

Investigation of membrane potential changes in *Nitellopsis obtusa* cells induced by blue and red light stimulation

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Light controls multiple physiological processes in plants. Membrane potential is widely used to report the early events associated with changes in ion permeability, thus the effect of monochromatic light on bioelectrical parameters of *Nitellopsis obtusa* cells was examined. In this study blue, red and far-red light-induced membrane potential changes, light-induced photoelectrical reaction and membrane excitability were investigated using K⁺-anaesthesia method. All investigated lights cause the same bioelectrical events in *Nitellopsis obtusa* cells. It can be concluded that all photoreception systems exert their effects by first altering the permeability of the plasma membrane to ions and later ion channels perform signal-transducing functions in the complex array of mechanisms that determine plant physiology.

Key words: plant photoreceptors, membrane potential, plant electrical signalling, plant photoelectrical reaction

INTRODUCTION

Light is a key factor for metabolism as well as a source of information in plant kingdom. Different light absorbing systems are reported to exist in plants. Aside from the light harvesting complexes that participate in photosynthesis, complex, multiple photoreception systems participate in physiological processes, and the major of them are red/far-red light-sensing phytochromes and blue

light-sensing phototropins and cryptochromes (Briggs and Olney, 2001). Photosynthetic pigments, mainly chlorophylls, can play a role as photoreceptors, too (Okazaki et al., 1994). Upon illumination, a complex interaction between these photoreceptors occurs (Živanović et al., 2005). During their “cross-talk” information on light quantity and quality is gained and eventually translated into signals controlling physiological functions (Franklin and Whitelam, 2004). However, the mechanisms by which these photoreceptors generate many diverse

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physiological responses remain unknown. It could be electrical signaling, because changes in plasma membrane potential (MP) are amongst the earliest cellular events in response to light (Szarek and Trebacz, 1999). It is suggested that light controls ion movements across the cell membrane (Shabala and Newman, 1999). To date, the link between light absorption by photoreception system and ion fluxes across the plasma membrane remains unclear. Among the factors which can participate in the transduction process changes in Ca^{2+} are often postulated (Ermolayeva et al., 1996). It is established that Ca^{2+} signaling and fluxes through ion channels are responsible for action potential generation (AP) and photoelectrical reaction in plants (Kurtyka et al., 2011). In *Characeae* cells the ionic mechanism causing AP was characterized in detail. According to the generally accepted model, an AP is initiated by calcium influx into the cytosol followed by Cl^- efflux (Lunevsky et al., 1983). Chloride ions leave the cell down their electrochemical potential gradient through Ca^{2+} activated anion channels. Repolarization occurs after opening of voltage-gated potassium channels allowing K^+ efflux (Thiel et al., 1997). It was suggested that supposedly the second stage of repolarization during AP is related to the operation of electrogenic H-pump in the plant excitable membrane (Sukhov et al., 2011). It has already been demonstrated that the membrane potential of *Characean* cells is very sensitive to light / dark and dark / light transition (so called photoelectrical reaction) and a typical reaction on this transition consists of two phases – a rapid transient depolarization of the MP followed by a slow membrane hyperpolarization (Shimmen et al., 1994). As the primary event, when an action potential (AP) is induced in *Characeae* cells is an increase in cytosolic Ca^{2+} level (Beilby, 2007), it is interesting to investigate *Characean* electrical excitability in monochromatic light. Light induced action potentials (APs) are known in excitable plants (Koselski et al., 2008). Moreover, shade induced APs were also reported in plants (Stahlberg, 2006), so it

is interesting to investigate light exposure on *Chlorophyceae* alga electrical events that may be recorded from individual cells. It was shown that some red-light effects, for example spore germination, are antagonistic to blue light, red and blue-light effects can also be the same or synergistic (Kagawa and Wada, 1996). So it is interesting to observe effect of monochromatic light on bioelectrical parameters because membrane potential is widely used to report for early events associated with changes in ion permeability. Electrophysiological techniques could be employed to characterize bioelectrical properties in *in vivo* conditions after monochromatic light irradiation.

