

## Chapter 2

# The Effect of Electrical Signals on Photosynthesis and Respiration

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**Abstract** Electrical signals are initial response of plant to the external stimuli. This type of signal may trigger different physiological responses. The most famous is the rapid leaf movement in carnivorous and sensitive plants. However, a lot of less visible changes in plant physiology may occur. This chapter focuses on the effect of action (APs) and variation potentials (VPs) on photosynthesis and respiration. First, experimental methods and setup for measurements of photosynthesis and respiration in response to electrical signals are described. Then detailed information about effect of AP and VP on CO<sub>2</sub> metabolism in different plant species are summarized. Both light and dark reactions of photosynthesis, as well as rate of respiration, are affected by electrical signals, but the effect is often adverse (from inhibition to stimulation of photosynthesis). In addition, the stomatal conductance ( $g_s$ ), an important component of gas exchange, is also differently affected by electrical signals. Summarizing the data from numerous authors, the hypothesis about mechanism underlying photosynthetic limitation and stimulation of respiration is proposed.

### Abbreviations

1-qP	excitation pressure at photosystem II
ABA	abscisic acid
AP	action potential
DCMU	3-(3',4'-dichlorophenyl)-1,1-dimethylurea
DW	dry weight
E	rate of transpiration
F <sub>0</sub>	minimal fluorescence level in dark-adapted leaves

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$F_0'$	minimal fluorescence level “in the light”
$F_m$	maximal fluorescence level from dark adapted leaves
$F_m'$	maximal fluorescence level in light-adapted leaves
$F_t$	steady-state fluorescence in the light
$F_v$	variable fluorescence
$F_v/F_m$	maximum quantum yield of photosystem II
$g_s$	stomatal conductance
IAA	indole-3-acetic acid
JA	jasmonic acid
NPQ	non-photochemical quenching
OEC	oxygen evolving complex
OPDA	12-oxo-phytodienoic acid
PAR	photosynthetic active radiation
Pheo	pheophytin
$P_G$	rate of gross photosynthesis
$P_N$	rate of net photosynthesis
PSI	photosystem I
PSII	photosystem II
PQ	plastoquinone pool
$Q_A$	plastoquinone A
$Q_B$	plastoquinone B
qE	energy-dependent quenching
qI	photoinhibitory quenching
qT	state-transition quenching
qP	photochemical quenching
$R_D$	rate of respiration
VP	variation potential
$\Phi_{PSII}$	effective photochemical quantum yield of photosystem II

## 2.1 Introduction

Life on Earth depends on energy derived from the sun. Photosynthesis is the only process of biological importance that can harvest this energy and provides building carbon blocks that plants depend on. On the other hand, respiration releases the energy stored in carbon compounds for cellular use. Although respiration is common for all eukaryotic organisms, the photosynthesis is confined only to the plant kingdom and also to some prokaryotic bacteria. Many environmental factors can affect rate of photosynthesis ( $P_N$ ) and respiration ( $R_D$ ). Concentration of  $O_2$ ,  $CO_2$ , temperature, water and nutrient supply are the most often designated (Taiz and Zeiger 2002). However, not very often mentioned, electrical signals generated by plants have also impact on both processes. And it is not only the case of sensitive (*Mimosa pudica*) and carnivorous plants (*Dionaea muscipula*,

*Aldrovanda vesiculosa*, *Drosera* spp.) but of all green plants around us. Besides photosynthesis and respiration, many others physiological responses to electrical signals have been reported, among them are, for example: decrease of elongation growth (Shiina and Tazawa 1986), changes in transpiration and stomata opening/closing (Koziolek et al. 2003; Hlaváčková et al. 2006; Kaiser and Grams 2006; Grams et al. 2009), phloem unloading and translocation (Fromm and Eschrich 1988a; Fromm 1991; Fromm and Bauer 1994), changes in transcription and translation (Davies and Schuster 1981; Graham et al. 1986; Wildon et al. 1992; Peña-Cortéz et al. 1995; Stankovič and Davies 1998), decrease of circumnutation rate in *Helianthus annuus* (Stolarz et al. 2010), induction of jasmonic acid (JA) and abscisic acid (ABA) synthesis (Fisahn et al. 2004; Hlaváčková et al. 2006) and rapid trap closure and secretion of digestive fluid in carnivorous plants (Affolter and Olivo 1975; Lichtner and Williams 1977). You may find detailed description of these responses in other chapters of this book. This chapter summarizes data on the effect of action (APs) and variation potentials (VPs) generated by different stimuli on photosynthesis and respiration in plants.

## 2.2 Methodology and Experimental Setup

The best way how to study the effect of electrical signals on photosynthesis is simultaneous measurements of electrical potential, gas exchange, and chlorophyll fluorescence. Such experimental setup together with modified inverted microscope for observation of stomata aperture has been used rarely (Kaiser and Grams 2006). If it is not possible to do simultaneous measurements, then the measurements of gas exchange and chlorophyll fluorescence can be done separately (e.g. Koziolek et al. 2003; Lautner et al. 2005; Hlaváčková et al. 2006). Because the techniques for measurements of electrical signals in plants are well summarized in Chaps. 7 (Jovanov and Volkov) and 8 (Fromm and Lautner) of this book, I will focus on measurement of gas exchange and chlorophyll fluorescence in response to electrical signals.

### 2.2.1 Gas Exchange Measurements

The most commonly used method of measuring the gas exchange of leaf is to enclose it in a cuvette, pass a known flow rate of air over the leaf, and measure the exchange in concentration of CO<sub>2</sub> and H<sub>2</sub>O in the air. Heteroatomic molecules such as CO<sub>2</sub>, H<sub>2</sub>O, NO, and NH<sub>3</sub> absorb infrared radiation in specific infrared wavebands. Gas molecules with two identical atoms (e.g. N<sub>2</sub>, O<sub>2</sub>, H<sub>2</sub>) do not absorb infrared radiation. A large number of infrared gas analyzers are available commercially for plant science application (Ciras-2, PP-Systems; LI-6400, Li-Cor Biosciences; LCpro, ADC Bioscientific). The most of analyzers act as

**Fig. 2.1** Experimental setup. The trap of Venus flytrap (*D. muscipula*) is enclosed in hermetically closed gas exchange cuvette with attached fluorocamera. The thin wire in the needle from syringe is hermetically sealed and used for trigger hair stimulation



absorptiometers measuring at  $\sim 4.26 \mu\text{m}$  ( $\text{CO}_2$ ) and  $\sim 2.60 \mu\text{m}$  ( $\text{H}_2\text{O}$ ). This means, that besides the rate of photosynthesis ( $P_N$ ) and respiration ( $R_D$ ), you can also measure the transpiration rate ( $E$ ) and stomatal conductance ( $g_s$ ). Absorption at any wavelength follows the Beer–Lambert Law so that if gas containing  $\text{CO}_2$  and  $\text{H}_2\text{O}$  is flushed through a tube with an infrared light source at one end and an infrared light detector at the other end, the signal from the detector will decline with increasing  $\text{CO}_2$  and  $\text{H}_2\text{O}$  concentration (Hunt 2003). The best equipment for such measurements is true differential system with four independent infrared gas analyzers, two for  $\text{CO}_2$  (one for analysis air from the cuvette with leaf and one for reference air) and two for  $\text{H}_2\text{O}$  (also one for analysis air from the cuvette and one for reference air) eliminating the problems associated with “gas switching.” The difference between analysis and reference air is used for calculation of  $P_N$ ,  $R_D$ ,  $g_s$  and  $E$ , in modern equipment this is automatically done by computer software (care must be taken, whether the leaf covers the entire cuvette area, otherwise the recalculation per area, or per dry weight is necessary). Equipments for high level research has usually independent automatic  $\text{CO}_2$  and  $\text{H}_2\text{O}$  control and self calibration checking facility as well as control of temperature, light and humidity with powerful, flexible software allowing simple, individual measurements to more complex, automated and preprogrammable measurements. Wide range of cuvettes and chambers are also commercially available. All modern automatic cuvettes are constructed from materials that have minimal absorption of  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , and have closed cell gaskets that give perfect hermetically leaf seal. Automatic cuvettes contain sensors to measure leaf and air temperature (these are important for calculation of parameters, e.g.  $g_s$ ) and photosynthetically active radiation (PAR). As a source of PAR, LED light sources produce very little heat and become increasingly popular. They usually produce light in narrow wave length, the most common are red and blue LEDs. It is very important, especially in studying the effect of electrical signals on photosynthesis, to take into account the time taken for gas to pass from the cuvette to the infrared gas analyzer, because the changes in  $\text{CO}_2$  and  $\text{H}_2\text{O}$  are recorded with constant delay at a given flow rate of air (Fig. 2.1).

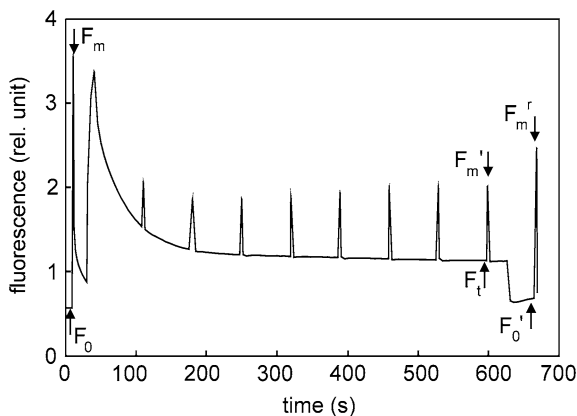
### 2.2.2 Chlorophyll Fluorescence Measurements

Gas-exchange measurements are powerful tool for studying respiration, the dark reaction of photosynthesis ( $\text{CO}_2$  assimilation) and water transpiration, the methods of chlorophyll fluorescence are useful for quantification of light reaction of photosynthesis occurring at the level of photosystems. Ground state molecules of chlorophylls in a leaf can absorb the light energy. Absorption of light excites the chlorophyll to a higher energy state, where it can be stable for a maximum of several nanoseconds ( $10^{-9}$  s) and soon return to its ground state. The energy difference between the ground and excited state can be used to drive photosynthesis (photochemistry), excess energy can be dissipated as heat or it can be reemitted as light (chlorophyll fluorescence). These three processes are in competition. Increase in the efficiency of one will results in a decrease in the yield of the other two. Under physiological condition, fluorescence signal is assumed to originate mainly from photosystem II (Maxwell and Johnson 2000).

Considering the competition of fluorescence with photochemistry, two extreme situations are possible. All reaction center can be open (i.e. electron acceptor plastoquinone A,  $\text{Q}_\text{A}$  is oxidized) or closed (i.e.  $\text{Q}_\text{A}$  is reduced), with fluorescence yield being minimal ( $F_0$ ) and maximal ( $F_\text{m}$ ), respectively. Prior to  $F_0$  measurement, it is important that the leaf sample was sufficiently dark-adapted (20–30 min). Then minimal fluorescence ( $F_0$ ) can be determined at very low photosynthetic active radiation (PAR) below  $0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. It is a fluorescence signal that comes from excited chlorophylls of light harvesting antenna of PS II before the excitation reaches the reaction center. Maximal fluorescence ( $F_\text{m}$ ) can be determined by strong saturation pulse of light (several thousand  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR) with duration around 1 s. This rise of fluorescence signal has been explained as a consequence of reduction of plastoquinone and in particular  $\text{Q}_\text{A}$ . Reduced  $\text{Q}_\text{A}$  decrease the capacity for PSII photochemistry almost to zero by inhibition of charge separation ( $\text{P680}^+ \text{Pheo}^-$ ) and an increase in a charge recombination (backward electron transport from  $\text{Pheo}^-$  to  $\text{P680}^+$ ) by electrostatic repulsion of the charge on  $\text{Q}_\text{A}^-$ . This means that under physiological condition, the rate of energy conversion at PSII reaction center is acceptor side limited [sufficient electrons are available from  $\text{H}_2\text{O}$  splitting oxygen evolving complex (OEC)] and, hence, fluorescence yield is controlled by the quencher  $\text{Q}_\text{A}$ . From  $F_0$  and  $F_\text{m}$  variable fluorescence ( $F_\text{v}$ ) can be calculated as  $F_\text{m} - F_0$  and maximum quantum efficiency of PSII photochemistry  $F_\text{v}/F_\text{m}$  as  $(F_\text{m} - F_0)/F_\text{m}$ , which is proportional to quantum yield of  $\text{O}_2$  evolution. Dark-adapted values of  $F_\text{v}/F_\text{m}$  are used as a sensitive indicator of plant photosynthetic performance, with optimal values of around 0.83 (Maxwell and Johnson 2000).

