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RESEARCH PAPER

On the mechanism underlying photosynthetic limitation upon trigger hair irritation in the carnivorous plant Venus flytrap (*Dionaea muscipula* Ellis)

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Abstract

Mechanical stimulation of trigger hairs on the adaxial surface of the trap of *Dionaea muscipula* leads to the generation of action potentials and to rapid leaf movement. After rapid closure secures the prey, the struggle against the trigger hairs results in generation of further action potentials which inhibit photosynthesis. A detailed analysis of chlorophyll *a* fluorescence kinetics and gas exchange measurements in response to generation of action potentials in irritated *D. muscipula* traps was used to determine the 'site effect' of the electrical signal-induced inhibition of photosynthesis. Irritation of trigger hairs and subsequent generation of action potentials resulted in a decrease in the effective photochemical quantum yield of photosystem II (Φ_{PSII}) and the rate of net photosynthesis (A_N). During the first seconds of irritation, increased excitation pressure in photosystem II (PSII) was the major contributor to the decreased Φ_{PSII}. Within ~1 min, non-photochemical quenching (NPQ) released the excitation pressure at PSII. Measurements of the fast chlorophyll *a* fluorescence transient (O-J-I-P) revealed a direct impact of action potentials on the charge separation–recombination reactions in PSII, although the effect seems to be small rather than substantial. All the data presented here indicate that the main primary target of the electrical signal-induced inhibition of photosynthesis is the dark reaction, whereas the inhibition of electron transport is only a consequence of reduced carboxylation efficiency. In addition, the study also provides valuable data confirming the hypothesis that chlorophyll *a* fluorescence is under electrochemical control.

Key words: Action potential, carnivorous plant, chlorophyll *a* fluorescence, *Dionaea muscipula*, electrical signal, O-J-I-P, photosynthesis, respiration.

Introduction

The endemic carnivorous plant Venus flytrap (*Dionaea muscipula* Ellis) produces a rosette of leaves, each divided into two parts: the lower part called the lamina and the upper part called the trap. The trap catches prey by very rapid movement of its bilobed halves that shut when the trigger hairs protruding from the upper leaf epidermis are stimulated by touch. At room temperature, two touches activate the trap, which snaps shut in a fraction of second (Juniper *et al.*, 1989). At higher temperature (35–40 °C) only one stimulus is required for trap closure (Brown and Sharp, 1910). The stimulation of trigger hairs activates mechanosensitive ion channels and generates a receptor

Abbreviations: 1–qP, excitation pressure in photosystem II; ΔG_0, Gibbs free energy difference; Φ_{PSII}, effective photochemical quantum yield of photosystem II; A_G, rate of gross photosynthesis; A_N, rate of net photosynthesis; C_i, intercellular CO_2 concentration; DW, dry weight; F_0, minimal fluorescence in the dark-adapted state; F_m, maximal fluorescence in the dark; F'_m, maximal fluorescence in the light; F_t, steady-state fluorescence in the light; F_v, variable fluorescence; F_v/F_m, maximum quantum yield of photosystem II photochemistry; g_s, stomatal conductance; NPQ, non-photochemical quenching; PAR, photosynthetically active radiation; Pheo, pheophytin; PSII, photosystem II; Q_A, plastoquinone A; Q_B, plastoquinone B; qE, energy state quenching; R_D, rate of respiration; Y^+_Z, secondary electron donor of photosystem II.

potential, which induces an action potential. Electrical signals are the immediate cause of the trap movements irrespective of the way in which the signal is triggered; for example by mechanical stimulation or by electrostimulation (Volkov *et al.*, 2007, 2008*a*, *b*, *c*, 2009*a*, *b*). In animals, the ionic mechanism of the action potential of axons depends on inward-flowing Na^+ (depolarization) and outward-flowing K^+ ions (repolarization), whereas the excitation of plant cells depends on Ca^{2+}, Cl^-, and K^+ ions (Fromm and Lautner, 2007). The action potentials in *Dionaea* have been extensively studied (e.g. Burdon-Sanderson, 1873; Affolter and Olivo, 1975; Hodick and Sievers, 1986, 1988, 1989; Sibaoka, 1991; Trebacz and Sievers, 1998; Krol *et al.*, 2006; Volkov *et al.*, 2007, 2008*a*, *b*, *c*, 2009*a*, *b*). They propagate from mechanosensitive trigger hairs of the lobe to the trap midrib, more rapidly across the lower (abaxial) surface than across the upper one, while they are not recorded in adjacent lamina (Burdon-Sanderson, 1873; Burdon-Sanderson and Page, 1876; Williams and Pickard, 1980; Volkov *et al.*, 2007). Volkov *et al.* (2007) found that the generated action potential had a duration of 1.5 ms and a velocity of 10 m s^{-1}. Trigger hair-induced generation of action potentials is not associated only with trap closure. The struggling of the entrapped prey in the closed trap results in generation of further action potentials which cease to occur when the prey stops moving. Over 100 action potentials were recorded in the trap with prey in the first 2 h and the mechanical stimulation triggered secretion of digestive fluid (Affolter and Olivo, 1975; Lichtner and Williams, 1977). In a previous study it was shown that repeated irritation of trigger hairs temporarily reduced the rate of photosynthesis (A_N) and the effective photochemical quantum yield of photosystem II (Φ_{PSII}), and stimulated the rate of respiration (R_D) in the traps but not in the adjacent laminae (Pavlovič *et al.*, 2010). These findings are not surprising because the inhibitory effect of electrical signals on A_N and Φ_{PSII} has also been well documented in non-carnivorous plants (Koziolek *et al.*, 2003; Lautner *et al.*, 2005; Bulychev and Kamzolkina, 2006*a*, b; Hlaváčková *et al.*, 2006; Kaiser and Grams, 2006; Fromm and Lautner, 2007; Krupenina and Bulychev, 2007, 2008; Grams *et al.*, 2009). However, the exact mechanism underlying the photosynthetic limitation caused by electrical signals is not yet known. It is difficult to conclude whether the changes in Φ_{PSII}, which measures the proportion of light absorbed by chlorophyll associated with PSII that is used in photochemistry (for definition, see Genty *et al.*, 1989; Maxwell and Johnson, 2000), are the reason for or just a consequence of decreased carboxylation efficiency. In fact, reduced carboxylation efficiency decreases Φ_{PSII}, which prevents overexcitation of PSII and protects it against photoinhibition (for a review, see Kramer *et al.*, 2004). It has been proposed that subcellular alternations in ion fluxes (e.g. Ca^{2+}) and pH may be involved in the photosynthetic responses, which modify the enzymatic activities in the cytoplasm or chloroplast (Lautner *et al.*, 2005; Bulychev and Kamzolkina, 2006*a*, *b*; Krupenina and Bulychev, 2007). Bulychev and Kamzolkina (2006*a*, *b*) found that the depression of electron transport after action potentials in cells of *Chara* was largely due to non-photochemical quenching (NPQ) in PSII. Koziolek *et al.* (2003) suggest that transient knockout of photosynthesis mediated by electrical signals in *Mimosa pudica* is too fast to be a result of zeaxanthin-dependent NPQ or chemical signals, as was later proposed by Hlaváčková *et al.* (2006), and propose that the rapid decline of Φ_{PSII} might result from direct interference with electron transport chains in chloroplasts through direct impact of electrical signals. In addition, a direct effect of the electrical field on charge separation and recombination in the reaction centre of PSII cannot be excluded (Meiburg *et al.*, 1983; Dau and Sauer, 1991, 1992; Bulychev and Vredenberg, 1999; Vredenberg and Bulychev, 2002, 2003; Vredenberg *et al.*, 2009). With the present state of knowledge it is still difficult to conclude what is the 'site effect' of electrical signal-induced inhibition of photosynthesis.