The technology based on light-emitting diodes (LEDs) expanded the possibilities to analyze the effects of different lighting spectra on plant physiological processes. The aim of this study was to examine blue, red and far-red light induced membrane potential changes and excitability in *Nitellopsis obtusa* cells.

MATERIALS AND METHODS

Cells of *Nitellopsis obtusa* were collected in November from Lake Siesartis (Lithuania) and were kept in a glass aquarium filled with ordinary room temperature water. Prior to the experiment, internodal cells (second or third internode below the tip of growing plants) were separated from neighboring cells.

The internodes were kept in the dark overnight in APW (control solution for electrophysiological testing) containing 0.1 mM KCl, 1.0 mM NaCl, 0.1 mM CaCl_2 , and 5 mM THS/RIS buffer, pH 7.2. Registration of membrane parameters is carried out according to K' -anaesthesia method (Kisnieriene et al., 2008).

Plexiglas chamber with Ag/AgCl₂ electrodes for cells stimulation and biopotential registration was used. One end of the cell is placed in the larger a-pool. The small segment (about 1 mm) of each cell is positioned in the common central c-pool, filled with 100 mM KCl solution. Electrical isolation between pools is achieved with 5% boric vaseline junctions as well as by an air gap between them (Fig. 1). The cell parts

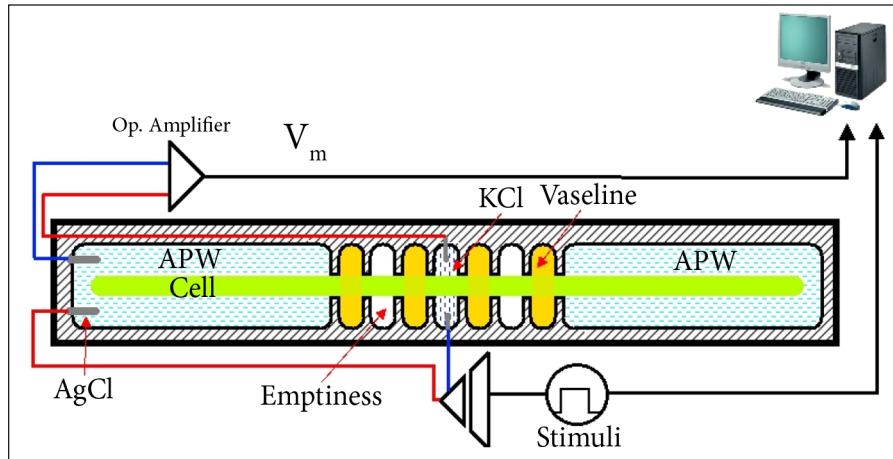


Fig. 1. Schematic diagram of the experimental set-up

in the pools are continuously bathed with APW solution. Bioelectrical signals are obtained from one general reference electrode placed in a reference pool, similar to an a-pool, and from appropriate measuring electrodes. The electrical contact between pools is enabled through the P-shaped glass tube bridge plugged with 3% (w/v) agar solidified in a 3M KCl solution.

Every 5 minutes three action potentials were elicited by injecting 0.1 s duration square pulse to threshold depolarizing current between two pools using Ag/AgCl wires. Two 12 bits analog outputs and 24 bits digital outputs of the “e-Biol” DAQ permit to easily implement any algorithm of stimulation. Signals were amplified with a WPI DAM50 preamplifier (input impedance – $10^{12} \Omega$, input leakage current – 50 pA, gain – 20x). Data were A/D

converted (12 bits, ADS7806P) and stored in the computer memory for later analysis of membrane parameters. As data acquisition device “e-Biol” system was used.

LED arrays ($10 \times 100 \times 1$ mm) constructed from 5 mm GaAlAs 18 mW/cm² power Roithner Lasertechnik GmbH, LED’s (430 nm, 660 nm ir 730 nm) were used as monochromatic light source. Light intensity was adjustable using potentiometer.

Photoelectric reaction was monitored 1 hour after light / dark and dark / light transition. Three AP were evoked every 5 minutes in the dark and after every illumination (Fig. 2).