After the initial rise of chlorophyll fluorescence signal, the fluorescence level typically starts to fall during few minutes and steady-state level is achieved ( $F_\text{t}$ ). This phenomenon termed fluorescence quenching (Kautsky curve) is explained in two ways. Photochemical quenching (qP) is process by which the electrons move away from PSII due to light-induced activation of enzymes involved in carbon

metabolism. Nonphotochemical quenching (NPQ) is increase in the efficiency with which the absorbed energy is converted to heat. Both qP and NPQ help to minimize production of triplet  $^3\text{Chl}^*$ , which can transfer energy to  $\text{O}_2$  to generate singlet oxygen ( $^1\text{O}_2^*$ ), an extremely damaging reactive oxygen species. Quenching analysis can distinguish between qP and NPQ. After initial assessment of  $F_0$  and  $F_m$  in dark-adapted samples actinic light is turn on, and at appropriate intervals, further saturation flashes are applied. It is recommended to use actinic light at low intensity ( $<100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR) for studying the electrical signals on chlorophyll fluorescence, because at higher PAR, electrical signals produce no additional fluorescence quenching as was found by Krupenina and Bulychov (2007). Thus, maximal fluorescence in light-adapted state ( $F_m'$ ) as well as steady-state fluorescence ( $F_t$ ) is determined. The value of  $F_m'$  is usually lower than  $F_m$  in dark-adapted sample, because NPQ is competing for the excited states and the difference between  $F_m'$  and  $F_m$  reflects fluorescence quenching due to heat dissipation. Finally, the minimal fluorescence in the light-adapted state ( $F_0'$ ) can be measured by turning off the actinic light or by applying a far-red light. From these data several parameters can be calculated. The most useful parameter is effective photochemical quantum yield of PSII ( $\Phi_{\text{PSII}}$ , but many alternative terms exist in literature, for overview see Roháček 2002), which is calculated as  $\Phi_{\text{PSII}} = (F_m' - F_t)/F_m'$ . This parameter measures the proportion of light absorbed by chlorophylls associated with PSII that is used in photochemistry and strong linear relationship exists between this parameter and  $P_N$ . Another parameter, qP =  $(F_m' - F_t)/(F_m' - F_0')$  measures the proportion of reaction centers that are open (i.e. the proportion of plastoquinone molecules that are oxidized). On the contrary,  $1 - \text{qP}$  gives an indication of reaction centers that are closed and is sometimes termed as excitation pressure at PSII. NPQ parameter can be calculated as  $(F_m - F_m')/F_m'$  and measures the thermal dissipation process at PSII. NPQ can be divided into at least three different components according to their relaxation kinetics. The major component of NPQ is pH-dependent or energy state quenching (qE) activated by light. Absorption of light that exceeds a plant capacity for  $\text{CO}_2$  fixation results in a build up of thylakoid  $\Delta\text{pH}$  that is generated by electron transport. Low pH activates violaxanthin de-epoxidase which transforms violaxanthin to zeaxanthin and harmlessly dissipates excess light energy as heat; however, zeaxanthin-independent quenching in PSII core and lutein may also contribute to heat dissipation (Finazzi et al. 2004; Johnson et al. 2009). A second component, state transition quenching (qT) is caused by uncoupling of light harvesting complexes from PSII. The third component of NPQ is related to photoinhibition (qI). The best way, how to distinguish among these three components, is relaxation kinetic measurements. Whereas, qE relaxes within seconds to minutes in the dark, qI shows very slow relaxation kinetics in the range of hours (for details see Müller et al. 2001). Sequence of a typical chlorophyll fluorescence trace is shown in Fig. 2.2. If the plant response to electrical signals is leaf folding (e.g. *Mimosa* and *Dionaea*), it is necessary to fix the leaf in proper position to avoid changes in light interception. Such changes may be source of errors in calculation of  $\Phi_{\text{PSII}}$  and other parameters and the changes in  $\Phi_{\text{PSII}}$  are then not caused by electrical signal

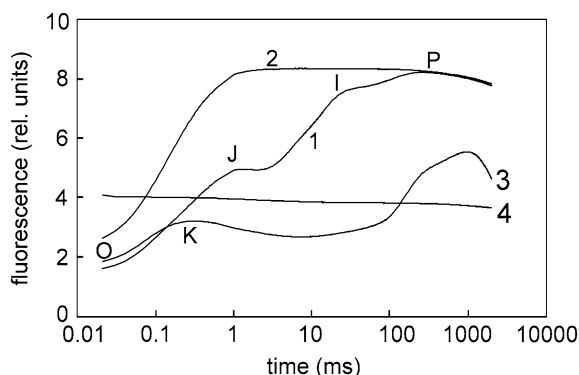


**Fig. 2.2** Sequence of a typical fluorescence trace. Minimum fluorescence ( $F_0$ ) is measured by weak light ( $<0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR). Then saturation flash is applied ( $4,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, 800 ms duration) and maximum fluorescence ( $F_m$ ) is measured. Actinic light is turn on (in this case  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR) and fluorescence is quenched to a new steady-state level in the light ( $F_t$ ). Simultaneously, saturation pulses of the same intensity and duration are given at regular interval. This allows the maximum fluorescence “in the light” ( $F_m'$ ) to be measured. Turning off the actinic light allows the minimum fluorescence “in the light” ( $F_0'$ ) to be estimated. Recovery of maximum fluorescence after switching off the light ( $F_m^r$ ) is measured by saturation pulse

itself, but by changes of leaf position and light interception. For more details about chlorophyll fluorescence measurements see Maxwell and Johnson (2000), Müller et al. (2001), Blankenship (2002), Roháček (2002) and Papageorgiou and Govindjee (2004).

On onset of illumination in dark-adapted leaf, fluorescence yield rises in two steps (J, I) from minimal level ( $F_0$ ) to a peak level ( $F_m$ ). These changes in fluorescence yield have been called fluorescence induction or fluorescence transient (O-J-I-P) and can be used as a quick monitor of the electron acceptor side reaction (Fig. 2.3). It has been proposed that O step (40–50  $\mu\text{s}$ , the same as  $F_0$ ) is the fluorescence signal coming from excited chlorophylls of light harvesting antenna before the excitations reach reaction center of PSII, J step (2–3 ms) reflects light-driven accumulation of  $Q_A^-$ , and I (30–50 ms) and P step (200–500 ms) reflect light-driven accumulation of  $Q_B^-$  and  $Q_B^{2-}$  respectively; however, several other explanations have been proposed, for example, electron transport reaction beyond PSII also effect the shape of induction curve (for review see: Lazár 2006, 2009; Vredenberg 2011). When a sample is treated with DCMU (3-(3',4'-dichlorophenyl)-1,1-dimethylurea; an inhibitor of electron transport between  $Q_A$  and  $Q_B$ ) fluorescence induction is characterized by a steep fluorescence increase, reaching maximal saturation level at the position of J step confirming that it is mainly  $Q_A^-$  that accumulates in the position of the J step (Strasser and Govindjee 1992). In the absence of DCMU,  $Q_A$  may not be fully reduced in the J step, because electrons move from  $Q_A^-$  further towards PSI, and fluorescence reaches maximum at the

**Fig. 2.3** Chlorophyll fluorescence rise on onset of illumination in dark-adapted control leaf (1), leaf incubated in 100 mM DCMU (2), heat-treated leaf (3) and chlorophyll solution in 80% acetone (no variable fluorescence is detected, 4). Five distinct phases is possible to recognize (O-K-J-I-P). The transient is plotted on logarithmic time scale



position of P step (Fig. 2.3). Apart from light-driven accumulation of reduced PQ pool, it has been proposed that electrical voltage across thylakoid membrane (thylakoid membrane potential) may affect I-P phase (Vredenberg and Bulychev 2002; Vredenberg 2004). This assumption is important because APs have significant effect on I-P rise of chlorophyll fluorescence as has been documented recently (Pavlovič et al. 2011). When the sample is treated with high temperature, a new step at 300–400  $\mu$ s denoted as K-step appears reflecting an inhibition of OEC or donor side of PSII (Srivastava et al. 1997). Chlorophyll in acetone solution does not show any variable fluorescence (Fig. 2.3). For details of chlorophyll a fluorescence transient see Schreiber (2004), Strasser et al. (2004) or Lazár (2006).

The experimental protocols developed for non-imaging fluorometry described above can be successfully adapted also to imaging fluorometers. Due to technical limitation the fast chlorophyll fluorescence transient (O-J-I-P) can not be measured by imaging fluorometers. Chlorophyll fluorescence imaging has the capacity to resolve photosynthetic performance over the leaf surface. Its application is growing rapidly including also plant electrophysiology. In contrast to non-imaging fluorometry, which measures an average fluorescence signals over the sample area, imaging fluorometry may reveal spatial heterogeneity in photosynthetic performance in response to electrical signals. Such spatial heterogeneity was documented, for example, in carnivorous Venus flytrap (*D. muscipula*) in response to trigger hair irritation (Pavlovič et al. 2010, 2011) as well as in maize (Grams et al. 2009) and *Mimosa* leaf in response to heat treatment (Koziolek et al. 2003; Kaiser and Grams 2006). Simultaneous measurement of chlorophyll fluorescence imaging and gas exchange is the best way how to monitor the photosynthetic response to electrical signals (Fig. 2.1). Fortunately, such systems are now commercially available (e.g. Photon System Instruments and PP-Systems). For details about chlorophyll fluorescence imaging see Nedbal and Whitmarsh (2004).



### 2.2.3 Polarographic $O_2$ Measurements

Not very often used in terrestrial but the indispensable method in aquatic plants or algae is the polarographic measurements of  $O_2$  evolution which come from photolysis of water (Hill reaction). Regarding studies on electrical signaling this method was successfully used by Alexander Bulychev group in *Chara corallina* (Bulychev and Kamzolkin 2006a, b). An  $O_2$  electrode (Clarke type) consists of a platinum cathode and a silver anode linked by an electrolyte. The current flowing between anode and cathode is directly proportional to the  $O_2$  concentration. For more details see Hunt (2003).

## 2.3 Effect of APs on Photosynthesis

APs are rapidly propagated electrical messages that are well known in animal kingdom. The first message about APs in plants comes from Burdon-Sanderson (1873) who conducted experiments with carnivorous plant Venus flytrap (*D. muscipula*) on Charles Darwin request. The APs are usually induced by non-damaging stimuli (e.g. cold, mechanical and electrical stimulation, irrigation etc.) and have three major features: (1) AP transmits at constant velocity and amplitude, (2) AP follows the all-or-none law, (3) after AP is generated, the cell membrane enters into refractory periods (Yan et al. 2009). While the ionic mechanism of APs in animals depends on inward-flowing  $Na^+$  (depolarization) and outward-flowing  $K^+$  ions (repolarization), excitation of plant cells depends on  $Ca^{2+}$ ,  $Cl^-$ , and  $K^+$  ions (Fromm and Lautner 2007). After perception of external stimulus,  $Ca^{2+}$  flow into the cell. The elevation of  $Ca^{2+}$  concentration in the cytoplasm activates the anion channel and  $Cl^-$  efflux depolarizes the plasma membrane. Then outward rectifying  $K^+$  channel would repolarize the membrane (Yan et al. 2009). In higher plants, the APs can travel over short as well as long distances through plasmodesmata and phloem, respectively (Sibaoka 1962, 1991; Fromm and Eschrich 1988b; Fromm and Lautner 2007).

