Variation potential-induced photosynthetic and respiratory changes increase ATP content in pea leaves

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Abstract

Local damage induces a physiological response in higher plants by means of generation and propagation of variation potential (VP). The response includes changes in photosynthesis and respiration. The aim of the present study was to investigate the effect of these changes on adenosine triphosphate (ATP) content in pea leaves. VP was induced by local heating of the first mature leaf and registered using extracellular and intracellular electrodes. Photosynthesis and respiration were measured using Dual-PAM-100 and GFS-3000. ATP content was determined using a bioluminescence-based ATP determination kit. Two non-stimulated leaves (second and fourth) were investigated. We showed that heating induced VP that propagated into the second mature leaf, but only a slight electrical reaction was registered in the fourth mature leaf. VP-induced inactivation of photosynthesis developed in the second leaf and included two stages: short- and long-term inactivation. Local heating also caused a two-stage increase in ATP content in the second leaf, which was connected with the photosynthetic responses. Changes in photosynthesis and ATP content were not observed in the fourth leaf. The effect of VP on respiration was investigated under dark conditions. We found that variation potential induced short-term activation of respiration in the second leaf. Local heating induced ATP content increase which included only one stage under dark conditions. Changes in ATP and respiration were absent in the fourth leaf under dark conditions. Thus, VP-induced photosynthetic and respiratory changes are likely to increase ATP content in pea leaves.

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in plants. Photosynthesis dark stage inactivation is likely the primary stage of photosynthetic response induced by AP and VP (Krupenina and Bulychev, 2007; Pavlovič et al., 2011; Sukhov et al., 2012, 2014a,b, 2015a; Sherstneva et al., 2015). Changes in light stage reactions are mainly connected with this inactivation. However, a direct influence of electrical signals on photosystems I (PSI) and II (PSII) is also possible (Sukhov et al., 2012, 2014a, 2015a; Vredenbg and Pavlovič, 2013). There are two potential ways that electrical signals initiate the photosynthetic response in plants. Investigation of AP influence on photosynthesis in Chara showed that Ca2+ influx is the potential initiator of photosynthetic changes (Krupenina and Bulychev, 2007). Analysis of VP influence on photosynthesis on higher plants has shown that the VP effect is associated with H+ influx (Grams et al., 2009; Sukhov et al., 2014a; Sherstneva et al., 2015). Additionally, the slow influence of VP on photosynthesis (tens of minutes) could be connected with increase of abscisic and jasmonic acids concentrations (Hlaváčková et al., 2006; Hlavinč et al., 2012). The mechanisms of electrical signal-induced activation of respiration are unclear.

Both photosynthesis electrical signals induced dark stage inactivation, which contributes to decreased ATP consumption in chloroplasts (Pavlovič et al., 2011), and respiration activation can potentially increase ATP content in leaves. Increased ATP may play an important role in plant adaptation to stress (Sukhov et al., 2014b). However, experimental data on the influence of electrical signals on ATP content in plants are contradictory. According to Pyatygin et al. (2008), AP induced multiphase changes in ATP content in phloem. However, leaf stimulation by ice water and cutting of the leaf tip did not induce significant changes in ATP concentration 15 min after the stress was applied (Fromm et al., 2013). These results may be caused by different dynamics of photosynthetic and respiratory responses, but connection of these responses with ATP content has not been investigated previously. The aim of the present work was to investigate the influence of variation potential-induced photosynthetic and respiratory changes on ATP content in pea leaves.

2. Materials and methods

2.1. Plant material

Pea (Pisum sativum L.) seedlings were cultivated hydroponically in a Binder KBW 240 plant growth chamber (Binder GmbH, Tutzing, Germany) at 24 °C under a 16/8 h (light/dark) photoperiod. Seedlings used in experiments were 14–21 days old.

2.2. Stimulation and electrical measurements

Stimulation and electrical measurements were carried out according to our previous work with pea seedlings (Sukhov et al., 2014a). VP was induced by heating ~1 cm² of a first mature leaf tip over a flame for 3–4 s (Fig. 1).