Here a detailed analysis of chlorophyll *a* fluorescence kinetics simultaneously with gas exchange measurements is provided during irritation of trigger hairs, which induce the generation of action potentials in *D. muscipula.* First, the relationship between electrical signals and chlorophyll fluorescence in the light at atmospheric CO_2 concentration was examined. In the second experiment, the Calvin cycle reactions were inhibited by lowering the CO_2 concentration to zero, while electrons still move on alternative electron acceptors, allowing determination of whether electrical signals have a direct impact on the electron transport chain. In the dark, the maximum quantum yield of PSII (F_v/F_m) together with fast chlorophyll fluorescence induction kinetics (O-J-I-P), reflecting the filling up of the PSII electron acceptor plastoquinone pools Q_A and Q_B, were measured in the presence of electrical signals. The main aim of the present study is to answer to the following question. Are the primary targets of electrical signal-induced inhibition dark or light reactions of photosynthesis?

Materials and methods

Plant culture and experimental set-up

Twenty 3- or 4-year-old *D. muscipula* J. Ellis plants were grown in a growth chamber at an irradiance of 150 µmol m^{-2} s^{-1} photosynthetic active radiation (PAR) and a 14/10 h light/dark period, in well-drained peat moss in plastic pots irrigated with distilled water. The trap was closed by mechanical stimulation and the leaf was cut near the base. The trap and thin wire (~0.1 mm diameter) placed in the closed trap were sealed into a leaf cuvette (PLC6, PP-systems, Hitchin, UK), which monitors CO_2 and H_2O exchange. The base of the lamina protruding outside the cuvette was submerged in distilled water in an Eppendorf tube to prevent it drying out. The trigger hairs in the closed trap in a hermetically closed cuvette were repeatedly stimulated for 15 s by moving the thin wire protruding outside. Movements of the wire in the empty closed cuvette had no effect on CO_2 and H_2O exchange, confirming that the movements of the wire had no effect on the gas-tight seal.

Simultaneous measurements of gas exchange and chlorophyll a fluorescence

Measurements of chlorophyll *a* fluorescence were performed with a Fluorcam FC-1000 LC (Photon Systems Instruments, Brno, Czech

Republic) attached to the PLC6 cuvette connected with a CIRAS-2 infrared gas analyser (PP-system, Hitchin, UK). Before each measurement the plant was dark adapted for 15 min. One leaf was cut and placed inside a PLC6 cuvette. Because the diameter of the cuvette window was 18 mm, the traps used in the experiments did not exceed this size. After 2 min, the minimal fluorescence (F_0) at a light intensity <0.1 μmol m^{-2} s^{-1} PAR was measured. Thereafter, the maximal fluorescence (F_m) was measured using a saturation pulse (light intensity 3000 μmol m^{-2} s^{-1} PAR, duration 800 ms). Then an actinic light was switched on (80 μmol m^{-2} s^{-1} PAR) and, after stabilization of the net photosynthetic rate (A_N), three saturation pulses were given every 60 s (3000 μmol m^{-2} s^{-1} PAR, 800 ms duration) for determination of the maximal fluorescence in the light-adapted state (F'_m) Afterwards the trigger hairs of the trap were mechanically stimulated by manipulation of the wire protruding outside the cuvette for 15 s. After 17 s a fourth saturation pulse was given followed by the remaining five pulses at regular 60 s intervals. In some experiments, after 17 s the actinic light was switched off and F'_0 was recorded for estimation of the excitation pressure (1–qP). Photosynthetic parameters (F_v/F_m, Φ_{PSII}, NPQ, and 1–qP) were calculated according to Maxwell and Johnson (2000). For definition of the parameters, see Roháček (2002). Simultaneously, the infrared gas analyser monitored CO_2 and H_2O exchange every 2 s at a leaf temperature of 22±1 °C, ambient CO_2 concentration of 380 μl l^{-1}, a relative air humidity of 60–70%, and a light intensity of 80 μmol m^{-2} s^{-1} PAR (red-emitting LEDs, λ=620 nm). In the second experiment the measurements were done in exactly the same way, but without CO_2 (~1 μl l^{-1}) in the cuvette. Finally, the whole experiment was performed in the dark at ambient CO_2 concentration of 380 μl l^{-1}. Changes in the CO_2 concentration in the measuring chamber were recorded with a constant delay of 9 s (the time taken for gas to pass from the cuvette to the infrared gas analyser). This delay was taken into account in figures presented here, and the bold lines on the *x*-axis were moved 9 s to the left. After measurements, the leaves were dried at 70 °C for 5 d, weighed, and the A_N and R_D were calculated in nmol CO_2 g^{-1} DW s^{-1}. Data shown are representative of a total of five independent measurements.

Measurements of fast chlorophyll a fluorescence induction kinetics (O-J-I-P)

At high excitation irradiance, dark-adapted leaves show characteristic polyphasic fluorescence kinetics with four distinct steps named O-J-I-P (for reviews, see Strasser *et al.*, 2004; Lazár, 2006). Because Vredenberg and Bulychev (2002) hypothesized that the I-P phase may be under photoelectrochemical control, the polyphasic increase in chlorophyll *a* fluorescence in the *D. muscipula* trap was measured in control (non-irritated) and irritated traps using a Fluorpen FP 100max (Photon Systems Instruments, Brno, Czech Republic). The fast increase in chlorophyll *a* fluorescence was measured over a time span of 10 μs to 1 s. Before the measurements, the trap was closed and dark adapted for 30 min. Then the fast chlorophyll *a* fluorescence induction kinetics were measured in non-irritated traps. After 30 min in the dark the same trap was stimulated by the thin wire protruding outside the trap for 15 s. After 17 s the saturation pulse was given (2000 μmol m^{-2} s^{-1} PAR, duration 1 s). The measurements were repeated three times on the same trap in the following order: control–irritated–control–irritated–control–irritated with a 30 min dark interval between measurements to ensure that the previous saturation pulse had no effect on the shape of the curves and the differences are caused by trigger hair irritation. The experiment was repeated on six traps (18 measurements altogether). Two-tailed paired *t*-test was used to find significant differences between the O, J, I, and P phases of the increase in chlorophyll *a* fluorescence (Statgraphics, Centurion XV).