It is known that electric signals arising at the plasma membrane are transmitted to the level of thylakoid membrane and may affect the photosynthetic reaction, but the mechanism is still not fully understood (Bulychev and Kamzolkin 2006a, b). Below you will find some typical examples for different group of plants and algae.

### 2.3.1 *Chara* cells

Internodal cells of characean algae represent a convenient model for studying plant cell excitability (Beilby 2007). The internodes of Characeae are suitable for exploring the interaction between electric excitation, photosynthesis, and ion

transport for the presence of single layer of chloroplasts in the peripheral part of cytoplasm. *Chara* cells exposed to illumination form domains with  $H^+$  extrusion and  $H^+$  sink that account for pH banding (Lucas 1975). The term pH banding designates the parts of cell producing alkaline and acid regions along the cell length in the outer medium. This spatial arrangement has significant effect on photosynthesis. Plieth et al. (1994) and Bulychev et al. (2001) found by using chlorophyll fluorescence imaging and microfluorometry, respectively, that electron transport was higher in the acid than in the alkaline bands. Polarographic measurements also revealed longitudinal profile of  $O_2$  evolution from Hill reaction. Plieth et al. (1994) suggested better supply of  $CO_2$  in the acid region, because equilibrium between  $CO_2$  and bicarbonate is shifted towards  $CO_2$ , in contrast to alkaline region, where is shifted toward bicarbonate, which poorly permeates through the membranes. In general, changing  $CO_2$  concentration modulates the activity of the Calvin cycle and magnitude of electron transport from PSII and thus chlorophyll fluorescence (Schreiber 2004).

APs have significant effect on pH banding. After electric stimulation and subsequent generation of AP, the pH decreases in alkaline regions resulting in the smoothening of the pH profile. The pH decrease in alkaline zone is accompanied by a reduction of  $F_m'$  and  $\Phi_{PSII}$  in contrast to the acid zone, where such changes were not observed (Bulychev et al. 2004; Bulychev and Kamzolkina 2006a, b). Whereas, AP develops on a time scale from milliseconds to seconds, subsequent pH and  $\Phi_{PSII}$  changes proceed within minutes. Krupenina and Bulychev (2007) called it as a long-lived state effect of AP on photosynthesis. Electrical excitation caused parallel decrease of  $\Phi_{PSII}$  and pH what disrupts the usual inverse relation between  $\Phi_{PSII}$  and pH found by Plieth et al. (1994), thus  $CO_2$  availability in ambient medium is unlikely to play any role in the AP-induced decrease of  $\Phi_{PSII}$  (Bulychev et al. 2001).

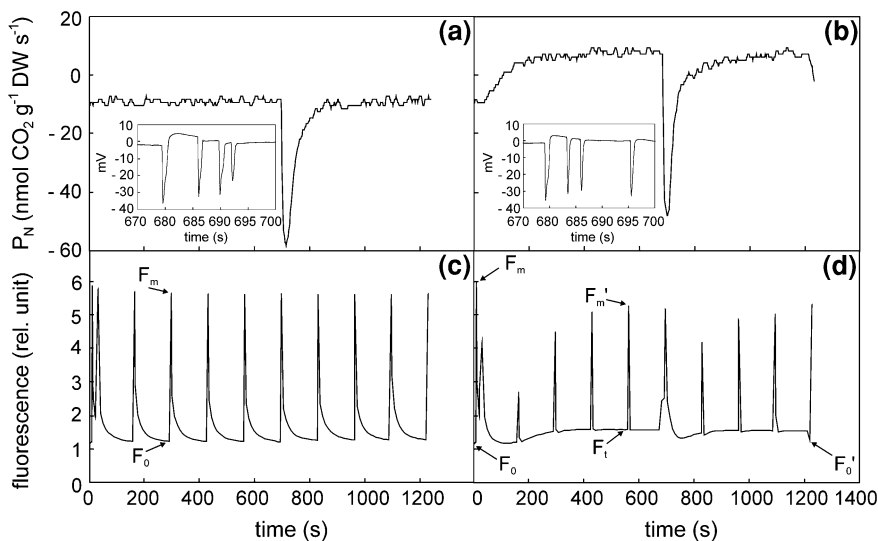
AP has no effect on  $F_m$  but have on  $F_m'$  indicating that excess energy absorbed by chlorophylls was dissipated by NPQ as heat. The effect of AP on  $\Phi_{PSII}$  and  $F_m'$  was most pronounced at light-limiting condition. The increase in light intensity elevated NPQ and diminished the AP-induced  $F_m'$  changes. Rapid dark relaxation of  $F_m'$  and experiments with protonophorous uncoupler monensin and nigericine confirmed that NPQ is related to the formation of thylakoid  $\Delta pH$  and qE and not to photoinhibition (Bulychev and Kamzolkina 2006a, b; Krupenina and Bulychev 2007). Application of ionophore A23187 which increase  $Ca^{2+}$  concentration in cytoplasm has similar effect as APs.  $Ca^{2+}$  plays a central role in a membrane excitation of plant cells and may be involved also in suppression of photosynthesis by indirect or direct way. Increase of cytosolic  $Ca^{2+}$  during AP may activate Ca-dependent protein kinases that modulate ion channels and pumps activity including transmembrane  $H^+$  flow. This may affect cytoplasmic pH and subsequently the function of chloroplasts by indirect way. However, it can not be excluded a direct role of  $Ca^{2+}$ . Chloroplast envelope and thylakoid membrane contain electrogenic  $Ca^{2+}$  pumps and  $Ca^{2+}/H^+$  antiporters which play role in light-induced depletion of cytosolic and stroma free  $Ca^{2+}$ , respectively (Kreimer et al. 1985; Ettinger et al. 1999). In chloroplast  $Ca^{2+}$  acts as cofactor in the OEC and may also influence the

activity of Calvin cycle enzymes. Suppression of Calvin cycle decreases ATP consumption. This would inhibit ATP synthesis, raise  $\Delta pH$  across the thylakoid membrane and activates NPQ (Bulychev et al. 2004; Krupenina and Bulychev 2007).

### 2.3.2 Carnivorous Plant Venus Flytrap (*D. muscipula*)

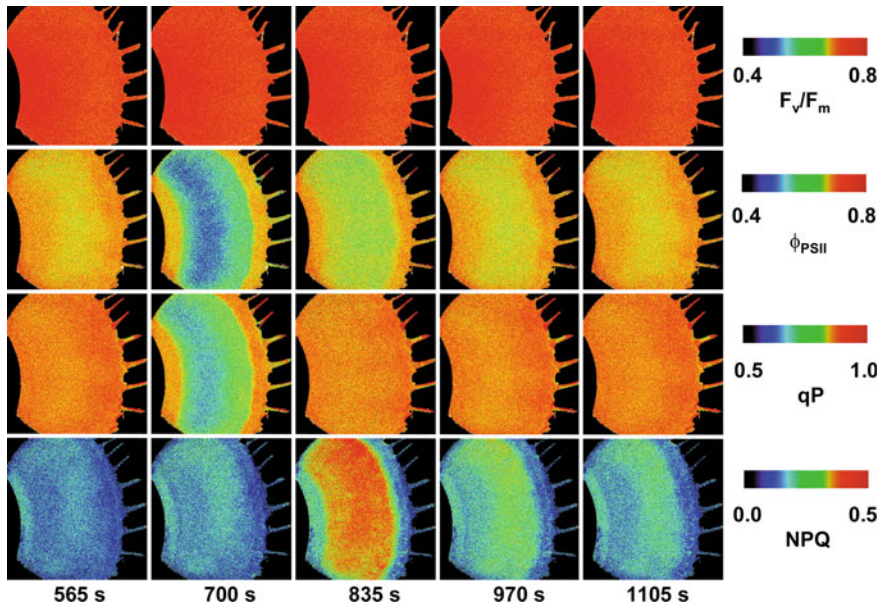
The most famous carnivorous plant, the Venus flytrap (*D. muscipula*), catches prey by rapid leaf movement. The plant produces a rosette of leaves, each divided into two parts: the lower is called the lamina and the upper is called the trap. The trap consists of two lobes, which close together after irritation of trigger hairs, which protrude from digestive zone of the trap. Two touches of trigger hairs activate the trap which snaps in a fraction of second at room temperature and AP play an indispensable role in this movement (Juniper et al. 1989). AP is generated at the base of the stimulated trigger hair after mechanical, electrical, or chemical stimulation and spreads in all directions over the whole surface of both lobes (Williams and Pickard 1980; Hodick and Sievers 1988; Sibaoka 1991; Volkov et al. 2008a, b). Volkov et al. (2007) found that the AP had duration 1.5 ms and velocity of  $10 \text{ m s}^{-1}$ . After the rapid closure secures the prey, struggling of the entrapped prey in the closed trap against trigger hairs results in generation of further APs, which cease to occur when the prey stop moving (Affolter and Olivo 1975).

APs spread over the trap surface and were not recorded in adjacent lamina (Volkov et al. 2007). This is in accordance with the observations that rapid changes of photosynthesis are confined to the digestive zone of trap and were not found in photosynthetic lamina (Pavlovič et al. 2010; Pavlovič and Mancuso 2011). Trigger hair irritation and rapid trap closure resulted in rapid inhibition of  $P_N$  and  $\Phi_{PSII}$ , whereas  $g_s$  was not affected. It can be argued that such changes may be associated with changes of trap geometry; however, the effect is similar and even stronger after repeated trigger hair irritation in closed trap. The changes in  $P_N$  and  $\Phi_{PSII}$  are rapid and occur usually within 2–4 s after irritation what is in accordance with rapid propagation of AP through the trap lobe (Pavlovič et al. 2010). A detailed analysis of chlorophyll a fluorescence kinetics revealed the possible mechanism underlying photosynthetic limitation. The decrease of  $\Phi_{PSII}$  is caused at first by increase in  $F_t$ , whereas  $F_m'$  was not affected immediately after irritation (Figs. 2.4d, 2.5). This indicates that plastoquinone pool became more reduced (lower qP) what results in a traffic jam of electrons and increase of excitation pressure at the PSII reaction center, promoting photoinhibition. Later  $F_t$  is quenched by NPQ as indicates large drop of  $F_m'$  thus preventing PSII against photoinhibition. Rapid relaxation of  $F_m'$  confirmed that the large drop of  $F_m'$  is associated with qE and not to qI. Uncoupling the light and dark enzymatic reaction of photosynthesis by unavailability of  $CO_2$  had no inhibition effect of AP on  $\Phi_{PSII}$ , indicating that changes in  $\Phi_{PSII}$  are mainly caused by inhibition of dark reaction of photosynthesis by feedback mechanism. Repeating the experiments in the dark,



**Fig. 2.4** Response of Venus flytrap (*D. muscipula*) to trigger hair irritation and subsequent generation of APs (figure insets) on gas exchange (a, b) and chlorophyll fluorescence (c, d). The trigger hairs were irritated between 670 and 700 s in the dark (a–c) and at light intensity  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR (b, d),  $F_0$ —minimal fluorescence in the dark adapted trap,  $F_m$ —maximum fluorescence in the dark after saturation pulse ( $4,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR),  $F_m'$ —maximum fluorescence in the light after saturation pulse ( $4,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR),  $F_i$ —steady-state fluorescence at light intensity  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR,  $F_0'$ —minimum fluorescence after light treatment measured in the dark at  $<0.1 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. a, c, and b, d measurements were recorded simultaneously in the dark and light, respectively. The APs were recorded by extracellular measurements as described in Pavlovič et al. (2011)

where the enzymes of Calvin cycle are inactivated, revealed that APs have only very small but significant effect on decrease of  $F_0$  and increase of  $F_m$  and thus increase of maximal quantum yield of PSII ( $F_v/F_m$ , Pavlovič et al. 2011). However, this assumption is valid only if we consider that changes in fluorescence yield are determined only by the redox-state of PSII reaction center (reduction of  $Q_A$ ) according to Strasser et al. (2004) what seems is not *a priori* true. A three state trapping model proposed by Vredenberg (2000, 2004) suggests that the saturation of photochemistry does not necessarily result in saturation of changes in fluorescence yield. Analysis of O-J-I-P curve after AP revealed that the decrease in the O-J-I rise and increase in the I-P rise found by Pavlovič et al. (2011) is in accordance with electrochemical stimulation of the fluorescence yield supplementary to qP (Vredenberg and Bulychiev 2002, 2003; Vredenberg 2004; Vredenberg et al. 2009). Electrical field in the vicinity of the reaction center can influence the chlorophyll fluorescence (Meiburg et al. 1983; Dau and Sauer 1991, 1992; Bulychiev and Vredenberg 1999; Vredenberg and Bulychiev 2002). Apart from  $Q_A$  oxidation, electrical field exerts its effect on charge separation and recombination reaction by decrease in Gibbs free-energy difference between the excited states in reaction center II and the charge separated state ( $P_{680}^+ \text{Pheo}^-$ , Dau and Sauer 1991, 1992).