Data are presented as mean and standard error. Statistical significance of differences was tested using paired T test and unpaired T test. All statements on statistical significance are

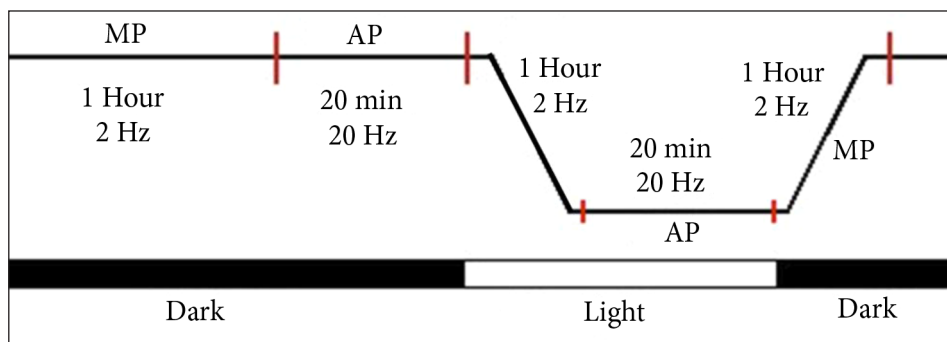


Fig. 2. The experimental protocol. Membrane potential (MP) recording without excitation continued 1 h at 2 Hz sampling frequency in the dark and after monochromatic illumination. Action potential (AP) recording continued 20 min at 20 Hz sampling frequency

based on confidence level of 95%. Calculations and statistical analysis were performed using Microcal ORIGIN 7.5, Statistica 6.0 (StatSoft).

RESULTS AND DISCUSSION

Light controls multiple physiological processes involving several light receptor systems. In this study we observed monochromatic light-induced photoelectrical reaction and membrane excitability in *Nitellopsis obtusa* cells. The effect of monochromatic blue, red and far-red light on membrane potential changes was evaluated using K⁺-anaesthesia method. Two processes of different polarity and time constants were observed in all cells when light was switched on and off. Illumination evokes a long-lasting hyperpolarization preceded by a small transient depolarization (Fig. 3). Switching off all investigated lights evokes long-lasting depolarization preceded by small transient hyperpolarization.

The rapid transient depolarization of membrane was observed in all investigated cells (8 cells in every light), but in some cells (3) after dark / red light transition depolarization amplitude was small. Statistical analysis

showed this effect to be not significant and every monochromatic light caused the same depolarization ($p > 0.5$). If the cell was irradiated by far-red light and then, after 2 hours of darkness, red light was switched on, we observed statistically significant increase in depolarization amplitude ($p < 0.01$). It is known that Ca²⁺ plays a role as a “second messenger” that mediates the light perception and it was shown that Ca²⁺ carries a significant portion of the depolarizing current (Ermolayeva et al., 1996). Other investigators have shown that blue light but not red light induces a calcium transient in the moss *Physcomitrella patens* (Russell et al., 1998). In *Vallisneria gigantea* Graebner mesophyll cells, red light irradiation induces cytoplasmic streaming by decreasing the Ca²⁺ concentration in the cytoplasm, while far-red light irradiation inhibits it by increasing the concentration (Shabala et al., 1999). Our results indicate that all investigated lights induce increment of cytoplasmic Ca²⁺ concentration in *Nitellopsis obtusa* cells.

Slow membrane hyperpolarization followed every light switching on. We could observe two patterns of MP changes (Fig. 3), but it depends not on the effect of monochromatic light, but