Measurements of action potentials

The extracellular electrical potential was measured with an intracellular electrometer (mod. 3100, A. M. Systems, Inc., Carlsborg, WA, USA) placed inside a Faraday cage. Each cut leaf was fixed inside a small measuring chamber so that the lamina was dipped in a mild saline solution (0.1 mM $CaCl_2$, 0.5 mM KCl), while the electrodes were placed on the abaxial surface of the closed trap. A glass micropipette containing an Ag/AgCl wire and filled with 3 M KCl was mounted with a half cell holder and connected to the headstage of the probe. An identical electrode was placed in the measuring chamber to serve as a reference electrode.

The electrodes were connected to the amplifier and the signal was recorded continuously during trap stimulation at a 1 kHz rate of sampling frequency with home-made lab-view software. The trap was stimulated for 15 s and the electrical signals were collected. The action potentials were measured in the light (80 μmol m^{-2} s^{-1} PAR) at ambient CO_2 concentration, in an atmosphere without CO_2 (~1 μl l^{-1}) and in darkness. Five traps from different plants were selected for each treatment; 10 measurements were performed. The statistical differences between treatments (amplitude and number of action potentials) were evaluated by Student *t*-test (Statgraphics, Centurion XV).

Results

Repeated 15 s irritation of trigger hairs in a closed trap decreased the effective photochemical quantum yield of PSII (Φ_{PSII}), indicating that linear electron transport was inhibited. After stopping the mechanical irritation, Φ_{PSII} started to recover. The inhibition of Φ_{PSII} was confined mainly to the digestive zone of the trap (Fig. 1). The rate of net photosynthesis (A_N) also dropped sharply (Fig. 2A). This rapid inhibition resulted in a transient increase in the intercellular CO_2 concentration (C_i; Fig. 2B), while the stomatal conductance (g_s) was not affected (data not shown; see Pavlovič *et al.*, 2010). During 15 s of irritation of trigger hairs in a closed trap, 3.7±0.7 (± 1 SE) (maximum 7) action potentials with an average amplitude of 40.1±3.5 mV (maximum 80 mV) were recorded (Fig. 2A, inset). The detailed analysis of chlorophyll *a* fluorescence kinetics in the digestive zone of the trap revealed that the decrease of Φ_{PSII} is caused at first by the increase in the steady-state fluorescence in the light (F_t), whereas maximal fluorescence in the light-adapted state (F'_m) was not affected immediately after irritation (Fig. 2C). This indicates that the plastoquinone pool became more reduced. The fluorescence increase upon reduction of plastoquinone is due to a decrease in the rate of radical pair formation (forward electron transfer) and an increase in the rate of radical pair recombination (backward electron transfer). The reduced plastoquinone pool results in increased excitation pressure at the PSII reaction centre, promoting photoinhibition (increased 1–qP; Fig. 2D) which is prevented by a series of down-regulatory processes known as NPQ. Within 1 min after irritation, F_t is quenched by NPQ as indicated by the large drop in F'_m (Fig. 2C, D). F'_m was rapidly reversed in darkness, indicating that it represents the fast relaxing energy state quenching (qE) rather than photoinhibitory quenching (qI; data not shown).

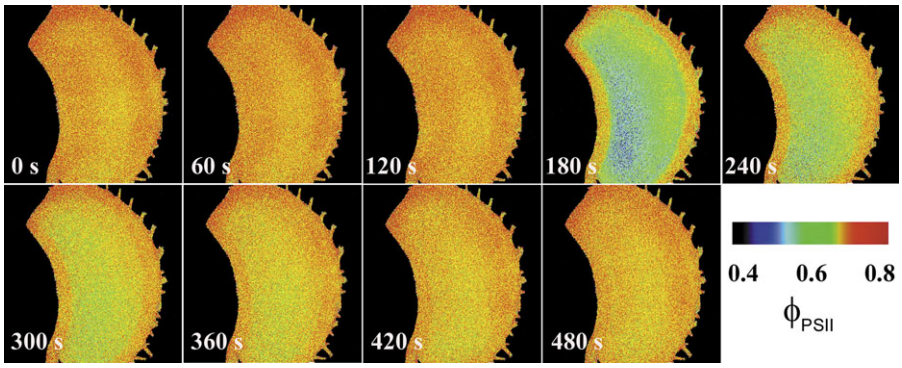

Fig. 1. Spatiotemporal changes of effective photochemical quantum yield of PSII (Φ_{PSII}) in a *D. muscipula* closed trap assessed by chlorophyll fluorescence imaging. The trap was irritated by a thin wire between 162 s and 177 s.

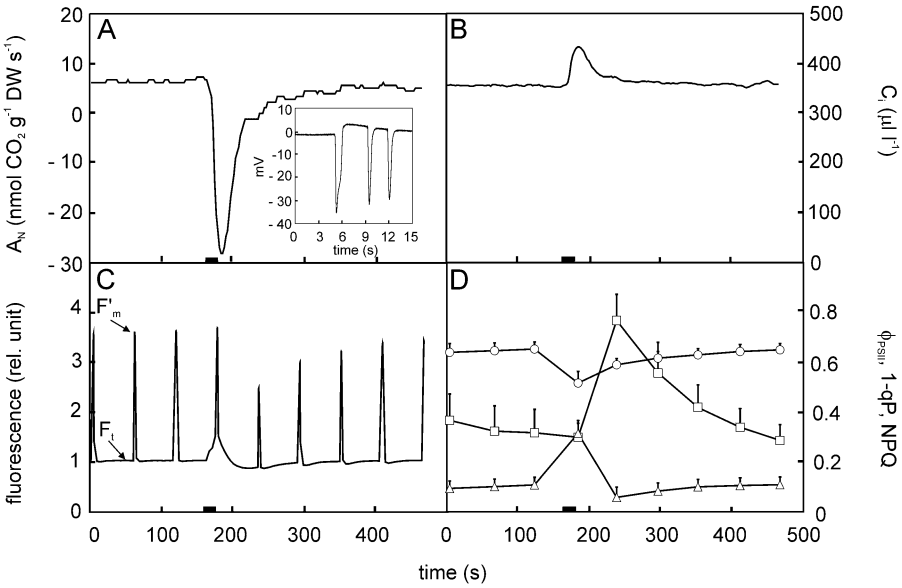

Fig. 2. Typical responses to trigger irritation in the Venus flytrap (*D. muscipula*) at a light intensity of 80 µmol m^{-2} s^{-1} PAR and an atmospheric CO_2 concentration of 380 µl l^{-1} at a leaf chamber temperature at ~22 °C. Net photosynthetic rate (A_N; A), electrical signals (A; inset), intercellular CO_2 concentration (C_i; B), chlorophyll fluorescence kinetics (C), and chlorophyll fluorescence parameters, means ±1 SE (D). Φ_{PSII}, the effective photochemical quantum yield of PSII, open circles; 1–qP, the excitation pressure, open triangles; NPQ, the non-photochemical quenching, open squares. The duration of trigger hair irritation is denoted as a bold line on the *x*-axis. Data shown are representative of a total of five measurements.