**Fig. 2.5** Chlorophyll fluorescence parameters showed in 2-D images from experiment presented in Fig. 2.4 in response to trigger hair irritation (between 670 and 700 s) in Venus flytrap (*D. muscipula*).  $F_v/F_m$ —maximum quantum yield of PSII,  $\Phi_{PSII}$ —effective photochemical quantum yield of PSII,  $qP$ —photochemical quenching,  $NPQ$ —nonphotochemical quenching

However, the shape of O-J-I-P curve, mainly I-P phase, is also influenced by reactions beyond PSII (cyclic electron flow around PSI, Schansker et al. 2005; Lazár 2009) so the effect of APs on PSI and cyclic electron flow can not be excluded. Indeed, Vredenberg and Bulychev (2010) and Vredenberg (2011) supposed that photoelectrical control is exerted by the PSI-powered proton pump associated with cyclic electron transport. These data indicate that besides the effect of APs on dark reaction of photosynthesis there is also a significant effect on light reactions (electron transport) but this will need further examination.

These results may have more general consequences in measurements of chlorophyll fluorescence. The pulse of light, which is commonly used during chlorophyll fluorescence measurement, may trigger AP at plasma membrane or even changes in thylakoid membrane potentials (Trebacz and Sievers 1998; Bulychev and Vredenberg 1999; From and Fei 1998; Pikulenko and Bulychev 2005). If changes of membrane potentials have effect on fluorescence yield then variable fluorescence in dark as well as light-adapted state are not exclusively related to quantum photochemical yield of PSII and  $qP$ , but incorporate also photoelectrochemical and photoelectrical component (Vredenberg et al. 2009; Vredenberg 2011). In other words, the  $F_m$  measured by saturation light pulse is different from the original hypothetical dark-adapted state, because pulse of light

triggers also changes in membrane potential in the vicinity of reaction center, which affect reaction in PSII. However, at high light intensity (routinely used for estimation of  $F_v/F_m$ ), the I-P phase contributes little to the variable fluorescence and its effect on quantum yield is not substantial.

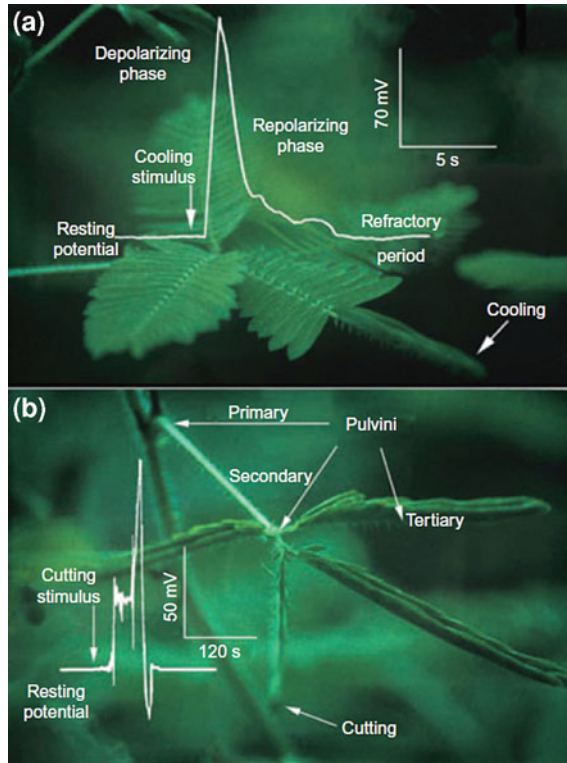
The above mentioned response to APs is direct and short term. However, it is tempting to assume that long term and AP-indirect inhibition of photosynthesis may occur in response to prey capture in Venus flytrap. Recently, Escalante-Pérez et al. (2011) found, that 30 min and 1 week after prey capture, the level of 12-oxo-phytodienoic acid (OPDA, precursor of JA) significantly increased. The increased level of OPDA is systemic and makes other traps more sensitive to mechanical irritation. Taken into account that JA inhibits photosynthesis (Herde et al. 1997), prey capture, retention and digestion may further inhibits photosynthetic assimilation even after the stop of prey moving. This will need further examination.

We can expect similar response of AP on photosynthesis also in related carnivorous plant *A. vesiculosa*; however, this plant species has not been investigated in this respect. Extensive electrical activity has also been recorded in the tentacle of carnivorous genus *Drosera*; however, the APs do not propagate to the photosynthetically active leaf lamina and have no effect on photosynthesis (Williams and Pickard 1972a, b, 1974, 1980; Williams and Spanswick 1976; Pavlovič 2010).

### 2.3.3 *Mimosa Pudica*

When the tip of leaf pinna in *M. pudica* is stimulated by cooling, touching, or electrically (Volkov et al. 2010) an AP is evoked and transmitted basipetally with a speed  $20\text{--}30\text{ mm s}^{-1}$ , what results in leaflet folding by a sudden loss of turgor pressure in the motor cells of the pulvinus (Fig. 2.6a, Fromm and Eschrich 1988c, Fromm and Lautner 2007). In contrast to *D. muscipula*, where all the major tissues of trap lobes (sensory cells of trigger hairs, upper, and lower epidermis, mesophyll cells) are excitable with similar resting potentials (150–160 mV, Hodick and Sievers 1988), excitable cells in *Mimosa* are found in vascular bundles and pulvinus (Sibaoka 1962; Samejima and Sibaoka 1983; Fromm and Eschrich 1988b). In *Mimosa*, the phloem and pulvinus is surrounded by sclerenchyma and collenchyma sheath to restrict electrical signaling to phloem and lateral propagation (Fleurad-Lessard et al. 1997; Fromm 2006; Fromm and Lautner 2007). Hoddinott (1977) found that following the mechanical stimulation and leaf folding,  $P_N$  declines by 40%. However, this declines is not caused by AP itself, but by leaf folding and reduced leaf area able to receive incident illumination. My unpublished results are in accordance with the observation that mesophyll cells of leaflets of *Mimosa* are probably not excitable in contrast to *Dionaea* and colling and touching of pinna has no inhibition effect on  $\Phi_{PSII}$  in fixed paired leaflets (in contrast to VP evoked by heating, Figs. 2.6, 2.7, 2.8).

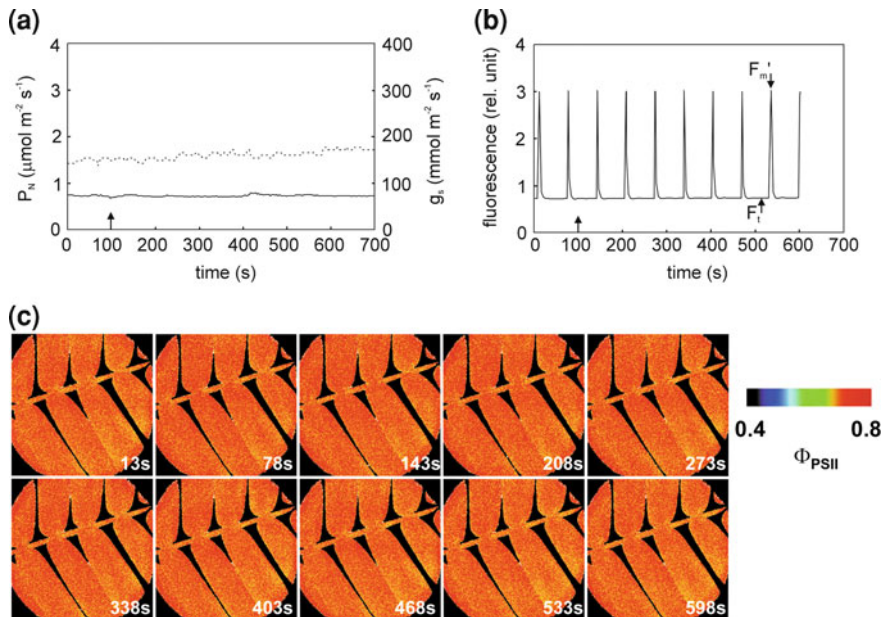




**Fig. 2.6** Electrical signaling in *M. pudica*. When the tip of a leaf pinna is stimulated by spontaneous cooling with ice water or mechanically by touch, an AP is evoked and transmitted basipetally within the rachis with the speed of  $20\text{--}30\text{ mm s}^{-1}$ . The tertiary pulvini at the base of the leaflets respond to the AP, causing ion and water fluxes that lead to leaf movements. This type of signal stops at the base of the pinna, and no further transmission occurs (a). When the leaf is stimulated by cutting or heating, a basipetally moving VP is generated in the rachis, irregular in shape, and long in duration. Its speed is slower ( $5\text{--}6\text{ mm s}^{-1}$ ) than that of the AP; however, it is able to pass through secondary pulvini at the base of the pinna and causes leaflet folding of neighbouring pinna, and also bending of the primary pulvinus at the base of the petiolus (from Fromm and Lautner 2007, courtesy of prof. Jörg Fromm and John Wiley and Sons) (b)

### 2.3.4 Other Plant Species

APs are not confined only to the carnivorous and sensitive plants but occur in all green plants around us. Whereas in *Chara* and *Dionaea* the APs generated by electrical or mechanical stimulation, respectively, had inhibition effect on photosynthesis, several other examples have been documented with no or even stimulatory effect on photosynthesis (Fromm and Fei 1998; Lautner et al. 2005; Grams et al. 2007). Such contrasting response is difficult to explain, but may be based on amplitude or on restriction of AP propagation mainly to phloem, without reaching

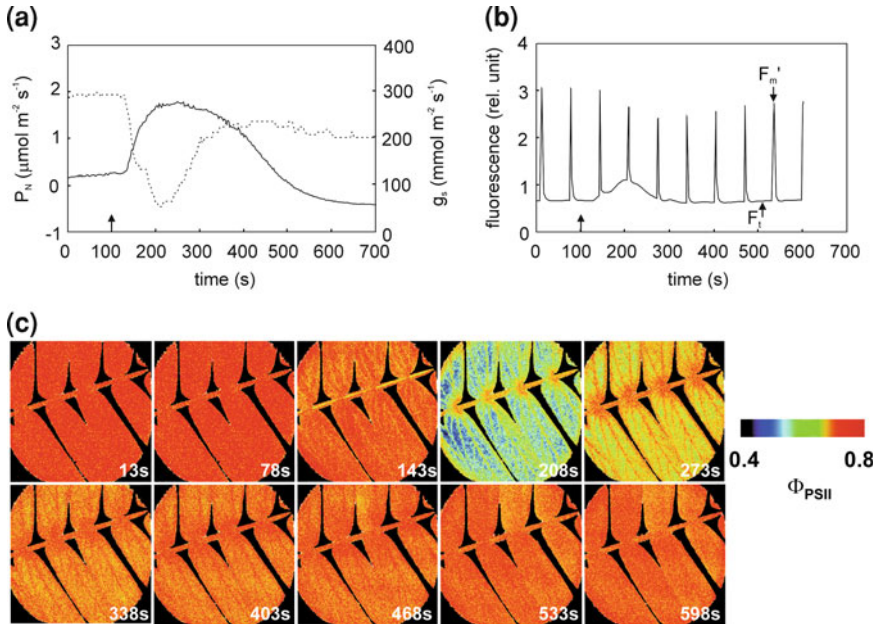


**Fig. 2.7** Photosynthetic response of *M. pudica* to cooling. The tip of pinna was cooled by ice water at time 100 s while the basal part of the same pinna was enclosed in gas exchange cuvette. Rate of net photosynthesis ( $P_N$ , dashed line), stomatal conductance ( $g_s$ , solid line) (a), trace of chlorophyll fluorescence (b), and spatio-temporal changes of effective photochemical quantum yield of PSII ( $\Phi_{\text{PSII}}$ ) calculated from fluorescence trace (c). Arrow on x-axis indicates the time of cooling.  $F_m'$ —maximum fluorescence in the light after saturation pulse ( $4,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR),  $F_t$ —steady-state fluorescence at light intensity  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR

the mesophyll cells (no response), or stimulation of stomata opening in drought stress plants with subsequent increase of intercellular  $\text{CO}_2$  concentration ( $c_i$ ) for photosynthesis and thus stimulation of photosynthesis.