The surface electrical potential was measured using Ag+/AgCl electrodes (RUE “Gomel Measuring Equipment Plant,” Gomel, Belarus), a high-impedance amplifier IPL-113 (Semic, Novosibirsk, Russia), and computer. The measurement electrodes contacted the non-stimulated leaf via ‘Uniigel’ conductive gel (Geltrek-Medica, Moscow, Russia). Two electrodes monitored electrical activity, with the first (E1) placed on the stem near the investigated leaf and the second (E2) connected with the center of its leaflet. The distance between the E2 and E1 was 3–5 cm. There were two variants of electrical activity assessment. In the first variant, electrical activity was registered in the second mature leaf (E3x and E3y). In the second variant, electrical activity was registered in the fourth mature leaf (E4x and E4y). The distance between the E3 site and the damaged area was 6–7 cm (second leaf) or 15–18 cm (fourth leaf). In both variants, the $E_R$ was placed in standard solution (1 mM KCl, 0.5 mM CaCl₂, and 0.1 mM NaCl), as above, surrounding the root.

The influence of a cold block (ice water) and block with metabolic inhibitor (1 and 5 mM NaNO₃) on VP propagation through the stem was investigated in separate series of experiments. The length of the zone of the block was about 3 cm. The zone was placed between the first and second mature leaves. Blocks were made before heating (20 min for ice water and 120 min for NaNO₃). The standard solution was used in the control.

For intracellular membrane potential ($E_M$) measurement in a leaf mesophyll cell, a microelectrode technique was used. The measuring system included a microscope SliceScope Pro 2000 (Scientifica, Uckfield, United Kingdom), amplifier Multiclamp 700 B (Molecular Devices, Sunnyvale, California, USA), a low-noise data acquisition system for electrophysiology Digidata 1550 (Molecular Devices, Sunnyvale, California, USA), and a PC. Micropipettes were fabricated using the Sutter Micropipette Puller P-97 (Sutter Instrument, Novato, California, USA). Micropipettes filled with 100 mM KCl were inserted into a mesophyll cell in the lamina of the second or fourth leaf. The reference electrode ($E_R$) was placed in standard solution in contact with the leaf.

2.3. Investigation of photosynthesis and respiration

Photosynthetic parameters in the second and fourth pea leaves were measured according to methods described previously (Sukhov et al., 2014a,b). A system composed of a GFS–3000 portable gas exchange measuring system, a Dual-PAM-100 measuring system for simultaneous assessment of P700 oxidation and chlorophyll fluorescence, and a measuring head Dual-PAM gas exchange Cuvette 3010-Dual (Heinz Walz GmbH, Effeltrich, Germany) was used for investigation of photosynthesis and respiration.

The initial parameters of PSI fluorescence, the dark fluorescence yield ($F_D$), and maximal fluorescence yield ($F_M$) were measured after dark adaptation for 20 min. The maximal change in the P700 signal ($F_m$) of PSI, reflecting maximal P700 oxidation, was measured after preliminary illumination by far-red light for 10 s. The steady-state ($F$) and maximal ($F_m$) fluorescence yields in light, steady-state ($P$), and maximal ($P_m$) signals in light were measured using generated periodical saturation pulses. Quantum yields of PSI ($ø_{PSI}$) and PSII ($ø_{PSII}$) were calculated using equations (Klughammer and Schreiber, 2008; Maxwell and Johnson, 2000). A non-photothermal quenching of photosystem II fluorescence (NPQ) was calculated using the equation (Maxwell and Johnson, 2000).

The CO₂ assimilation rate ($A$, µmol CO₂ m⁻² s⁻¹) was found using the GFS–3000 and its software, and the parameter programmatically calculated according to Von Caemmerer and Farquhar (1981).

The external CO₂ concentration, relative air humidity, and leaf temperature were 360 µmol mol⁻¹, 70%, and 23 °C, respectively. The blue actinic light (460 nm, 239 µmol m⁻² s⁻¹) was used in photosynthetic investigations.

Respiration was investigated under dark conditions. Respiration rate in the dark ($R_d$) has been calculated as $-A$.