Because light and dark reactions of photosynthesis are coupled together by the production and consumption of ATP and NADPH, from the above-mentioned results it is difficult to conclude whether the decreased Φ_{PSII} is a reason for or just a consequence of reduced A_N. Transiently increased 1–qP before induction of NPQ indicates that a traffic jam of electrons in the electron transport chain occurred. This is probably due to a decreased concentration of the oxidized form of $NADP^+$ (an electron acceptor from PSI) determined by a decreased activity of the Calvin cycle. Therefore, the concentration of CO_2 was decreased, to inhibit the dark reactions of photosynthesis, allowing the electrons to move on alternative electron acceptors (e.g. O_2, activation of cyclic electron flow and photorespiration, N metabolism) to observe the direct impact of action potentials on Φ_{PSII}. The experiment in a CO_2-free atmosphere (~1 µl l^{-1}) showed the reverse effect of trigger hair irritation on Φ_{PSII}, despite the same efflux of CO_2 (Fig. 3A, B). Trigger hair irritation decreased F_t and increased F'_m, and thus Φ_{PSII} slightly increased (Fig. 3C, D). Within a few minutes the changes recovered. The absence of CO_2 had not significant effect on action potentials, as the amplitude and number of pulses were comparable with those in the previous experiment (number 3.2±0.5, P=0.545; amplitude 37.2±3.2 mV, P=0.485, Fig. 3A inset).

In the dark-adapted leaf the plastoquinone pool is oxidized—that is, the reaction centres are open and Calvin cycle enzymes are inactivated, allowing estimatation of the maximal photochemical activity of PSII (F_v/F_m). Trigger hair irritation resulted in transient efflux of CO_2 from the trap, indicating that the increased respiration rate (R_D) is the major contributor to the decreased A_N [A_N is a function of R_D and gross photosynthesis (A_G) (Fig. 4A, B)]. Trigger hair irritation had the opposite effect on fluorescence in

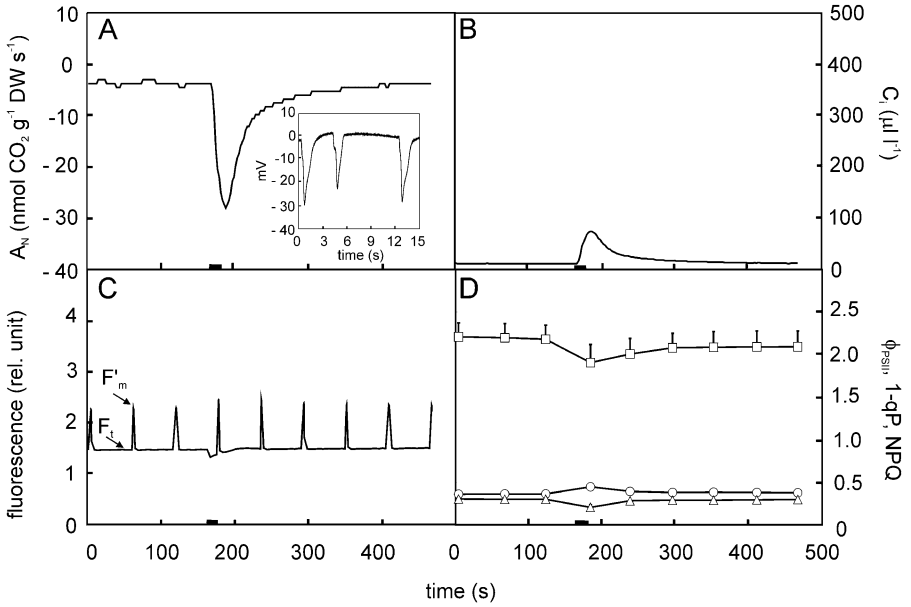

Fig. 3. Typical responses to trigger hair irritation in the Venus flytrap (*Dionaea muscipula*) at a light intensity of 80 μmol m^{-2} s^{-1} PAR and an atmospheric CO_2 concentration of ~1μl l^{-1} at a leaf chamber temperature of ~22 °C. Net photosynthetic rate (A_N; A), electrical signals (A; inset), intercellular CO_2 concentration (C_i; B), chlorophyll fluorescence kinetics (C), and chlorophyll fluorescence parameters, means ±1 SE (D). Φ_{PSII}, the effective photochemical quantum yield of PSII, open circles; 1–qP, the excitation pressure, open triangles; NPQ, the non-photochemical quenching, open squares. The duration of trigger hair irritation is denoted as a bold line on the *x*-axis. Data shown are representative of a total of five measurements.

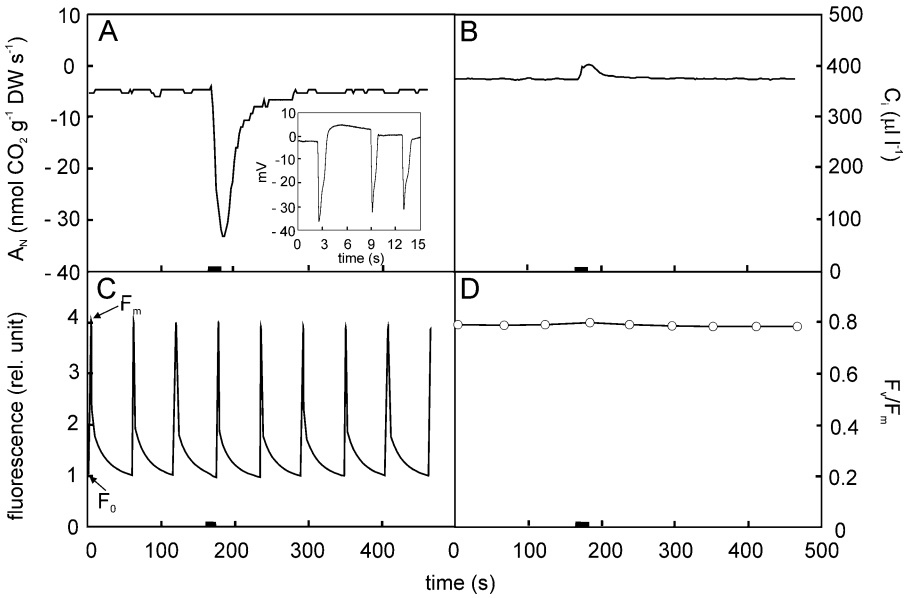

Fig. 4. Typical responses to trigger hair irritation in the Venus flytrap (*Dionaea muscipula*) in darkness and an atmospheric CO_2 concentration of 380 μl l^{-1} at a leaf chamber temperature of ~22 °C. Net photosynthetic rate (A_N; A), electrical signals (A; inset), intercellular CO_2 concentration (C_i; B), chlorophyll fluorescence kinetics (C), and chlorophyll fluorescence parameter, means ±1 SE (D). F_v/F_m, the maximal quantum yield of PSII photochemistry, is shown by open circles. The duration of trigger hair irritation is denoted as a bold line on the *x*-axis. Data shown are representative for total of five measurements.