Stimulation of leaf or stem with ice water in poplar tree resulted in generation of typical APs with amplitude around 25 mV in phloem (measured using aphid stylet); however, no photosynthetic response in leaves was found (Lautner et al. 2005). After watering the roots of maize in drying soil, an AP with average amplitude of 50 mV was evoked and measured on the leaf surface and phloem. At the same time the  $P_N$  and  $g_s$  began to rise. Application of dye solution and microscopic observations revealed that changes in gas exchange were not triggered by water ascent but by AP (Fromm and Fei 1998). After watering the plant, VPs are also often generated as a result of pressure increase in the xylem (Stahlberg and Cosgrove 1997; Mancuso 1999). To eliminate the effect of hydraulic signals, Grams et al. (2007) applied compensatory pressure to the root system of maize and found similar effect of AP on  $P_N$  and  $g_s$  as Fromm and Fei (1998). It is interesting that  $\Phi_{\text{PSII}}$  did not change significantly. Similarly, application of auxin (IAA) and cytokinins triggered AP and 3 min later the  $P_N$  and  $g_s$  increased in willow (*Salix*





**Fig. 2.8** Photosynthetic response of *M. pudica* to heating by flame. The tip of pinna was heated by flame at time 100 s while the basal part of the same pinna was enclosed in gas exchange cuvette. Rate of net photosynthesis ( $P_N$ , dashed line), stomatal conductance ( $g_s$ , solid line) (a), trace of chlorophyll fluorescence (b), and spatio-temporal changes of effective photochemical quantum yield of PSII ( $\Phi_{\text{PSII}}$ ) calculated from fluorescence trace (c). Arrow on x-axis indicates the time of heating.  $F_m'$ —maximum fluorescence in the light after saturation pulse ( $4,000 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR),  $F_t$ —steady-state fluorescence at light intensity  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR

*viminalis*, Fromm and Eschrich 1983). Authors believe that the response is too fast to be explained by moving a signaling substance to the leaves via transpiration stream.

The response described above is in contrast to the response found in *D. muscipula*, where AP generation has no effect on  $g_s$  and inhibits  $P_N$  and  $\Phi_{\text{PSII}}$  (Pavlovič et al. 2010; 2011). To explain the adverse effect of APs on photosynthesis in different plant species need further examination.

## 2.4 Effect of VPs on Photosynthesis

VPs are generated in response to damaging stimuli (wounding, organ excision, flaming etc.) Many studies have suggested a chemical means of propagation in which wounding releases a chemical substance Ricca's factor, which is moved by the xylem flow causing electrical changes along the path (Ricca 1916). Published

evidences indicate that hydraulic event produced by wounding is the signal responsible for systemic induction of the VP (Malone and Stankovič 1991). The hydraulic surge precedes the electrical changes, thus VP seems to be a local response to the passage of an hydraulic wave (Stahlberg and Cosgrove 1997; Mancuso 1999). In contrast to APs, magnitude and shape of VPs vary with the intensity of the stimulus and decrease with increasing distance from the injured site, have prolonged repolarization and are able to pass through dead plant tissue (Mancuso 1999; Fromm and Lautner 2007; Yan et al. 2009). The ionic mechanism differs from APs and involves a transient inactivation of proton pumps in the plasma membrane (Stahlberg and Cosgrove 1996). Sometimes APs and VPs may be confused and in some cases their affiliation is difficult, not to mention that are often generated together (Mancuso 1999; Grams et al. 2009).

In contrast to APs, where different response to photosynthesis has been recorded, in the case of VPs only inhibition of photosynthesis has been documented. Below you will find same typical effects of VP on photosynthesis.

### 2.4.1 *Mimosa Pudica*

The best documented effect of VPs on photosynthesis is in the sensitive plants *M. pudica*. In contrast to the AP, which is generated by touching or cooling and is stopped at the base of pinna, VP generated by cutting or heating passes through the secondary pulvinus at the base of a pinna and moves into three neighboring pinna and causes a bending movement (Fig. 2.6b). Further, the VP propagates basipetally through petiolus causes a bending movement of the primary pulvinus and pulvini from distant leaves (Fromm and Lautner 2007). Heat stimulation by the flame of one pinna resulted in generation of VP, which spread with velocity of  $4.0\text{--}8.0\text{ mm s}^{-1}$  to neighboring fixed pinna where decrease of  $\Phi_{\text{PSII}}$  was recorded starting 90–170 s after heat stimulation of the first pinna. This inhibition propagated acropetally throughout the pinna. The decrease of  $\Phi_{\text{PSII}}$  has spatio-temporal distribution, as chlorophyll fluorescence imaging indicates. In the intervein area, where the photosynthetically most active mesophyll is located, the strongest response was detected, but delayed in comparison to leaflet vein, what indicates that the VP spread via the veins. After 450 s  $\Phi_{\text{PSII}}$  reached a similar level as before the stimulus. Concurrently, the  $P_N$  sharply dropped to about compensation point and  $g_s$  first rapidly increased and then declined to a lower values, indicating that stomata did not contribute to rapid suppression of  $P_N$ . When the leaflets of investigated pinna were allowed to close, the first rapid increase of  $g_s$  was not recorded (Koziolek et al. 2003). The microscopic observations and stomata aperture measurements revealed a fast stomata opening movement leading to a doubling of aperture within 1–2 min after heat stimulation and followed by a closing movements, which was completed within 4–5 min after heat treatment (Fig. 2.8; Kaiser and Grams 2006). The authors concluded that the fast opening movements is not osmotically driven but caused by a sudden loss of epidermal

turgor. The most likely explanation is that epidermal cells, similar to the pulvinar motor cells, respond to depolarization and decrease a turgor with subsequent hydropassive stomata opening. On the other hand, the second phase—stomata closure can be caused either by a decrease in guard cell turgor or a recovery of epidermal turgor. Besides direct effect of electrical signals on guard cells, it is also possible that stomata closure is triggered indirectly, by increase  $c_i$  during inhibition of  $P_N$ . To exclude the possibility that chemical signals are responsible for such rapid response in *Mimosa*, Koziol et al. (2003) determined the speed of a chemical signals by exposing the part of pinna to  $^{14}\text{CO}_2$ . Then the translocation rate of  $^{14}\text{CO}_2$  was observed.  $^{14}\text{CO}_2$  signal in neighboring pinna was detected after 12 h, confirming that chemical signals are too slow to account for the photosynthetic response.

### 2.4.2 Other Plant Species

Flame stimulation of poplar leaf evoked a VP that was recorded in stimulated as well as distant leaf. Signals moving in basipetal direction change toward negative direction (hyperpolarization), which contrasts with the acropetally traveling signals (depolarization). Application of  $\text{K}^+$  channel by  $\text{TEA}^+$  resulted in inhibition of basipetally transmitted signals, indicating that  $\text{K}^+$  efflux caused the hyperpolarization of the plasma membrane. Both, hyperpolarization as well as depolarization had significant effect on photosynthesis. Flaming of the leaf caused substantial decrease of  $\Phi_{\text{PSII}}$  after 80 s at a distance 3 cm from stimulated leaf tip as well as in distant leaf after 240 s. In stimulated leaf,  $\Phi_{\text{PSII}}$  decreased in veins and intervein regions simultaneously, in distant leaf the response was delayed by about 60 s in intervein regions in comparison to veins, indicating that long-distance signaling occurs through the veins. Rate of net photosynthesis decreased; however,  $g_s$  was not affected in stimulated and distant leaf. Calcium deficiency, as well as chilling the stem with ice water, disrupted VP transition and photosynthetic response, indicating the direct role of electrical signaling in regulation of photosynthesis (Lautner et al. 2005).

Hlaváčková et al. (2006) measured gas exchange and chlorophyll fluorescence induction curves on the fifth leaf after local burning of the sixth leaf in tobacco. The VPs were evoked with amplitude 10–25 mV which preceded the response in photosynthetic parameters in distant leaf. Five minutes following the burning of the sixth leaf, the  $g_s$  and  $P_N$  started to decline in fifth leaf and continued for an hour after burning. No changes in  $c_i$  were observed indicating that reduction of  $\text{CO}_2$  concentrations due to stomatal closure were balanced by reduction of  $P_N$ . No changes in O-J-I-P transients were recorded, indicating that local burning did not influence the linear electron transport in PSII directly. Within 8 min after burning, ABA concentration increased and then decreased between 16 and 60 min in fifth leaf. However, JA concentration increased after 15 min of local burning and concentration of salicylic acid (SA) concentration did not change. It is known that

both ABA and JA induce stomata closure and inhibition of photosynthesis (Herde et al. 1997). Because electrical signals VP precede the systemic increase in the endogenous concentration of ABA and JA, Hlaváčková et al. (2006) believe that electrical signals (induced by propagating hydraulic signals) first triggered stomata closure directly by ionic fluxes across the membrane of the guard cells with some participation of chemical signals ABA and JA may play a role in the continuing process of stomatal closure in the second phase. However, the signaling pathway leading from electrical signals to the accumulation of ABA and JA is unknown. The external application of an electrical current was able to initiate systemic ABA and JA accumulation, indicating the involvement of ion fluxes in triggering hormonal response (Herde et al. 1996, 1999b). Then ABA may have a stimulatory effect on JA accumulation (Peña-Cortéz et al. 1995), but local burning can apparently activate JA biosynthesis independently (Herde et al. 1996, 1999b). These results provide confirming evidences that electrical and chemical signals interactively participate in photosynthetic responses to local burning (Hlaváčková et al. 2006).

Inhibition of  $P_N$  after heat treatment in tomato plants is accompanied also by changes in pigment composition. Increase in zeaxanthin and decrease of violaxanthin concentrations 5 h after heat treatment indicates involvement of xanthophyll cycle in deexcitation of excess absorbed light by NPQ and protection of photosynthetic apparatus against photoinhibition. No changes in chlorophyll concentrations were found confirming that such photoprotection is sufficient and no photo-bleaching occurred (Herde et al. 1999a).