2.4. Measurement of ATP content in leaves

The sensitive, bioluminescence-based ATP determination kit (Biafin GmbH & Co KG, Kassel, Germany) was used for the measurement of ATP content in plant leaves. A cut leaf was immediately weighed and homogenized in 5% ice trichloroacetic acid (Larsson and Olsson, 1979). After 15 min, the homogenate was neutralized by a small volume of 3 M KOH and diluted 12 times, before ATP content measurement. Every replication included calibration samples,
a control sample (leaf from a non-damaged plant), and experimental samples (leaf from a damaged plant). Light sums (during 15 min after sample addition) of all samples were measured simultaneously using Synergy 2 Multi-Mode Reader (BioTek Instruments, Inc., Winooski, VT, USA). ATP content was measured in the second and fourth mature leaves under light and in the second mature leaf under dark conditions. Each variant included a control (without VP induction) and experiments (in 5, 10, 20, 30, 40, 50, and 60 min after VP induction).

ATP content measurements were highly variable. Therefore, we used two approaches to decrease variability: (i) ATP content in leaves ([ATP]) at rest was measured in all control plants \((n = 14–26)\), and (ii) changes in ATP content \((\Delta[ATP])\) after VP induction were investigated using the difference between ATP concentration in the experimental sample and the concentration in the control sample \((n = 5–9)\).

2.5. Statistics

Each measurement was performed on a separate plant. Representative records, mean values, and standard errors were determined and are presented in the figures. Significant differences were determined according to the Student’s \(t\)-test.

3. Results

3.1. Propagation of variation potential induced by local heating

Local heating of the first mature leaf induced VP generation in pea seedlings (Fig. 2), which has been shown using measurements of surface membrane potential. In stems, VP amplitudes were about 67 mV near the second leaf and about 43 mV near the fourth leaf; therefore, decrement of variation potential was about 6.4% cm\(^{-1}\). However, the moderate decrease of VP amplitude in stems is likely to strongly influence VP in leaves located proximal to the induction site. VP amplitude in the second leaf was about 38 mV, which was in a good agreement with our previous results (Sukhov et al., 2014a,b, 2015a,b). VP amplitude in the fourth leaf was only about 8 mV. Velocities of VP propagation were 0.5–3.2 mm s\(^{-1}\) between \(E_r^2\) and \(E^4\), and 0.1–0.6 mm s\(^{-1}\) between \(E^4\) and \(E^4\).

We also show that VP amplitudes in the stem were 36 ± 3 mV in the control (standard solution), 41 ± 4 mV after the cold block (ice water), 43 ± 2 and 32 ± 8 mV after the block with metabolic inhibitor (1 and 5 mM NaN\(_3\), respectively); i.e. stem’s blocks did not suppress propagation of variation potential. It is known (Fromm and Lautner, 2007) that VP is able to pass through dead tissue, and our results supported this ability. Thus, inhibition of VP propagation could not be used in further analysis.

Measurements of intracellular membrane potential in a leaf mesophyll cell showed similar results (Fig. 3). Heating-induced VP was considerable in the second leaf and was minor in the fourth leaf. VP amplitudes were about 50 and 7 mV in the second and fourth leaves, respectively.

3.2. Local heating-induced changes in photosynthesis and ATP content under light

Local heating of the first mature leaf induced inactivation of photosynthesis in the second leaf, but weakly influenced photosynthesis in the fourth leaf (Fig. 4). Photosynthesis inactivation in the second leaf included decreased quantum yields of PSI and PSII, reduced the CO\(_2\) assimilation rate, and increased non-photochemical quenching of photosystem II fluorescence. There were two components in the dynamics of these changes. First, inactivation peaked about 5 min after heating and, second, inactivation peaked again about 40–60 min later. VP amplitudes in
the leaflet of the second leaf were strongly correlated with magnitudes of the first component of heating-induced changes in $A$ ($-0.63$, $p<0.05$), $\Phi_{PSI}$ ($-0.74$, $p<0.05$), $\Phi_{PSII}$ ($-0.77$, $p<0.05$), and $NPQ$ ($0.77$, $p<0.05$).

ATP content was about 77 nmol g$^{-1}$ fresh wt in the second leaf and 64 nmol g$^{-1}$ fresh wt in the fourth leaf (Fig. 5a). Data from the literature (Lüttge and Ball, 1976; Gaff and Ziegler, 1989; Teixeira et al., 2005; Fromm et al., 2013; Li et al., 2013) showed that ATP content in leaves varied widely and usually equaled about 40–180 nmol g$^{-1}$ fresh wt. Our results were in accordance with the data and were included in this range. Local heating of the first mature leaf induced an increase in ATP content in the second leaf but not in the fourth leaf (Fig. 5b). ATP content began to increase ($\Delta[ATP]$) in the second leaf within 5 min after heating. The
Fig. 4. Heating-induced photosynthetic responses in the second (a) and fourth (b) leaves (n = 6–13). Average dynamics of photosynthetic responses (black solid lines) and standard errors (gray shadows) are presented. Arrow indicates local heating of the first mature leaf.