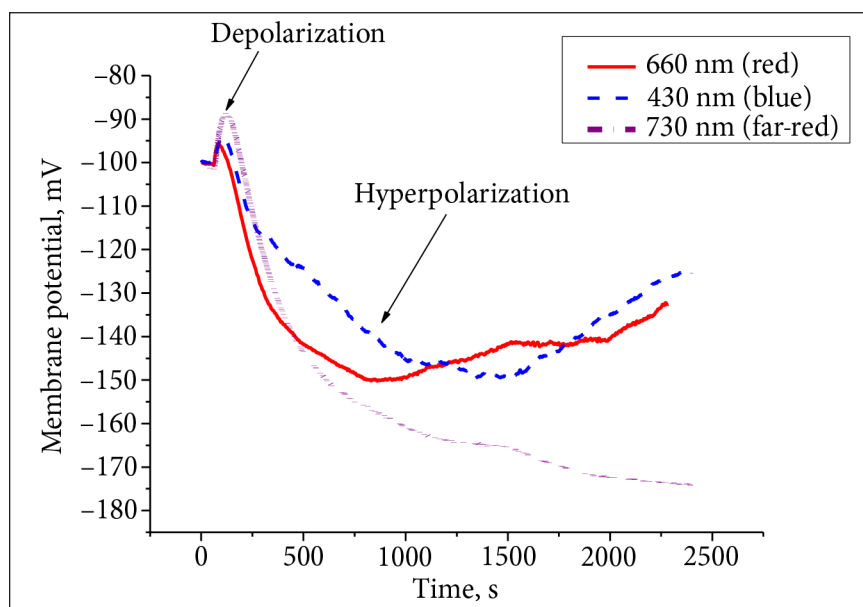


Fig. 3. Typical example of light-induced membrane potential changes after different monochromatic light illumination. Arrows show depolarization and hyperpolarization

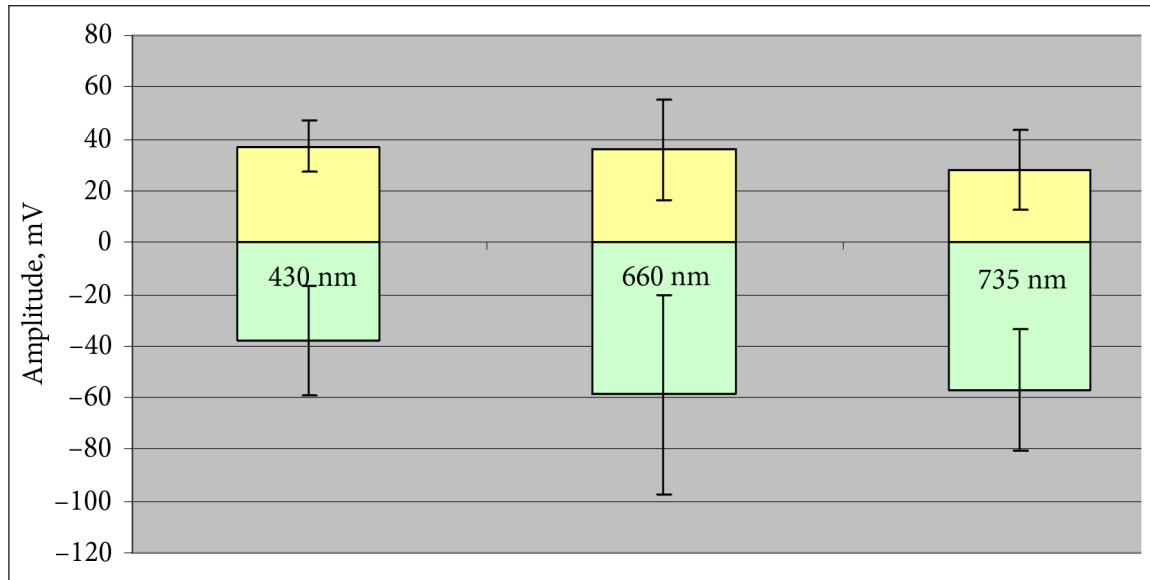


Fig. 4. Averaged long-lasting cell responses to the on / off effect. Hyperpolarization was observed when the test light was switched on, depolarization – when the test light was switched off

on the individual cell properties and statistical analysis reveals the same hyperpolarization process after dark / monochromatic light transition. Accordingly, light / dark transition causes similar responses but in the opposite direction. Statistical analysis did not show significant differences after different illumination (Fig. 4).