VP and AP are often generated together, but AP usually precedes the VP due to the faster rate of propagation, not to mention that the changes in membrane potentials referred as AP by some authors may be interpreted as VP by other researchers (Stahlberg and Cosgrove 1997; Mancuso 1999). Grams et al. (2009) were not able to affiliate electrical signals evoked by heating in maize leaf. Heat-induced electrical signals are usually VP; however, Grams et al. (2009) reported short repolarization period similar to AP. Also Pyatygin et al. (2008) reported generation of APs in response to burning in 2-week-old pumpkin seedlings. Heat stimulation of the leaf tip of maize plants resulted in transient decline of  $P_N$  in central part of leaf lamina. While  $P_N$  declined,  $g_s$  first rapidly increased and then declined. The changes in fluorescence yield were observed by simultaneous measurements of quantum yields of PSI (excitation at  $\lambda = 715$  nm) and PSII (excitation at  $\lambda = 440$  nm). Because of chloroplast dimorphism in  $C_4$  plants with bundle sheath chloroplast lacking grana and PSII, simultaneous measurements of PSI and PSII chlorophyll fluorescence may provide further evidence that the electrical signals propagate via the veins. And this was confirmed, heat stimulation affected quantum yield of PSI before PSII. Simultaneously pH was measured using a microprobe. Acidification of cytoplasm and alkalization of apoplast during propagation of electrical signals may trigger the photosynthetic response, as isolated chloroplast showed strong dependence of  $\Phi_{PSII}$  on pH (Grams et al. 2009).

Current injection and mechanical wounding resulted in two major responses in tobacco and potato plants: a transient stomatal closure within 2–3 min and a more pronounced closure at 10 min with simultaneous decrease in  $P_N$ . The membrane potential kinetics resembles the gas exchange kinetics (Peña-Cortéz et al. 1995; Herde et al. 1998). The authors suggested that the fast response is triggered by AP and the second more pronounced response is triggered by VP and subsequently by plant hormones (ABA, JA).

## 2.5 Possible Mechanism Underlying Photosynthetic Limitation upon Impact of Electrical Signals

Without a doubt, the electrical signals have significant impact on photosynthesis. Whereas the VPs have usually uniform negative impact on  $P_N$ , the response of APs range from stimulation, no response to inhibition of photosynthesis. With the current stage of knowledge it is difficult to explain such different reaction in response to APs, especially when it is believed that AP in plants is a nonspecific bioelectric signal which does not carry any stimulus-specific information, but it gives a signal to resting tissue and organs about the onset of adverse conditions in some region (Pyatygin et al. 2008). On the other hand, some results strongly suppose the view that different stimulus-dependent APs cause specific response (Fromm et al. 1995). The different responses to AP may lay in the restriction of AP propagation in phloem without reaching the mesophyll cells (no response), or stimulation of opening closed stomata and thus increase of  $P_N$  (e.g. in drought-stressed plants), but this will need further examination. In contrast, this is not the case in VPs which have ability the spread even over dead plant region (Mancuso 1999), thus there is no restriction in propagation and only the negative impact on photosynthesis has been recorded. It can be suggested that AP and VP may have similar mechanism of action on photosynthesis, although both signals depends on different ion translocation. Whereas the APs depends on  $Ca^{2+}$ ,  $Cl^-$  and  $K^+$  flows, the VPs depends on  $H^+$  flow (Mancuso 1999; Fromm and Lautner 2007). However, active transport of  $H^+$  across the plasma membrane could also play an essential role in generation of APs (Opritov et al. 2002; Bulychev and Kamzolkina 2006a, b) and may be responsible for downregulation of photosynthetic assimilation. The chlorophyll fluorescence trace in response to AP and VP is similar (compare Figs. 2.4d and 2.8b). Thus the mechanism underlying photosynthetic limitation on impact of AP and VP is may be similar, dependent on changes in pH in cytoplasm (Grams et al. 2009) although the role of  $Ca^{2+}$  can not be excluded. Taken together, the negative impact on photosynthesis is predominant and further I try to outline the possible mechanism underlying photosynthetic limitation on impact of electrical signals.

The first barrier in the pathway of  $CO_2$  molecules into the leaf is stomata aperture. In most cases VPs induce stomata closure (Koziolek et al. 2003;

Hlaváčková et al. 2006; Kaiser and Grams 2006; Grams et al. 2007), but may also induce rapid hydropassive stomata opening in fixed leaflets of *Mimosa*; however, fixed leaflets are not typical for plants in natural habitat (Koziolek et al. 2003; Kaiser and Grams 2006). No response was also recorded (Lautner et al. 2005). On the other hand, APs have no effect on  $g_s$  (Pavlovič et al. 2010, 2011) or even induce stomata opening (Fromm and Fei 1998; Grams et al. 2007). Kaiser and Grams (2006) explained such different response by either the decrease of epidermal cells turgor (similar to the pulvinar motor cells) and subsequent rapid hydropassive stomata opening or by decrease of guard cell turgor and subsequent stomata closure. Stomata closure is triggered by ionic fluxes across the plasma membrane of guard cells and the ionic channels participating in this process are activated by membrane depolarization and increase  $Ca^{2+}$  concentration (Hlaváčková et al. 2006). Since guard cells are sensitive to  $CO_2$  concentration and APs induce increase in  $c_i$  (Pavlovič et al. 2010, 2011) it can not be excluded that stomata closure is driven indirectly by increase  $CO_2$  concentration (Kaiser and Grams 2006). Nevertheless, it seems that stomata limitation of photosynthesis does not occur, because  $c_i$  is usually constant during the course of stomata closure (Hlaváčková et al. 2006). The plant hormones (ABA, JA) play later also indispensable role in stomata closure (see discussion in Sect. 2.4.2., Hlaváčková et al. 2006).

It has been hypothesized that the primary target of electrical signals on photosynthesis is in dark reactions (Bulychev and Kamzolkin 2006a, b; Grams et al. 2009; Pavlovič et al. 2011); however, some authors do not exclude direct interference of electron transport chains through direct impact by the electrical signals (Koziolek et al. 2003). It seems that both sites are targeted by electrical signals, but the major impact is in the dark reaction of photosynthesis (Pavlovič et al. 2011). It has been hypothesized that changes in  $Ca^{2+}$  or  $H^+$  concentration are involved in inhibition of photosynthesis. For example, a key Calvin cycle enzyme Fru-1,6-bisphosphatase, is  $Ca^{2+}$  dependent enzyme inhibited by high  $Ca^{2+}$  concentration in chloroplastidic stroma (Hertig and Wolosiuk 1983; Kreimer et al. 1988). During a day, the light-stimulated  $Ca^{2+}/H^+$  antiporter pumps  $Ca^{2+}$  from stroma into the thylakoid lumen (where is necessary for OEC assembly) and prevents  $Ca^{2+}$ -mediated inhibition of  $CO_2$  fixation in the light (Ettinger et al. 1999; Sai and Johnson 2002). It is possible that  $Ca^{2+}$  efflux into the cytoplasm during AP may disrupt these fluxes which maintain low stroma  $Ca^{2+}$  concentration, and thus inhibit the enzymatic reactions of photosynthesis. However, Grams et al. (2009) found no changes in  $\Phi_{PSII}$  with increasing  $Ca^{2+}$  concentration in external solution in isolated chloroplasts, but the  $\Phi_{PSII}$  was sensitive to changes in pH. They supposed that, for example, carbonic anhydrase, a pH dependent enzyme, may be strongly involved in photosynthetic limitation. However, one may suppose that changes in pH are caused by the increase in  $Ca^{2+}$  level in cytoplasm. This rise may initiate the reaction sequences that perturb  $H^+$  flows across the plasma membrane (Bulychev and Kamzolkin 2006b). Whatever ion ( $Ca^{2+}$  or  $H^+$ ) is responsible for altering the photosynthetic reactions its major primary target seems is in enzymatic reaction of photosynthesis. This is supported by observations, that first traffic jam

of electrons is generated and increase excitation pressure at PSII (1–qP) occurred, and later acidification of thylakoid lumen triggered NPQ and subsequently the excess excitation energy is quenched by harmless manner (Fig. 2.5, Pavlovič et al. 2011).

Besides dark reaction of photosynthesis, the electrical signals also interfered with photochemical reaction in thylakoid membrane. The changes in ionic environment and photoelectrochemical field may influence the charge separation and recombination reactions in PSII (Dau and Sauer 1991, 1992; Meiburg et al. 1983; Pavlovič et al. 2011). In comparison to dark enzymatic reactions, this effect seems to be rather minor than substantial; however, provide important evidence that variable chlorophyll fluorescence is under photoelectrochemical and photoelectrical control (Vredenberg and Bulychiev 2002, 2010; Vredenberg 2004; Pavlovič et al. 2011).

## 2.6 Effect of Electrical Signalling on Respiration

Effect of electrical signalling on respiration has been much less studied than effect on photosynthesis. Since photosynthesis and respiration occur simultaneously, gas exchange measurements can not partition between these two processes. Carbon dioxide flux in gas exchange system in the light corresponds to net photosynthesis ( $P_N$ ): the rate of  $\text{CO}_2$  fixation during photosynthesis (gross photosynthesis,  $P_G$ ) minus the rate of  $\text{CO}_2$  simultaneously lost during respiration ( $R_D$ ). However, by measuring the gas exchange in response to electrical signals in the dark, it is possible to determine the  $\text{CO}_2$  lost by respiration. Figure 2.4a, b document that the  $R_D$  is the major contributor to the  $\text{CO}_2$  lost in response to APs at least in Venus flytrap ( $R_D$  before stimulus in the dark is  $\sim 9 \text{ nmol CO}_2 \text{ g}^{-1} \text{ DW s}^{-1}$  and after stimulus at low light intensities dropped to  $\sim 50 \text{ nmol CO}_2 \text{ g}^{-1} \text{ DW s}^{-1}$ , and even to  $\sim 60 \text{ nmol CO}_2 \text{ g}^{-1} \text{ DW s}^{-1}$  in the dark, indicating rapid stimulation of  $R_D$ ). This was also documented in the work published by Pavlovič et al. (2010, 2011). This documents the importance of using chlorophyll fluorescence technique in studying the effect of electrical signals on photosynthesis, because only changes in chlorophyll fluorescence indicate that besides  $R_D$  also  $P_N$  is affected. The separated tentacles of carnivorous *Drosera prolifera* showed many times greater  $R_D$  in comparison with photosynthetic lamina. If we take into account extensive electrical activity in *Drosera* tentacles (Williams and Pickard 1972a, b, 1974, 1980; Williams and Spanswick 1976), it is tempting to assume that the electrical irritability is responsible for such high  $R_D$  (Adamec 2010).

Besides Venus flytrap, rapid increase of  $R_D$  was documented in *Conocephalum conicum* in response to cut or electrical stimulus (Dziubinska et al. 1989). Filek and Kościelniak (1997) showed increased shoot  $R_D$  in response to wounding the roots by high temperature in *Vicia faba*. The response was hindered by local cooling of the stem or by treatment with sodium azide which blocked the change in the electric potential. Fromm et al. (1995) found increased  $R_D$  in response to



stigma stimulation by pollen but decreased  $R_D$  in response to cooling and heating in *Hibiscus* plant. It is interesting that pollen as well as cooling trigger APs (heating triggered VPs), which have different effect on  $R_D$ . This indicates that stimulus-dependent electrical signals cause specific responses in ovarian metabolism. Application of killed pollen, which was heated at 150°C or pollen of other species trigger no electrical signal and did not affect  $R_D$ . Ten minutes after pollination the amount of ATP increased by 12% but cold shocked and wounded flowers have lower concentration of ATP by 19 and 22%, respectively. This is consistent with the data from respiration measurements. Moreover starch level increased 15-fold after pollination, indicating that female reproductive system prepares for the following fertilization by increasing its metabolism.

Propagation of electrical signals in plants is costly. In *M. pudica*, the ATP level is much higher in pulvini than in the tissue between pulvini and stimulation causes consumption of ATP with simultaneous increase in ADP content (Lyubimova et al. 1964; Fromm and Eschrich 1988b). During the 1–3 s required for trap closure in *D. muscipula* 29% of ATP is lost (Jaffe 1973; Williams and Bennett 1982). Fromm and Eschrich (1988b) concluded that ATP is consumed for extrusion of positive charges during reestablishing or original resting potential and this is associated with the increase of  $R_D$  found by Dziubińska et al. (1989). Increase cellular level of ADP after AP may stimulate  $R_D$ . ADP initially regulates the rate of electron transport, which in turn regulates citric acid cycle activity, which, finally, regulates the rate of the glycolytic reactions. Thus, plant  $R_D$  is controlled from the “bottom up” by the cellular level of ADP (for overview see Taiz and Zeiger 2002), concentration of which is increased after APs.