dark-adapted and light-adapted traps at ambient CO_2 concentration. The irritation slightly decreased the minimal fluorescence (F_0). This small change in fluorescence intensity is not very obvious in Fig. 4C, but can be seen in Table 1 (O=F_0). This indicates that the F_v/F_m, which is proportional to the quantum yield of O_2 evolution from PSII, was slightly higher after irritation (Fig. 4D, Table 1). However, the effect of action potentials on the fluorescence in dark-adapted traps was much less obvious (changes in F_0 up to 4%) than in light-adapted traps (changes in F_t up to 60%, and of in F'_m up to 35%; compare Figs 2C and 4C); therefore, the changes in F_m were omitted in the calculation

Table 1. Data from chlorophyll a fluorescence transient (O-J-I-P)

Values are the means ±1 SE from 18 measurements. Statistical differences were evaluated by two-tailed paired Student *t*-test; significant differences before and after irritation of the same trap (paired data) are in bold.

	Non-irritated trap	Irritated trap	*P*
O (50 μs)	4155±116	3985±101	**<0.001**
J (2 ms)	13 712±521	12 884±527	**<0.001**
I (60 ms)	19 686±417	19 625±430	0.413
P (F_m)	22 441±357	22 766±350	**<0.001**
F_v/F_m	0.815±0.003	0.825±0.002	**<0.001**

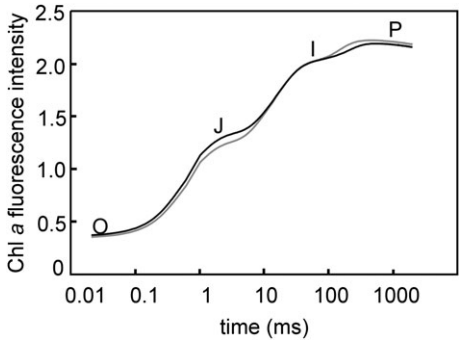

Fig. 5. Chlorophyll *a* fluorescence transients in non-irritated (black line) and irritated (grey line) *D. muscipula* traps given on a logarithmic time scale. Data shown are representative for a total of 18 measurements.

of NPQ. The amplitude and number of action potentials were comparable with our previous experiments, indicating that darkness had no effect on electrical signalling (number 3.8±0.3, P=0.837; amplitude 45.9±5.1 mV, P=0.332; Fig. 4A inset).

Measurements of the polyphasic increase in chlorophyll *a* fluorescence (O-J-I-P) in dark-adapted leaves have advantages over a single parameter such as the well known F_v/F_m. Trigger hair irritation had an effect on the shape of the induction curve. The decrease in the fluorescence rise in the 20–200 ms time range (O-J-I rise) was compensated by an increase in the rise in the 20–200 ms time range (I-P rise; Fig. 5) in irritated traps. The values of four distinct phases of the increase in chlorophyll *a* fluorescence (O-J-I-P) are summarized in Table 1. Significant differences were found in O, J, and P phases of the increase in chlorophyll *a* fluorescence. The 'I phase' was not significantly affected (paired *t*-test).

Discussion

The results reported here and in a previous study (Pavlovič *et al.*, 2010) confirmed that the irritation of the trigger hairs and the subsequent generation of action potentials in the digestive zone of the closed trap of *D. muscipula* resulted in a transient decrease in Φ_{PSII} and A_N (Figs 1, 2). The generation of action potentials and their negative effect on photosynthesis were confined to the trap and were not recorded in the adjacent lamina (Volkov *et al.*, 2007, 2008*a*; Pavlovič *et al.*, 2010). Convincing evidence on the role of the electrical signals in the regulation of photosynthesis has been described by numerous authors (Herde *et al.*, 1999; Koziolek *et al.*, 2003; Lautner *et al.*, 2005; Bulychev and Kamzolkina, 2006*a*, *b*; Hlaváčková *et al.*, 2006; Kaiser and Grams, 2006; Fromm and Lautner, 2007; Krupenina and Bulychev, 2007, 2008; Grams *et al.*, 2009). In accordance with Krupenina and Bulychev (2007), the inhibition of Φ_{PSII} and the rapid efflux of CO_2 are longer than the duration of the action potential itself. These authors called it the 'long-lived state' effect of action potentials on photosynthesis. However, the mechanism underlying photosynthetic limitation upon electrical signals is not known. It was suggested by Grams *et al.* (2009) that if the electrical signals have an impact on cytosolic pH, changes in enzyme activity might play a role in photosynthetic limitation (e.g. carbonic anhydrase, a pH-dependent enzyme important in the regulation of mesophyll conductance). Bulychev and Kamzolkina (2006*a*, *b*) proposed that action potentials suppress the Calvin cycle reactions by increasing $[Ca^{2+}]$ in chloroplast stroma. In contrast, Grams *et al.* (2009) found in isolated chloroplasts that the increase of $[Ca^{2+}]$ had no effect on the Φ_{PSII}. Lautner *et al.* (2005) suggested direct involvement of increased $[Ca^{2+}]$ in O_2 formation of PSII, and Koziolek *et al.* (2003) proposed that the rapid decline in Φ_{PSII} might result from an interference of the electron transport chains in chloroplasts through the direct impact of electrical signals. Hlaváčková *et al.* (2006) suggested that an increased level of jasmonic acid and abscisic acid had a direct inhibitory effect on the photosynthetic apparatus and stomata closure, respectively, in response to electrical signals evoked by local burning in tobacco. However, chemical signals are too slow to account for the photosynthetic response in sensitive plants (e.g. *Mimosa* or *Dionaea*), as was concluded by Koziolek *et al.* (2003). In *Mimosa*, tobacco, and poplar, electrical signals also induce changes in g_s (Koziolek *et al.*, 2003; Lautner *et al.*, 2005; Hlaváčková *et al.*, 2006; Kaiser and Grams, 2006). However no changes in g_s were found during irritation of the trap in this and a previous study, and the stomatal limitation of photosynthesis in carnivorous *D. muscipula* can be excluded (Pavlovič *et al.*, 2010).