Fig. 5. Stationary ATP content (a, n = 14–26) under light and its changes (Δ[ATP]) after local heating (b, n = 5–9) in the second and fourth leaves. Stationary ATP content was measured in intact leaves. Δ[ATP] was calculated for each repetition and averaged for each experimental point. Zero point on time axis indicates local heating of the first mature leaf. The first point of dynamics of changes in ATP concentration was control Δ[ATP] which equals to zero. *Δ[ATP] is significantly differed from zero (p < 0.05), Student t-test.

dynamics of Δ[ATP] included two maximums where Δ[ATP] was significantly more than zero. The first was about 10 min after heating and equaled 40 nmol g\(^{-1}\) fresh wt; the second peak occurred 30–50 min after heating and equaled 18–23 nmol g\(^{-1}\) fresh wt.
3.3. Local heating-induced changes in respiration and ATP content under dark conditions

Local heating of the first mature leaf and heating-induced VP transiently increased the respiration rate of the second leaf in pea seedlings (Fig. 6a). Maximum respiration activation was observed about 5 min after heating and this timing was similar with the first photosynthetic inactivation. However, the magnitude of the change in CO$_2$ exchange was about 0.74 µmol m$^{-2}$ s$^{-1}$, which was only 37% of the VP-induced changes in A under light (1.98 µmol m$^{-2}$ s$^{-1}$). VP amplitudes in the leaflet of the second leaf were strongly correlated with magnitudes of first component of heating-induced changes in $R_{L}$ (0.82, p < 0.05). Local heating of the first mature leaf did not influence the respiration rate of the fourth leaf in pea seedlings (Fig. 6b).

Analysis of stationary ATP content in the second and fourth leaves under dark conditions showed that ATP was not essentially changed without light, equaling 65 and 57 nmol g$^{-1}$ fresh wt (Fig. 7a), i.e., about 85–90% of the light level ATP content in the leaves. This weak decrease of stationary ATP content was in accordance with literature data. It was shown that stationary ATP content under dark conditions was about 60–90% of this content under light (Lütting and Ball, 1976; Roesse and Chollet, 1989) that was connected with respiration (Lütting and Ball, 1976). VP induced an increase in ATP content in the second leaf, reaching a maximum 5 min after heating (Fig. 7b). The magnitude of $\Delta [\text{ATP}]$ was about 14 nmol g$^{-1}$ fresh wt., equaling about 35% of the VP-induced increase of [ATP] ($\Delta [\text{ATP}]$) under light. Thus, VP-induced changes in respiration moderately increased ATP content in the leaf. Significant $\Delta [\text{ATP}]$ changes in the fourth leaf were not observed.

4. Discussion

Our results showed that local heating can induce changes in ATP content in the undamaged leaves. The changes have first and second maximums that are in accordance with the multiphase dynamics of ATP content in phloem exudate observed after electrical signal induction (Pyatygin et al., 2008).

It is likely that local heating-induced photosynthetic inactivation, which was observed in the present work as well as in other experiments (Sukhov et al., 2014a,b) is the main mechanism behind the increase in ATP concentration. There are several arguments supporting this hypothesis: (i) photosynthetic responses and changes in ATP content in the second leaf have very similar dynamics, but the first inactivation of photosynthesis is formed faster than the first increase of ATP concentration in the leaf; (ii) both photosynthetic and ATP content changes in the fourth leaf are absent; and (iii) magnitudes of ATP content increase are drastically decreased under dark conditions. VP propagation is probable mechanism of local heating-induced photosynthetic response (Grams et al., 2009; Sukhov et al., 2012, 2014a,b). Our current results support VP participation in induction of photosynthetic response because magnitudes of local heating-induced changes in photosynthetic parameters in the second leaf are strongly correlated with VP amplitudes in this leaf. Moreover, photosynthetic inactivation is absent in the fourth leaf, where only weak electrical reactions were observed. The last result is in good agreement with our previous data (Sukhov et al., 2012), which show that a weak electrical reaction with low amplitude cannot induce a photosynthetic response.