## 2.7 Conclusions

Although plants have never developed the same degree of neuronal network complexity as in animals, electrical signaling plays an important role in their life. Numerous physiological responses of plant excitation have been reported (for review see Fromm and Lautner 2007) which help the plants survive in changing environment. If you look at carnivorous Venus flytrap growing in poor soil catching its prey rich in nutrients, without doubt you must think that electrical signaling is beneficial for plants (up to 75% of leaf nitrogen is taken up from insect prey, Schulze et al. 2001). Regarding carbon metabolism, we can just speculate whether the effect of electrical signals on photosynthesis and respiration has some purpose or it is just a negative consequence of ion interaction. Soma data may support the view about the usefulness of electrical signals in communication regarding photosynthesis (e.g. stomata opening after irrigation of roots in water-stressed plants, Fromm and Fei 1998; Grams et al. 2007) other seems to be without any meaning (e.g. inhibition of photosynthesis in response to trigger hair irritation in carnivorous plant Venus flytrap, Pavlovič et al. 2010, 2011). All three processes, photosynthesis, respiration and plant cell excitability, are dependent on ion fluxes. Therefore, it is tempting to



assume that ion fluxes during propagation of electrical signals may interfere with electron transport chain directly by repulsion of charges or indirectly by changes in ionic environment and thus affecting enzymatic activity. Electrical signals are also costly in term of consumption of ATP and the changes of the level of this important molecule may regulate photosynthesis and respiration.

Further studies would be directed towards better understanding the mechanisms underlying photosynthetic response, i.e., which ion triggers the response, what are the primary targets of electrical signals on metabolism, how can different electrical signals triggered different response (e.g. stimulation vs. inhibition of photosynthesis and respiration). Recent studies also reveal that interference between electrical signals and photosynthesis is suitable model for studying the photoelectrochemical and photoelectrical component of variable chlorophyll a fluorescence—a parameter which is widely used in plant physiology. Further studies are needed for complex understanding of electrical signal-mediated response on the light and dark reaction of photosynthesis.

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## References

- Adamec L (2010) Dark respiration of leaves and traps of terrestrial carnivorous plants: are there greater energetic costs in traps. *Cent Eur J Biol* 5:121–124
- Affolter JM, Olivo RF (1975) Action potentials in Venus's-flytraps: long term observations following the capture of prey. *Am Midl Nat* 93:443–445
- Beilby MJ (2007) Action potential in charophytes. *Int Rev Cytol* 257:43–82
- Blankenship RE (2002) Molecular mechanisms of photosynthesis. Blackwell Science, MPG Books Ltd, Cornwall
- Bulychev AA, Kamzolkina NA (2006a) Differential effects of plasma membrane electric excitation on  $H^+$  fluxes and photosynthesis in characean cells. *Bioelectrochemistry* 69:209–215
- Bulychev AA, Kamzolkina NA (2006b) Effect of action potential on photosynthesis and spatially distributed  $H^+$  fluxes in cells and chloroplasts of *Chara corallina*. *Russ J Plant Physiol* 53:1–9
- Bulychev AA, Vredenberg WJ (1999) Light-triggered electrical events in the tylakoid membrane of plant chloroplast. *Physiol Plant* 105:577–584
- Bulychev AA, Cherkashin AA, Rubin AB, Vredenberg VS, Zykov VS, Mueller SC (2001) Comparative study on photosynthetic activity of chloroplasts in acid and alkaline zones of *Chara corallina*. *Bioelectrochemistry* 53:225–232
- Bulychev AA, Kamzolkina NA, Luengviriya J, Rubin AB, Müller SC (2004) Effect of a single excitation stimulus on photosynthetic activity and light-dependent pH banding in *Chara* cells. *J Membr Biol* 2:11–19
- Burdon-Sanderson JS (1873) Note on the electrical phenomena which accompany stimulation of the leaf of *Dionaea muscipula*. *Proc R Soc* 21:495–496
- Dau H, Sauer K (1991) Electrical field effect on chlorophyll fluorescence and its relation to photosystem II charge separation reactions studied by a salt-jump technique. *BBA-Bioenerg* 1098:49–60
- Dau H, Sauer K (1992) Electrical field effect on the picosecond fluorescence of photosystem II and its relation to the energetics and kinetics of primary charge separation. *BBA-Bioenerg* 1102:91–106

- Davies E, Schuster A (1981) Intercellular communication in plants: evidence for a rapidly generated, bidirectionally transmitted wound signal. *Proc Nat Acad Sci USA* 78:2422–2426
- Dziubińska H, Trębacz K, Zawadski T (1989) The effect of excitation on the rate of respiration in the liverwort *Conocephalum conicum*. *Physiol Plant* 75:417–423
- Escalante-Pérez M, Krol E, Stange A, Geiger D, Al-Rasheid KAS, Hause B, Neher E, Hedrich R (2011) A special pair of phytohormones controls excitability, slow closure, and external stomach formation in the Venus flytrap. *Proc Nat Acad Sci USA* 108:15492–15497
- Ettinger WF, Clear AM, Fanning KJ, Peck ML (1999) Identification of a  $\text{Ca}^{2+}/\text{H}^{+}$  antiport in the plant chloroplast thylakoid membrane. *Plant Physiol* 199:1379–1385
- Filek M, Kościelniak J (1997) The effect of wounding the roots by high temperature on the respiration rate of the shoot and propagation of electric signal in horse bean seedlings (*Vicia faba* L. minor). *Plant Sci* 123:39–46
- Finazzi G, Johnson GN, Dall'Osto L, Joliot P, Wollman FA, Bassi R (2004) A zeaxanthin-independent nonphotochemical quenching mechanism localized in the photosystem II core complex. *Proc Nat Acad Sci USA* 101:12375–12380
- Fisahn J, Herde O, Willmitzer L, Peña-Cortés H (2004) Analysis of the transient increase in cytosolic  $\text{Ca}^{2+}$  during the action potential of higher plants with high temporal resolution: requirement of  $\text{Ca}^{2+}$  transients for induction of jasmonic acid biosynthesis and PINII gene expression. *Plant Cell Physiol* 45:456–459
- Fleurat-Lessard P, Bouché-Pillon S, Leloup C, Bonnemain JL (1997) Distribution and activity of the plasma membrane  $\text{H}^{+}$ -ATPase in *Mimosa pudica* L. in relation to ionic fluxes and leaf movements. *Plant Physiol* 113:747–754
- Fromm J (1991) Control of phloem unloading by action potentials in *Mimosa*. *Physiol Plant* 83:529–533
- Fromm J (2006) Long-distance electrical signalling and its physiological functions in higher plants. In: Volkov AG (ed) *Plant electrophysiol*. Springer, Berlin
- Fromm J, Bauer T (1994) Action potentials in maize sieve tubes change phloem translocation. *J Exp Bot* 45:463–469
- Fromm J, Eschrich W (1983) Electric signals released from roots of willow (*Salix viminalis* L) change transpiration and photosynthesis. *J Plant Physiol* 141:673–680
- Fromm J, Eschrich W (1988a) Transport processes in stimulated and non-stimulated leaves of *Mimosa pudica*: I. The movement of  $^{14}\text{C}$ -labelled photoassimilates. *Trees* 2:7–17
- Fromm J, Eschrich W (1988b) Transport processes in stimulated and non-stimulated leaves of *Mimosa pudica*: II. Energetics and transmission of seismic stimulation. *Trees* 2:18–24
- Fromm J, Eschrich W (1988c) Transport processes in stimulated and non-stimulated leaves of *Mimosa pudica*: III. Displacement of ions during seismonastic leaf movements. *Trees* 2:65–72
- Fromm J, Fei H (1998) Electrical signaling and gas exchange in maize plants of drying soil. *Plant Sci* 132:203–213
- Fromm J, Lautner S (2007) Electrical signals and their physiological significance in plants. *Plant, Cell Environ* 30:249–257
- Fromm J, Hajirezaei M, Wilke I (1995) The biochemical response of electrical signalling in the reproductive system of *Hibiscus* plant. *Plant Physiol* 109:375–384
- Graham JS, Hall G, Pearce G, Ryan CA (1986) Regulation of synthesis of proteinase inhibitors I and II mRNAs in leaves of wounded tomato plants. *Planta* 169:399–405
- Grams TEE, Koziolek C, Lautner S, Matyssek R, Fromm J (2007) Distinct roles of electric and hydraulic signals on the reaction of leaf gas exchange upon re-irrigation in *Zea mays* L. *Plant, Cell Environ* 30:79–84
- Grams TEE, Lautner S, Felle HH, Matyssek R, Fromm J (2009) Heat-induced electrical signals affect cytoplasmic and apoplastic pH as well as photosynthesis during propagation through the maize leaf. *Plant, Cell Environ* 32:319–326
- Herde O, Atzorn R, Fisahn J, Wasternack C, Willmitzer L, Peña-Cortés H (1996) Localized wounding by heat initiates the accumulation of proteinase inhibitor II in abscisic acid-deficient plants by triggering jasmonic acid biosynthesis. *Plant Physiol* 112:853–860

- Herde O, Peña-Cortéz H, Willmitzer L, Fisahn J (1997) Stomatal responses to jasmonic acid, linolenic acid and abscisic acid in wild-type and ABA-deficient tomato plants. *Plant, Cell Environ* 20:136–141
- Herde O, Peña-Cortéz H, Willmitzer L, Fisahn J (1998) Remote stimulation by heat induces characteristic membrane-potential responses in the veins of wild-type and abscisic acid-deficient tomato plants. *Planta* 206:146–153
- Herde O, Peña-Cortéz H, Fuss H, Willmitzer L, Fisahn J (1999a) Effects of mechanical wounding, current application and heat treatment on chlorophyll fluorescence and pigment composition in tomato plants. *Physiol Plant* 105:179–184
- Herde O, Peña-Cortéz H, Wasternack C, Willmitzer L, Fisahn J (1999b) Electric signaling and *pin2* gene expression on different abiotic stimuli depend on a distinct threshold level of endogenous abscisic acid in several abscisic acid-deficient tomato mutants. *Plant Physiol* 119:213–218
- Hertig CM, Wolosiuk RA (1983) Studies on the hysteretic properties of chloroplast fructose-1,6-bisphosphatase. *J Biol Chem* 258:984–989
- Hlaváčková V, Krcňák P, Nauš J, Novák O, Špundová M, Strnad M (2006) Electrical and chemical signals involved in short-term systemic photosynthetic responses of tobacco plants to local burning. *Planta* 225:235–244
- Hoddinott J (1977) Rates of translocation and photosynthesis in *Mimosa pudica*. *New Phytol* 79:269–272
- Hodick D, Sievers A (1988) The action potential of *Dionaea muscipula* ellis. *Planta* 174:8–18
- Hunt S (2003) Measurements of photosynthesis and respiration in plants. *Physiol Plant* 117:314–325
- Jaffe MJ (1973) The role of ATP in mechanically stimulated rapid closure of the Venus's-flytrap. *Plant Physiol* 51:17–18
- Johnson MP, Pérez-Bueno ML, Zia A, Horton P, Ruban AV (2009) The zeaxanthin-independent and zeaxanthin-dependent qE components of nonphotochemical quenching involve common conformational changes within the photosystem II antenna in *Arabidopsis*. *Plant Physiol* 149:1061–1075
- Juniper BE, Robins RJ, Joel DM (1989) The carnivorous plants. Academic, London
- Kaiser H, Grams TEE (2006) Rapid hydropassive opening and subsequent active stomatal closure follow heat-induced electrical signals in *Mimosa pudica*. *J Exp Bot* 57:2087–2092
- Koziolek C, Grams TEE, Schreiber U, Matyssek R, Fromm J (2003) Transient knockout of photosynthesis mediated by electrical signals. *New Phytol* 161:715–722
- Kreimer G, Melkonian M, Latzko E (1985) An electrogenic uniport mediates light-dependent Ca<sup>2+</sup> influx into intact spinach chloroplasts. *FEBS Lett* 180: 253–258
- Kreimer G, Melkonian M, Holtum JAM, Latzko E (1988). Stromal free calcium concentration and light-mediated activation of chloroplast fructose-1,6-bisphosphatase. *Plant Physiol* 86: 423–428
- Krupenina NA, Bulychev AA (2007) Action potential in a plant cell lowers the light requirement for non-photochemical energy-dependent quenching of chlorophyll fluorescence. *Biochim Biophys Acta* 1767:781–788
- Lautner S, Grams TEE, Matyssek R, Fromm J (2005) Characteristics of electrical signals in poplar and responses in photosynthesis. *Plant Physiol* 138:2200–2209
- Lazár D (2006) The polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light. *Funct Plant Biol* 33:9–30
- Lazár D (2009) Modelling of light-induced chlorophyll a fluorescence rise (O-J-I-P transient) and changes in 820 nm-transmittance signal of photosynthesis. *Photosynthetica* 47:483–498
- Lichtner FT, Williams SE (1977) Prey capture and factors controlling trap narrowing in *Dionaea* (Droseraceae). *Am J Bot* 64:881–886
- Lucas WJ (1975) The influence of light intensity on the activation and operation of the hydroxyl efflux system of *Chara corallina*. *J Exp Bot* 26:347–360
- Lyubimova MN, Demyanovskaya NS, Fedorovich IB, Homlenskite IV (1964) Participation of ATP in the motor function of the *Mimosa pudica* leaf. *Transl Biokhim* 29:774–779