In this study evidence is provided that the decrease in Φ_{PSII} is a consequence of reduced activity of enzymes involved in the dark reaction of photosynthesis, due to a feedback mechanism of CO_2 assimilation on electron transport rather than a direct effect of electrical signals on the light reaction, which seems not to be affected substantially. This assumption is supported by the observation that during the first seconds after trigger hair irritation in the light, the electron transfer chain became over-reduced (increased 1–qP) before the lumen could be sufficiently acidified to initiate NPQ (Fig. 2D). Rapid relaxation of NPQ in the dark indicates that energy state quenching (qE) is the major contributor to NPQ (data not shown). The release of NPQ by nigericine and the rapid reversal of

action potential-triggered NPQ in darkness in *Chara* cells also indicates NPQ's relationship to qE (Bulychev and Kamzolkina, 2006*a*, *b*; Krupenina and Bulychev, 2007). A correlation between NPQ and zeaxanthin accumulation after 5 h in response to current application in *Solanum lycopersicum* was also found (Herde *et al.*, 1999). Zeaxanthin dissipates excess excitation energy as heat and prevents photoinhibition of PSII (Pospíšil, 1997). Absorption of sunlight that exceeds a plant's capacity for CO_2 fixation results in a build up of the thylakoid ΔpH that is generated by photosynthetic electron transport. The lumen acidification and subsequent activation of violaxanthin de-epoxidase, which catalyses the conversion of violaxanthin first to antheraxanthin and then to zeaxanthin and is connected to qE, might be explained by the decreasing ATP consumption in the Calvin–Benson cycle. Cyclic electron flow around PSI may also contribute to lumen acidification, because a role in down-regulation of PSII via production of ΔpH and subsequent activation of qE has been proposed (Müller *et al.*, 2001; Kramer *et al.*, 2004; Niyogi *et al.*, 2005). However, a zeaxanthin-independent NPQ mechanism localized in the PSII core complex or the role of lutein cannot be excluded; both are also activated by generation of ΔpH and are rapidly relaxed in darkness. These types of quenching form rapidly and may precede zeaxanthin-dependent quenching (Ruban and Horton, 1999; Finazzi *et al.*, 2004; Johnson *et al.*, 2009).

It seems that electron transport is not directly inhibited by electrical signals. In the absence of CO_2, when Calvin cycle reactions are inhibited by unavailability of CO_2 substrate, trigger hair irritation did not decrease Φ_{PSII} (Fig. 3D). Even a slight decrease in F_t and increase in F'_m in the light resulted in higher Φ_{PSII} (Fig. 3C, D). It is tempting to assume that a transient increase of C_i after irritation, as a result of transiently increased R_D, decreased 1–qP and NPQ and slightly and transiently increased Φ_{PSII} by the stimulation of the Calvin cycle which consumes NADPH and restores the oxidized form of $NADP^+$, an electron acceptor from PSI (Fig. 3B, D). However, the possibility of a direct impact of the electrical signals on the charge separation–recombination reaction in PSII and subsequent increased fluorescence yield also cannot be excluded, as discussed below.

The changes in chlorophyll *a* fluorescence in dark-adapted traps and F_v/F_m are not so obvious as the changes in the light-adapted state (with or without CO_2) and are rather minor (Fig. 4C, D). The polyphasic increase in chlorophyll *a* fluorescence has advantage over a single parameter such as the well known F_v/F_m and takes into account all the steps of sequential fluorescence increase upon sudden illumination. Quantitative models enable calculation of the energy cascade from PSII light absorption to electron transport using O-J-I-P curves (for a review, see Strasser *et al.*, 2004). It has been proposed that the O step is the fluorescence signal coming from excited chlorophylls of light-harvesting antenna before the excitations reach the reaction centre of PSII, the J step reflects light-driven accumulation of Q_A^-, and steps I and P reflect light-driven accumulation of Q_B^- and Q_B^{2-}, respectively; however, several other explanations have been proposed (for reviews, see Lazár, 2006, 2009). At first glance, it seems that trigger hair irritation and subsequent generation of action potentials in *D. muscipula* resulted in an increase in F_v/F_m and thus increased photochemical efficiency of PSII (Table 1). However, care must be taken in the interpretation of the results, because the models relating variable PSII fluorescence and energy trapping are based on the assumption that the energetic state of PSII reaction centres is determined and quantified by the redox state of Q_A (two-state trapping model). A three-state trapping model, proposed by Vredenberg (2000, 2004), suggests that the saturation of photochemistry does not necessarily result in saturation of the changes in fluorescence yield, as pheophytin (Pheo) and oxidized secondary donor tyrosine (Y^+_Z) may also act as efficient fluorescence quenchers of PSII. Therefore, any calculations of energy fluxes in PSII according to the two-state trapping model (Strasser *et al.*, 2004) were avoided and only the differences at four distinct steps of the increase in chlorophyll *a* fluorescence were quantified (Table 1). The decrease in the O-J-I rise and increase in the I-P rise in an irritated trap is in accordance with electrochemical stimulation of the fluorescence yield supplementary to photochemical quenching (Fig. 5, Pospíšil and Dau, 2002; Vredenberg and Bulychev, 2002, 2003; Vredenberg, 2004; Vredenberg *et al.*, 2009). It was proposed that an electric field in the vicinity of the reaction centre could influence the chlorophyll fluorescence (Meiburg *et al.*, 1983; Dau and Sauer, 1991, 1992; Bulychev and Vredenberg, 1999; Vredenberg and Bulychev, 2002; Vredenberg, 2004; Vredenberg *et al.*, 2009). Apart from the influence of Q_A^- oxidation, the electrical field may exerts its effect on recombination of charges in PSII by decreasing the Gibbs free energy difference (ΔG_0) between the excited states in the reaction centre of PSII and the charge-separated state ($P680^+$ $Pheo^-$). As far as is known, this is the first time that the generation of action potentials has impact on yield of chlorophyll *a* fluorescence during the O-J-I-P transient, and provides convincing evidence that the fluorescent rise is under electrochemical control. Hlaváčková *et al.* (2006) found no changes in the fluorescence induction in response to variation potentials generated by tobacco leaf in response to local burning.

It seems that the donor side inhibition of photosynthesis (electrons from water) was not significantly affected as the K step in the increase in chlorophyll *a* fluorescence has not appeared. Srivastava *et al.* (1997) concluded that a typical K step in the increase in chlorophyll fluorescence is due to the decrease in the continuous supply of electrons to the reaction centre of PSII from water. Because the effect of electrical signals on the fluorescence yield of PSII in a dark-adapted trap of *D. muscipula* is relatively small, it is suggested that the main 'site effect' of electrical signals on inhibition of photosynthesis is in dark reactions. For better understanding, Fig. 6 summarizes the hypothesis about the target of electrical signals on photosynthesis in *D. muscipula*.