It is known that photosynthetic inactivation induced by electrical signals in higher plants is mainly connected with a decrease in photosynthesis dark stage activity (Pavlović et al., 2011; Sukhov et al., 2012, 2014a,b, 2015a; Sherstnev et al., 2015). In particular, this mechanism has been shown for photosynthetic responses in pea plants (Sukhov et al., 2014a,b, 2015a). Inactivation of dark stage photosynthesis (Pavlović et al., 2011; Sukhov et al., 2012, 2014a) decreases consumption of ATP and reduced nicotinamide adenine dinucleotide phosphate (NADPH) in the Calvin cycle, and, thereby, must increase ATP and NADPH content in chloroplast stroma. Excess ATP and NADPH can be used by different shuttle systems for the transfer of reductant out of the chloroplast that contributes to ATP concentration increase in the cytoplasm (Hoefnagel et al., 1998; Noctor and Foyer, 2000).

It should be noted that our observation of local heating-induced changes in ATP content is not in agreement with data of Fromm et al. (2013). Fromm et al. (2013) showed that ATP content in wounded leaves 15 min after stimulation was not significantly different from that in control leaves of maize plants. However, wound induced only slow inactivation of photosynthesis, which developed 24–30 min after stimulation. Therefore, it is possible that an increase in ATP content may occur in 24–30 min after wounding in maize plants.

The local heating-induced increase of ATP content under dark conditions in the second leaf shows that activation of respiration can also participate in the increase in the leaf ATP concentration. Activation of respiration after electrical signals has been shown in some plants (Hlaváčková et al., 2006; Filek and Koscielniak, 1997; Pavlović et al., 2011; Sukhov et al., 2012; Sherstnev et al., 2015), including pea (Sukhov et al., 2014a). Our results also show that local heating induces respiratory activation in the second leaf, and magnitudes of $R_{L}$ changes are strongly correlated with VP amplitudes. The similar dynamics of respiration activation and ATP increase in the second leaf provide additional support for the participation of respiration in the ATP content increase under dark conditions. Both photosynthetic and ATP content changes in the fourth leaf are absent, also showing participation of respiratory responses in ATP content increase.

Thus, our results show that VP-induced inactivation of photosynthesis and activation of respiration increase ATP content in pea leaves. The mechanism of electrical signal influence on photosynthesis and respiration could be connected with changes in concentrations of ions during electrical reaction development (Pyatygin et al., 2008). As for VP-induced photosynthetic responses in higher plants, this mechanism is likely related to cell H$^+$ influx (Grams et al., 2009; Sukhov et al., 2014a; Sherstnev et al., 2015). Also, we cannot exclude the possibility that VP-induced slow changes in ATP content and photosynthesis could be connected with increase of abscisic and jasmonic acids concentrations (Hlaváčková et al., 2006; Hlavinka et al., 2012). VP-induced activation of respiration can potentially have similar mechanisms, but this question requires further study.

The physiological role of the VP-induced ATP content increase requires further analysis. We speculate that an increase in ATP concentration participates in an increase of plant resistance to stressors (Retivin et al., 1997; Moussavi et al., 2013; Sukhov et al., 2015b). In particular, the electrical signals increase resistance of photosynthetic machinery (Retivin et al., 1999; Sukhov et al., 2014b, 2015b; Surova et al., 2016). Alternatively, an increase in ATP content could contribute to numerous adaptation reactions, including an increase of photosynthetic machinery resistance to stressors (Allakhverdiev et al., 2008).

5. Conclusion

Our results show that local heating of the leaf can induce an increase in ATP content in undamaged pea leaves. The increase is caused by photosynthesis inactivation and respiratory activation, which are induced by local heating. Variation potential, which is induced by local heating and can propagate through plant, is the likely mechanism of induction of photosynthetic and respiratory
responses. Thus, variation potential-induced photosynthetic and respiratory changes increase ATP content in pea leaves. It is possible that the ATP content increase can further participate in plant adaptation to environmental stressors.

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References