- Malone M, Stankovič B (1991) Surface potentials and hydraulic signals in wheat leaves following localised wounding by heat. *Plant, Cell Environ* 14:431–436
- Mancuso S (1999) Hydraulic and electrical transmission of wound-induced signals in *Vitis vinifera*. *Aust J Plant Physiol* 26:55–61
- Maxwell K, Johnson GN (2000) Chlorophyll fluorescence—a practical guide. *J Exp Bot* 51:659–668
- Meiburg RF, Van Gorkom HJ, Van Dorssen RJ (1983) Excitation trapping and charge separation in photosystem II in the presence of an electrical field. *BBA-Bioenerg* 724:352–358
- Müller P, Li X-P, Niyogi KK (2001) Non-photochemical quenching. A response to excess light energy. *Plant Physiol* 125:1558–1566
- Nedbal L, Whitmarsh J (2004) Chlorophyll fluorescence imaging of leaves and fruits. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a fluorescence a signature of photosynthesis*. Springer, Dordrecht
- Oprittov VA, Pyatygin SS, Vodeneev VA (2002) Direct coupling of action potential generation in cells of a higher plant (*Cucurbita pepo*) with the operation of an electrogenic pump. *Russ J Plant Physiol* 49:142–147
- Papageorgiou GC, Govindjee (2004) *Chlorophyll a fluorescence: a signature of photosynthesis*. Springer, Dordrecht
- Pavlovič A (2010) Spatio-temporal changes of photosynthesis in carnivorous plants in response to prey capture, retention and digestion. *Plant Signal Behav* 5:1–5
- Pavlovič A, Mancuso S (2011) Electrical signalling and photosynthesis: can they co-exist together? *Plant Signal Behav* 6:840–842
- Pavlovič A, Demko V, Hudák J (2010) Trap closure and prey retention in Venus flytrap (*Dionaea muscipula*) temporarily reduces photosynthesis and stimulates respiration. *Ann Bot* 105:37–44
- Pavlovič A, Slováková L', Pandolfi C, Mancuso S (2011) On the mechanism underlying photosynthetic limitation upon trigger hairs irritation in carnivorous plant Venus flytrap (*Dionaea muscipula* ellis). *J Exp Bot* 62:1991–2000
- Peña-Cortés H, Fisahn J, Willmitzer L (1995) Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. *Proc Nat Acad Sci USA* 92:4106–4113
- Pikulencko MM, Bulychev AA (2005) Light-triggered action potentials and changes in quantum efficiency of photosystem II in *Athoceros* cells. *Russ J Plant Physiol* 52:584–590
- Plieth C, Tabrizi H, Hansen U-P (1994) Relationship between banding and photosynthetic activity in *Chara corallina* as studied by the spatially different induction curves of chlorophyll fluorescence observed by an image analysis system. *Physiol Plant* 91:205–211
- Pyatygin SS, Oprittov VA, Vodeneev VA (2008) Signaling role of action potential in higher plants. *Russ J Plant Physiol* 55:312–319
- Ricca U (1916) Soluzione d'un problema di fisiologia: la propagazione di stimulo nella *Mimosa*. *Nuovo G Bot Ital* 23:51–170
- Roháček K (2002) Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationship. *Photosynthetica* 40:13–29
- Sai J, Johnson CH (2002) Dark-stimulated calcium ion fluxes in the chloroplast stroma and cytosol. *Plant Cell* 14: 1279–1291
- Samejima M, Sibaoka T (1983) Identification of the excitable cells in the petiole of *Mimosa pudica* by intracellular injection of procion yellow. *Plan Cell Physiol* 24:33–39
- Schansker G, Tóth SZ, Strasser RJ (2005) Methylviologen and dibromothymoquinone treatments of pea leaves reveal the role of photosystem I in the chl *a* fluorescence rise. *Biochim Biophys Acta* 1706:250–261
- Schreiber U (2004) Pulse-amplitude-modulation (PAM) fluorometry and saturation pulse method: an overview. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a fluorescence: a signature of photosynthesis*. Springer, The Netherlands
- Schulze W, Schulze ED, Schulze I, Oren R (2001) Quantification of insect nitrogen utilization by the Venus flytrap *Dionaea muscipula* catching prey with highly variable isotope signatures. *J Exp Bot* 52:1041–1049

- Shiina T, Tazawa M (1986) Action potential in *Luffa cylindrica* and its effects on elongation growth. *Plant Cell Physiol* 27:1081–1089
- Sibaoka T (1962) Excitable cells in *Mimosa*. *Science* 137:226
- Sibaoka T (1991) Rapid plant movements triggered by action potentials. *Bot Mag Tokyo* 104:73–95
- Srivastava A, Guissé B, Greppin H, Strasser RJ (1997) Regulation of antenna structure and electron transport in photosystem II of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll a fluorescence transient: OKJIP. *Biochim Biophys Acta* 1320:95–106
- Stahlberg R, Cosgrove DJ (1996) Induction and ionic basis of slow wave potentials in seedlings of *Pisum sativum* L. *Planta* 199:416–425
- Stahlberg R, Cosgrove DJ (1997) The propagation of slow wave potentials in pea epicotyls. *Plant Physiol* 113:209–217
- Stanković B, Davies E (1998) The wound response in tomato involves rapid growth and electrical responses, systemically up-regulated transcription of proteinase inhibitor and calmodulin and down-regulated translation. *Plant Cell Physiol* 39:268–274
- Stolarz M, Król E, Dziubinska H, Kurenda A (2010) Glutamate induces series of action potentials and a decrease in circumnutation rate in *Helianthus annuus*. *Physiol Plant* 138:329–338
- Strasser RJ, Govindjee (1992) On the O-J-I-P fluorescence transient in leaves and D1 mutants of *Chlamydomonas reinhardtii*. In: Murata M (ed) *Research in photosynthesis*, vol 2. Kluwer Academic Publishers, Dordrecht
- Strasser RJ, Tsimilli-Michael M, Srivastava A (2004) Analysis of the chlorophyll a fluorescence transient. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a fluorescence: a signature of photosynthesis*. Springer, Dordrecht
- Taiz L, Zeiger E (2002) *Plant physiology*, 3rd edn. Sinauer Associates Inc., Publishers, MA, p 690
- Trebacz K, Sievers A (1998) Action potentials evoked by light in traps of *Dionaea muscipula* ellis. *Plant Cell Physiol* 39:369–372
- Volkov AG, Adesina T, Jovanov E (2007) Closing of Venus flytrap by electrical stimulation of motor cells. *Plant Signal Behav* 2:139–145
- Volkov AG, Adesina T, Jovanov E (2008a) Charge induce closing of *Dionaea muscipula* ellis trap. *Bioelectrochemistry* 74:16–21
- Volkov AG, Adesina T, Markin VS, Jovanov E (2008b) Kinetics and mechanism of *Dionaea muscipula* trap closing. *Plant Physiol* 146:694–702
- Volkov AG, Foster JC, Ashby TA, Walker RK, Johnson JA, Markin VS (2010) *Mimosa pudica*: electrical and mechanical stimulation of plant movements. *Plant, Cell Environ* 33:163–173
- Vredenberg WJ (2000) A three-state model for energy trapping and chlorophyll fluorescence in photosystem II incorporating radical pair recombination. *Biophys J* 79:26–38
- Vredenberg WJ (2004) System analysis and photo-electrochemical control of chlorophyll fluorescence in terms of trapping models of photosystem II: a challenging view. In: Papageorgiou GC, Govindjee (eds) *Chlorophyll a fluorescence: a signature of photosynthesis*. Springer, Dordrecht
- Vredenberg WJ (2011) Kinetic analyses and mathematical modeling of primary photochemical and photoelectrochemical processes in plant photosystems. *Biosystems* 103:138–151
- Vredenberg WJ, Bulychiev AA (2002) Photo-electrochemical control of photosystem II chlorophyll fluorescence in vivo. *Bioelectrochemistry* 57:123–128
- Vredenberg WJ, Bulychiev AA (2003) Photoelectric effects on chlorophyll fluorescence of photosystem II in vivo. Kinetics in the absence and presence of valinomycin. *Bioelectrochemistry* 60:87–95
- Vredenberg WJ, Bulychiev AA (2010) Photoelectrochemical control of the balance between cyclic- and linear electron transport in photosystem I. algorithm for P700<sup>+</sup> induction kinetics. *Biochim Biophys Acta* 1797:1521–1532
- Vredenberg WJ, Durchan M, Prášil O (2009) Photochemical and photoelectrochemical quenching of chlorophyll fluorescence in photosystem II. *Biochim Biophys Acta* 1787:1468–1478

- Wildon DC, Thain JF, Minchin PEH, Gubb IR, Reilly AJ, Skipper YD, Doherty HM, O'Donnel J, Bowles DJ (1992) Electrical signaling and systemic proteinase inhibitor induction in the wounded plant. *Nature* 360:62–65
- Williams SE, Bennett AB (1982) Leaf closure in the Venus flytrap: an acid growth response. *Science* 218:1120–1121
- Williams SE, Pickard BG (1972a) Receptor potentials and action potentials in *Drosera* tentacles. *Planta* 103:193–221
- Williams SE, Pickard BG (1972b) Properties of action potentials in *Drosera* tentacles. *Planta* 103:222–240
- Williams SE, Pickard BG (1974) Connections and barriers between cells of *Drosera* tentacles in relation to their electrophysiology. *Planta* 116:1–16
- Williams SE, Pickard BG (1980) The role of action potentials in the control of capture movements of *Drosera* and *Dionaea*. In: Skoog F (ed) *Plant growth substances*. Springer, Berlin
- Williams SE, Spanswick RM (1976) Propagation of the neuroid action potential of the carnivorous plant *Drosera*. *J Comp Physiol* 108:211–223
- Yan X, Wang Z, Huang L, Wang C, Hou R, Xu Z, Qiao X (2009) Research progress on electrical signals in higher plants. *Prog Nat Sci* 19:531–541



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