In photosynthetic plant cells, the light can rapidly modulate the PM H⁺-ATPase activity and voltage-dependent ion channels. The evidence indicates that blue light activates the plasma membrane H⁺-ATPase, producing an outward current that hyperpolarizes the membrane. Blue light directly activates the proton pump (Elzenga et al., 1995), but red light might affect the activity of the pump via calcium and potassium entering through channels in the plasma membrane (Ermolayeva et al., 1996). We observed that onset of every illumination causes a prolonged hyperpolarization which could be explained by activation of the plasma membrane H⁺-pump. H⁺ pumping hyperpolarizes the membrane potential, thus creating an electrical gradient (inside negative) required for passive K⁺ uptake through the K⁺-selective channels (Roelfsema et al., 2001). It was shown that light stimulates potassium

uptake, which increases the osmotic potential of the cytoplasm and precedes cell expansion (Živanović et al., 2005). In our experiments we observed some depolarization after long-lasting hyperpolarization (Fig. 3), so we suggested that K⁺ might act as a charge balancing ion to compensate for H⁺ extrusion.

If an increase in Ca²⁺ and depolarization are sufficient to activate anion channels, action potentials are generated (Sukhovet et al., 2011). AP generation depends on cell excitation threshold. In our cases it was averaged -80 ± 10 mV, and light induced depolarization amplitude was too small to reach the excitation threshold. In contrast to the report of light induced AP in *Physcomitrella patens* (Koselski et al., 2008) we recorded action potentials only once in *Nitellopsis obtusa* cells after red light illumination. It might be related to light intensity, so further investigations are required. However, we could elicit action potentials using electrical stimulation which is typical in excitable plants (Fig. 5).

Amplitude and duration of electrically evoked AP in each illumination were evaluated. We found that at all tested lights irradiation amplitudes did not differ significantly ($p > 0.1$, $n = 6$) (Fig. 6, A). However, we found the effect

of different lighting on AP duration (Fig. 6, B). Duration of AP was evaluated as width of AP half amplitude. We found that AP duration in blue light was 35% longer than in far-red light ($p < 0.03$).

It is known that repolarization occurs after the opening of outward rectifying K^+ channels and activation of the electrogenic proton pump (Beilby, 2007). It was shown that K^+ channels and H^+ -ATPase can be responsible for the differences in AP duration in light- and dark-induced action potentials in *Physcomitrella patens* (Koselski, 2008). Our results suggest activation of proton pump in far-red light, so significant AP duration difference can be related to more negative MP in this light. Future studies could be performed to investigate the effect of monochromatic light on separate ion transport systems using a voltage clamp method.

Membrane potential changes induced by red light were not reversed or inhibited by far-red light in our investigated cells. Moreover, blue and red light cause the same bioelectrical events in *Nitellopsis obtusa* cells. It can be concluded that all photoreception systems exert their effects by first altering the permeability of the plasma membrane to ions and later ion channels perform signal-transducing functions in the complex array of mechanisms that determine plant photomorphogenesis.

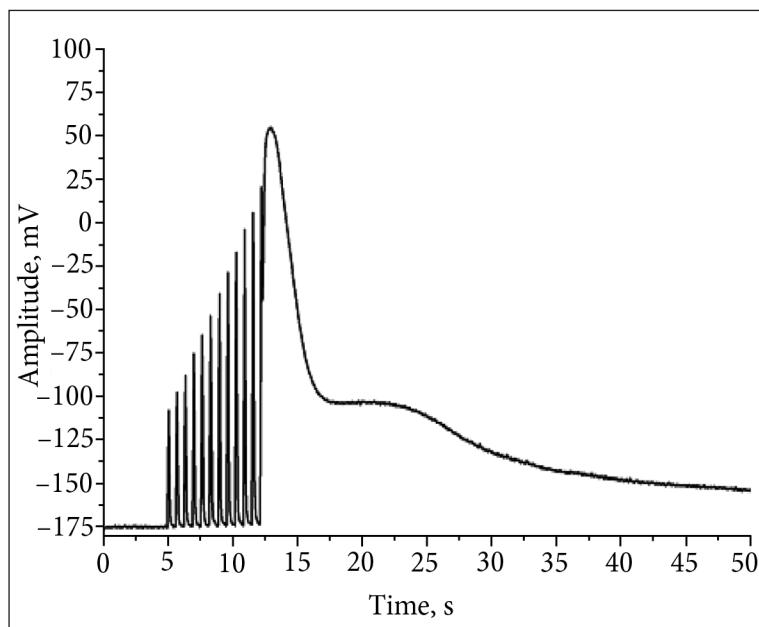


Fig. 5. Typical example of electrically evoked AP of *Nitellopsis obtusa* cells in blue light