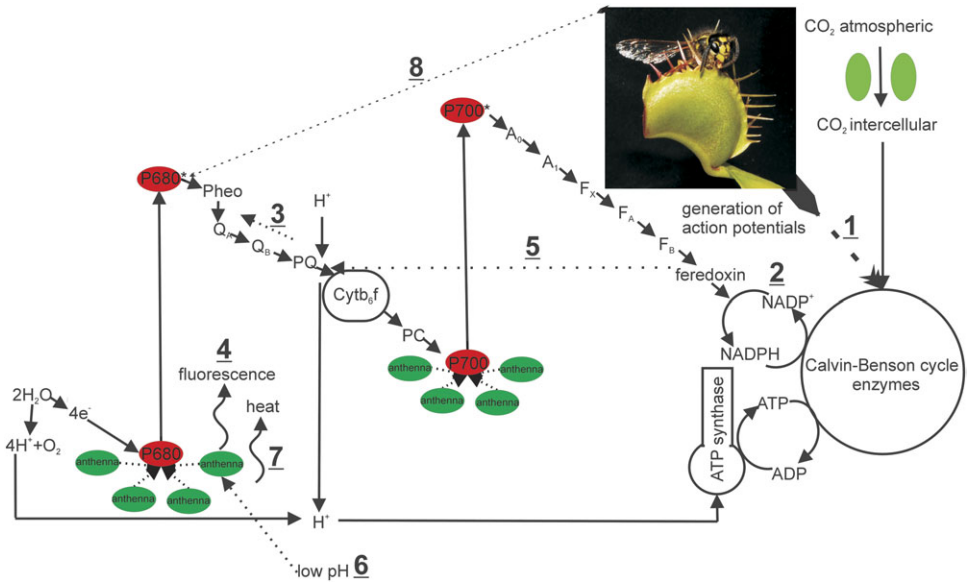

Fig. 6. Hypothesis about the effect of action potentials on photosynthesis in *Dionaea muscipula* upon trigger hair irritation. In accordance with the present results, it is supposed that the main targets of action potential-induced inhibition of photosynthesis are dark reactions of photosynthesis (1). Stomatal conductance is not affected (Pavlovič *et al.*, 2010). Suppression of Calvin cycle reactions or CO_2 availability by inhibition of carbonic anhydrase (an important part of the regulation of mesophyll conductance) may decrease ATP and $NADPH^+$ consumption. Unavailability of ADP and the oxidized form of $NADP^+$ inhibits ATP synthesis and linear electron transport (2). This results in increased excitation pressure at PSII (3) due to the accumulation of the reduced plastoquinone pool and increased emission of fluorescence (4). Inhibition of ATP synthesis and probably also enhanced cyclic electron flow around PSI (5) decrease the pH in the thylakoid lumen (6). The decrease in pH within the thylakoid lumen is an immediate signal of excessive light and activates qE. Excess excitation energy is dissipated as heat, and chlorophyll fluorescence is quenched (7). The result also support the hypothesis about the direct effect of electrical signals on charge separation–recombination reactions in PSII (8), although the effect seems to be small rather than substantial.

The results of the experiment performed in the dark indicate that rapid efflux of CO_2 originates not only from inhibition of photosynthesis but also from stimulation of respiration (Fig. 4A). A transient rise in R_D after generation of action potentials was also documented in the liverwort *Conocephalum conicum* (Dziubinska *et al.*, 1989). The results suggest that at least some of the energy connected with the rise of R_D is utilized for the restoration of the state of the ionic balance (i.e. restores the resting state). Jaffe (1973) and Williams and Bennet (1982) found that during trap closure in *D. muscipula*, 29% of ATP is lost. Subsequent availability of an increased concentration of ADP may stimulate enzymes in early steps of the respiration pathway (for an overview, see Taiz and Zeiger, 2002). However, the role of ATP is not only in rapid closure of the trap, but also in generation of action potentials, as suggested by Dziubinska *et al.* (1989), because repeated mechanical irritation in a closed trap resulted in transient stimulation of R_D (Fig. 4A).

In conclusion, the action potentials generated by trigger hair irritation in the carnivorous plant *D. muscipula* have an impact on both light and dark reactions of photosynthesis as chlorophyll *a* fluorescence measurements indicate. However, the changes in the yield of chlorophyll *a* fluorescence in dark-adapted traps are small in comparison with the changes in the light-adapted state. It is concluded that the main target of action potential-induced inhibition of photosynthesis is in the dark reaction, whereas the decreased electron transport (expressed as Φ_{PSII}) is only a consequence of impaired CO_2 assimilation, preventing photooxidative damage of PSII by dissipation of excitation energy via NPQ. The action potentials may also have direct impact on charge separation–recombination reactions in PSII; in this case the effect is small rather than substantial but provides important and convincing evidence about the electrochemical component of chlorophyll *a* fluorescence *in vivo*.

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References

Affolter JM, Olivo RF. 1975. Action potentials in Venus's-flytraps: long term observations following the capture of prey. *American Midland Naturalist* **93,** 443–445.

Brown WH, Sharp LW. 1910. The closing response in *Dionaea*. *Botanical Gazette* **49,** 290–302.

Bulychev AA, Kamzolkina NA. 2006*a*. Differential effects of plasma membrane electric excitation on H^+ fluxes and photosynthesis in characean cells. *Bioelectrochemistry* **69,** 209–215.

Bulychev AA, Kamzolkina NA. 2006*b*. Effect of action potential on photosynthesis and spatially distributed H^+ fluxes in cells and chloroplasts of *Chara corallina*. *Russian Journal of Plant Physiology* **53,** 1–9.

Bulychev AA, Vredenberg WJ. 1999. Light-triggered electrical events in the thylakoid membrane of plant chloroplast. *Physiologia Plantarum* **105,** 577–584.

Burdon-Sanderson JS. 1873. Note on the electrical phenomena which accompany stimulation of the leaf of *Dionaea muscipula*. *Proceedings of the Royal Society* **21,** 495–496.

Burdon-Sanderson JS, Page JFM. 1876. On the mechanical effects and on the electrical disturbance consequent on excitation of the leaf of *Dionaea muscipula*. *Proceedings of the Royal Society* **25,** 411–434.

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. *Biochimica et Biophysica Acta* **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. *Biochimica et Biophysica Acta* **1102,** 91–106.

Dziubińska H, Trębacz K, Zawadzki T. 1989. The effect of excitation on the rate of respiration in the liverwort *Conocephalum conicum*. *Physiologia Plantarum* **75,** 417–423.

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. *Proceedings of the National Academy of Sciences, USA* **101,** 12375–12380.

Fromm J, Lautner S. 2007. Electrical signals and their physiological significance in plants. *Plant, Cell and Environment* **30,** 249–257.

Genty B, Briantais J-M, Baker NR. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990,** 87–92.

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 and Environment* **32,** 319–326.

