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

Andrej Pavlović1,*, L’udmla Slováková1, Camilla Pandolfi2,3 and Stefano Mancuso2,3

1 Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina B2, 842 15, Bratislava, Slovakia
2 Department of Horticulture, University of Florence, Viale delle Idee 30, 50019 Sesto Fiorentino, Italy
3 International Plant Neurobiology Laboratory, Viale delle Idee 30, 50019 Sesto Fiorentino, Italy

* To whom correspondence should be addressed. E-mail: pavlovic@fns.uniba.sk

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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 (\(\Phi_{\text{PSII}}\)) 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 \(\Phi_{\text{PSII}}\). 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...
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, 2008a, b, c, 2009a, 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 Ca2+, 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, 2008a, b, c, 2009a, 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 lamina (Pavlović 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, 2006a, 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. Ca2+) 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, 2006a, b; Krupenina and Bulychev, 2007). Bulychev and Kamzolkina (2006a, 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 CO2 concentration was examined. In the second experiment, the Calvin cycle reactions were inhibited by lowering the CO2 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_Fm/Fm) 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 CO2 and H2O 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 CO2 and H2O 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
Photosynthetic limitation upon impact of electrical signals in Venus flytrap

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 CaCl₂, 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 CO₂ concentration, in an atmosphere without CO₂ (~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 (ΦPSII), indicating that linear electron transport was inhibited. After stopping the mechanical irritation, ΦPSII started to recover. The inhibition of ΦPSII was confined mainly to the digestive zone of the trap (Fig. 1). The rate of net photosynthesis (Aₜ) also dropped sharply (Fig. 2A). This rapid inhibition resulted in a transient increase in the intercellular CO₂ concentration (Cᵢ; Fig. 2B), while the stomatal conductance (gs) was not affected (data not shown; see Pavlović 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 ΦPSII is caused at first by the increase in the steady-state fluorescence in the light (Fᵢ), whereas maximal fluorescence in the light-adapted state (Fm') 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 PSIII 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, Fᵢ is quenched by NPQ as indicated by the large drop in Fm' (Fig. 2C, D). Fm' was rapidly reversed in darkness, indicating that it represents the fast relaxing energy state quenching (qE) rather than photoinhibitory quenching (qI; data not shown).

Measurements of fast chlorophyll a fluorescence induction kinetics

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; Lázár, 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 800 ms). Then an actinic light was switched on (80 μmol m⁻² s⁻¹ PAR) and, after stabilization of the net photosynthetic rate (Aₜ), 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 (Fm'). For determination of the maximal fluorescence in the light-adapted state (Fm'), the polyphasic increase of chlorophyll a fluorescence in the light (80 μmol m⁻² s⁻¹ PAR) at ambient CO₂ concentration was measured over a time span of 10 s. 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 CO₂ and H₂O exchange every 2 s at a leaf temperature of 22±1 °C, ambient CO₂ 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 CO₂ and H₂O exchange every 2 s at a leaf temperature of 22±1 °C, ambient CO₂ concentration of 380 μl l⁻¹, a relative air humidity of 60–70%.
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 $\text{U}_{\text{PSII}}$ is a reason for or just a consequence of reduced $A_{\text{N}}$. Transiently increased $1-q_P$ 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 $\Phi_{\text{PSII}}$. The experiment in a CO$_2$-free atmosphere (~1 $\mu$l $\text{l}^{-1}$) showed the reverse effect of trigger hair irritation on $\Phi_{\text{PSII}}$, despite the same efflux of CO$_2$ (Fig. 3A, B). Trigger hair irritation decreased $F_t$ and increased $F_m'$, and thus $\Phi_{\text{PSII}}$ 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, $P=0.485$; 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 estimation 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 $A_{\text{N}}$ [($A_{\text{N}}$ is a function of $R_D$ and gross photosynthesis ($A_{\text{G}}$) (Fig. 4A, B)]. Trigger hair irritation had the opposite effect on fluorescence in...
dark-adapted and light-adapted traps at ambient CO₂ 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
The results reported here and in a previous study (Pavlovicˇ 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 $\Phi_{PSII}$ 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, 2008a; Pavlovicˇ 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, 2006a, 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 $\Phi_{PSII}$ 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 (2006a, b) proposed that action potentials suppress the Calvin cycle reactions by increasing [Ca$^{2+}$] in chloroplast stroma. In contrast, Grams et al. (2009) found that isolated chloroplasts that the increase of [Ca$^{2+}$] had no effect on the $\Phi_{PSII}$. 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 $\Phi_{PSII}$ 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 stomatal 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 (Pavlovicˇ et al., 2010).

In this study evidence is provided that the decrease in $\Phi_{PSII}$ 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

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

<table>
<thead>
<tr>
<th></th>
<th>Non-irritated trap</th>
<th>Irritated trap</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>O (60 μs)</td>
<td>4155 ± 116</td>
<td>3985 ± 101</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>J (2 μs)</td>
<td>13 712 ± 521</td>
<td>12 884 ± 527</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>I (60 ms)</td>
<td>19 668 ± 417</td>
<td>19 625 ± 430</td>
<td>0.413</td>
</tr>
<tr>
<td>$P$ ($F_m$)</td>
<td>22 441 ± 357</td>
<td>22 766 ± 350</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$F_o/F_m$</td>
<td>0.815 ± 0.003</td>
<td>0.825 ± 0.002</td>
<td>&lt;0.001</td>
</tr>
</tbody>
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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.
action potential-triggered NPQ in darkness in *Chara* cells also indicates NPQ's relationship to qE (Bulychev and Kamzolkina, 2006a, 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 CO₂ 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; Finazzi 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 CO₂, when Calvin cycle reactions are inhibited by unavailability of CO₂ substrate, trigger hair irradiation did not decrease Φₚₛₛᵢᵢ in the light resulted in higher Φₚₛₛᵢᵢ (Fig. 3C, D). It is tempting to assume that a transient increase of Ci after irradiation, as a result of transiently increased Rd, decreased 1–qP and NPQ and slightly and transiently increased Φₚₛₛᵢᵢ by the stimulation of the chlorophyll fluorescence 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 *Fₛₛᵢᵢ/Fₚₛₛᵢᵢ* are not so obvious as the changes in the light-adapted state (with or without CO₂) 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 *Fₛₛᵢᵢ/Fₚₛₛᵢᵢ* 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 Qₐ, and steps I and P reflect light-driven accumulation of Qₐ and Q₂, 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 *Fₛₛᵢᵢ/Fₚₛₛᵢᵢ* 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 QA (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 (*Y⁷*₂) 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-P 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 QA oxidation, the electrical field may exerts its effect on recombinant changes of charges in PSII by decreasing the Gibbs free energy difference (∆Gₒ) 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*. 

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**Table 1.** 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-P 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 QA oxidation, the electrical field may exerts its effect on recombinant changes of charges in PSII by decreasing the Gibbs free energy difference (∆Gₒ) 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.
The results of the experiment performed in the dark indicate that rapid efflux of CO₂ 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 $\Phi_{PSII}$) is only a consequence of impaired CO₂ assimilation, preventing photodamage of PSII by dissipation of excitation energy via NPQ. The action potentials may also have direct impact on charge separation–recombination reactions in PSII, although the effect seems to be small rather than substantial.

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**References**


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