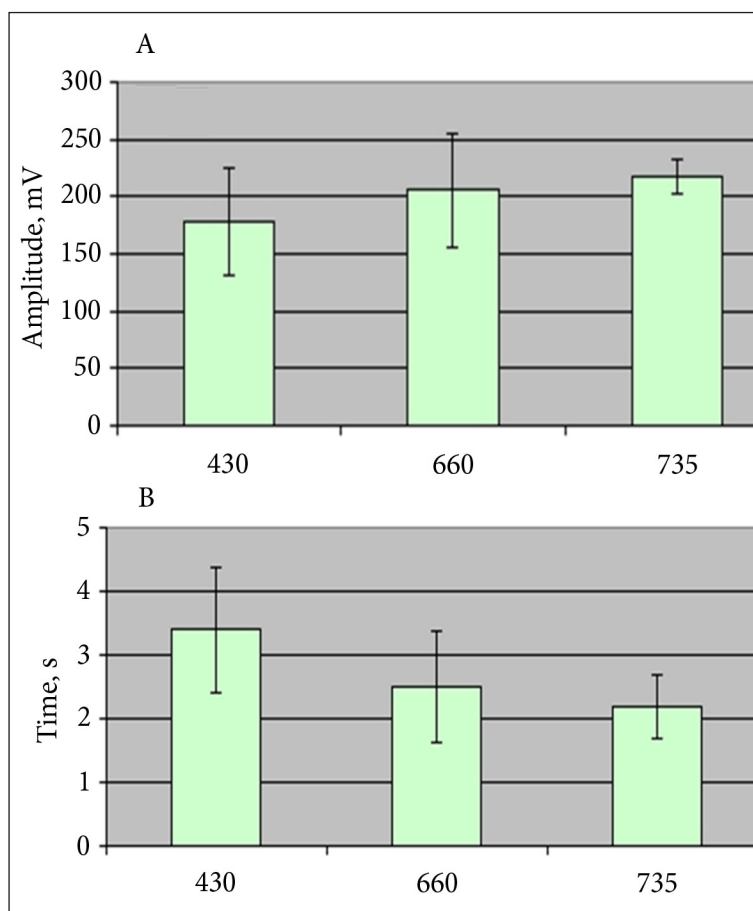


Fig. 6. Amplitude (A) and duration (B) of electrically evoked AP after blue (430 nm), red (660 nm) and far-red (735 nm) light irradiation

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**MĖLYNOS, RAUDONOS IR TOLIMOS
RAUDONOS ŠVIESOS POVEIKIO
NITELLOPSIS OBTUSA LĄSTELIŲ
MEMBRANINIAM POTENCIALUI TYRIMAS**

Santrauka

Šviesa kontroliuoja įvairius augalų fiziologinius procesus. Membraninio potencialo pokyčiai atspindi ląstelių jonų pernašos sistemų veiklą, todėl šiame darbe buvo tiriamas monochromatinės šviesos poveikis *Nitellopsis obtusa* ląstelių elektrofiziologiniams parametrams. Membraninio potencialo pokyčiai buvo registruojami naudojant ekstraląstelinį „K⁺ anestezija“ metodą. Mėlynos, raudonos ir tolimos raudonos šviesos šaltiniais pasirinktos trys 430 nm, 660 nm ir 735 nm bangos ilgių šviestukų matricos. Buvo vertinama fotoelektrinė reakcija ir ląstelių sužadinimas. Nustatyta, kad visų tirtų bangos ilgių šviesos įjungimas ir išjungimas daro tokį patį tiesioginį poveikį membranai potencialui, veikimo potencialo generavimui bei jo parametrams.

Raktažodžiai: augalų fotoreceptoriai, membraninis potencialas, augalų elektriniai signalai, augalų fotoelektrinė reakcija