicate analyses were made of each sample.

| Measurement | Duration of $N_2$-breathing | | | | |
|---|---|---|---|---|---|
| | *Caiman crocodilus* | | *Chrysemys picta* | | |
| | 0 min | 30 min | 0 min | 30 min | 720 min |
| *p*H | 7.54 | 7.58 | 7.78 | 7.86 | 7.12 |
| Glucose (mmole/liter) | 7.8 | 10.6 | 5.4 | 8.1 | 53.3 |
| Lactate (meq/liter) | 5.4 | 8.5 | 1.1 | 4.2 | 22.7 |