Herde O, Peña-Cortes H, Fuss H, Willmitzer L, Fisahn J. 1999. Effect of mechanical wounding, current application and heat treatment on chlorophyll fluorescence and pigment composition in tomato plants. *Physiologia Plantarum* **105,** 179–184.

Hlaváčková V, Krchňák 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.

Hodick D, Sievers A. 1986. The influence of Ca^{2+} on the action potential in mesophyll cells of *Dionaea muscipula* Ellis. *Protoplasma* **133,** 83–84.

Hodick D, Sievers A. 1988. The action potential of *Dionaea muscipula* Ellis. *Planta* **174,** 8–18.

Hodick D, Sievers A. 1989. On the mechanism of trap closure of Venus flytrap (*Dionaea muscipula* Ellis). *Planta* **179,** 32–42.

Jaffe MJ. 1973. The role of ATP in mechanically stimulated rapid closure of the Venus's-flytrap. *Plant Physiology* **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 Physiology* **149,** 1061–1075.

Juniper BE, Robins RJ, Joel DM. 1989. *The carnivorous plants*. London: Academic Press.

Kaiser H, Grams TEE. 2006. Rapid hydropassive opening and subsequent active stomatal closure follow heat-induced electrical signals in *Mimosa pudica*. *Journal of Experimental Botany* **57,** 2087–2092.

Koziolek C, Grams TEE, Schreiber U, Matyssek R, Fromm J. 2003. Transient knockout of photosynthesis mediated by electrical signals. *New Phytologist* **161,** 715–722.

Kramer DM, Avenson TJ, Kanazawa A, Cruz JA, Ivanov B, Edwards GE. 2004. The relationship between photosynthetic electron transfer and its regulation. In: Papageorgiou GC, Govindjee, eds. *Chlorophyll a fluorescence: a signature of photosynthesis*. Berlin: Springer, 251–278.

Krol E, Dziubinska H, Stolarz M, Treabacz K. 2006. Effects of ion channel inhibitors on cold- and electrical-induced action potential in *Dionaea muscipula*. *Biologia Plantarum* **50,** 411–416.

Krupenina NA, Bulychev AA. 2007. Action potential in a plant cell lowers the light requirement for non-photochemical energy-dependent quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **1767,** 781–788.

Krupenina NA, Bulychev AA. 2008. Effect of action potential on photosynthesis and proton transport in the plant cell. In: Allen JF, Gantt E, Golbeck JH, Osmond B, eds. *Photosynthesis: energy from the sun: 14th International Congress on Photosynthesis*. Berlin: Springer, 1299–1302.

Lautner S, Grams TEE, Matyssek R, Fromm J. 2005. Characteristics of electrical signals in poplar and responses in photosynthesis. *Plant Physiology* **138,** 2200–2209.

Lazár D. 2006. The polyphasic chlorophyll a fluorescence rise measured under high intensity of exciting light. *Functional Plant Biology* **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). *American Journal of Botany* **64,** 881–886.

Maxwell K, Johnson GN. 2000. Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany* **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. *Biochimica et Biophysica Acta* **724,** 352–358.

Müller P, Li X-P, Niyogi KK. 2001. Non-photochemical quenching. A response to excess light energy. *Plant Physiology* **125,** 1558–1566.

Niyogi KN, Li X-P, Rosenberg V, Jung H-S. 2005. Is PsbS the site of non-photochemical quenching in photosynthesis? *Journal of Experimental Botany* **56,** 375–382.

Pavlovič A, Demko V, Hudák J. 2010. Trap closure and prey retention in Venus flytrap (*Dionaea muscipula*) temporarily reduces photosynthesis and stimulates respiration. *Annals of Botany* **105,** 37–44.

Pospíšil P. 1997. Mechanisms of non-photochemical chlorophyll fluorescence quenching in higher plants. *Photosynthetica* **34,** 343–355.

Pospíšil P, Dau H. 2002. Valinomycin sensitivity proves that light induced thylakoid voltages result in millisecond phase of chlorophyll fluorescence transients. *Biochimica et Biophysica Acta* **1554,** 94–100.

Roháček K. 2002. Chlorophyll fluorescence parameters: the definitions, photosynthetic meaning, and mutual relationship. *Photosynthetica* **40,** 13–29.

Ruban AV, Horton P. 1999. The xanthophyll cycle modulates the kinetics of nonphotochemical energy dissipation in isolated light-harvesting complexes, intact chloroplasts, and leaves of spinach. *Plant Physiology* **119,** 531–542.

Sibaoka T. 1991. Rapid plant movements triggered by action potentials. *Botanical Magazine of 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. *Biochimica et Biophysica Acta* **1320,** 95–106.

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*. Berlin: Springer, 321–362.

Taiz L, Zeiger E. 2002. *Plant physiology*, 3rd edn. Sunderland, MA: Sinauer Associates.

Trebacz K, Sievers A. 1998. Action potentials evoked by light in traps of *Dionaea muscipula* Ellis. *Plant and Cell Physiology* **39,** 369–372.

Volkov AG, Adesina T, Jovanov E. 2007. Closing of Venus flytrap by electrical stimulation of motor cells. *Plant Signaling and Behavior* **2,** 139–145.

Volkov AG, Adesina T, Jovanov E. 2008*a*. Charge induced closing of *Dionaea muscipula* Ellis trap. *Bioelectrochemistry* **74,** 16–21.

Volkov AG, Adesina T, Markin VS, Jovanov E. 2008*b*. Kinetics and mechanism of *Dionaea muscipula* trap closing. *Plant Physiology* **146,** 694–702.

Volkov AG, Carrell H, Baldwin A, Markin VS. 2009*a*. Electrical memory in Venus flytrap. *Bioelectrochemistry* **75,** 142–147.

Volkov AG, Carrell H, Markin VS. 2009*b*. Biologically closed electrical circuits in Venus flytrap. *Plant Physiology* **149,** 1661–1667.

Volkov AG, Coopwood KJ, Markin VS. 2008*c*. Inhibition of the *Dionaea muscipula* Ellis trap closure by ion and water channels blockers and uncouplers. *Plant Science* **175,** 642–649.

Vredenberg WJ. 2000. A three-state model for energy trapping and chlorophyll fluorescence in photosystem II incorporating radical pair recombination. *Biophysical Journal* **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*. Berlin: Springer, 133–172.

Vredenberg WJ, Bulychev AA. 2002. Photo-electrochemical control of photosystem II chlorophyll fluorescence *in vivo*. *Bioelectrochemistry* **57,** 123–128.

Vredenberg WJ, Bulychev 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, Durchan M, Prášil O. 2009. Photochemical and photoelectrochemical quenching of chlorophyll fluorescence in photosystem II. *Biochimica et Biophysica Acta* **1787,** 1468–1478.

Williams SE, Bennett AB. 1982. Leaf closure in the Venus flytrap: an acid growth response. *Science* **218,** 1120–1121.

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*. Berlin: Springer, 471–480.