The mechanism of propagation of variation potentials in wheat leaves

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Here we examined the mechanism of propagation of variation potential (VP) induced by burning in wheat leaves. Participation of hydraulic and chemical mechanisms in VP transmission was analyzed by optical coherent tomography and a radioactive tracer method, respectively. The speed of the hydraulic signal considerably exceeded the VP velocity. Investigation of a chemical substance spreading from the zone of local wounding was based on experimental data for radioactive marker transmission derived with a one-dimensional diffusion equation. The speed of the marker transmission was in accordance with VP velocity. The elimination of the potential transmission of a chemical signal by a timed severing of the leaf between the burn site and the recorded site blocked VP propagation. We suggest that a VP is formed by the transmission of a wound substance, the velocity of which is likely increased by hydraulic wave propagation.

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Introduction

External stimuli lead to formation of a systemic response in plants manifested as changes in important physiological processes, such as growth, respiration, photosynthesis, and gene expression (Léon et al., 2001; Baluska and Mancuso, 2009). A key process in stress sensing and systemic response development is the formation and transmission of an electrical signal from localized stimuli to distant non-irrigated parts of a plant (Fromm and Lautner, 2007; Pyatygin et al., 2008). Plant electrical signals are represented by two types of reactions in accordance with the nature of the stimulation: an action potential (AP) induced by non-damaging factors and a variation potential (VP) caused by damaging factors.

Generation of AP is connected with changes in membrane permeability by activation of ionic channels and passive influxes of Ca²⁺, K⁺ and Cl⁻ ions (Trebcz et al., 2006; Davies, 2006; Fromm and Lautner, 2007). The ionic nature of VP generation differs from the AP signal. VPs are considered to reflect a transient inactivation of the proton pump at the plasma membrane. Several types of evidence provide support for such a mechanism, which includes reduction of VP depolarization by the use of metabolic inhibitors (Julien et al., 1991; Stahlberg and Cosgrove, 1992) and no measurable changes in the cell input resistance at VP formation (Stahlberg and Cosgrove, 1992; Stahlberg et al., 2006). It cannot be discounted that VP has a complex nature, and in particular ionic channels activation could also participate in its formation (Stahlberg et al., 2006; Vodeneev et al., 2011).

The mechanism of AP propagation is similar to that of nerve impulse transmission (Trebcz et al., 2006; Davies, 2006; Fromm and Lautner, 2007). Although some studies suggest that VP also has an electrotonic mechanism of transmission (Tsaplev and Zatepina, 1980), a considerable body of experimental data discounts this possibility. Such data, in particular, concern dependence of VP propagation velocity on distance from a zone of wounding and its ability to pass through killed or poisoned stalk sites (Stahlberg et al., 2006). Therefore, VP is considered to be a local electrical response to the propagated signal, the nature of which is discussed below.

The prevalent hypothesis of VP propagation relates to its induction by localized damage hydraulic waves, which cause generation of an electrical response in plant cells (Malone and Stankovic, 1991; Stahlberg and Cosgrove, 1992; Mancuso, 1999). As described in a number of studies, localized damage induces VP generation that is preceded by reversible changes in leaf or stem thickness that reflect the propagation of a high-pressure wave (Malone and Stankovic, 1991; Stahlberg and Cosgrove, 1992; Mancuso, 1999). Some studies have shown that VP can be induced by the application of positive, not negative, steps in xylem pressure. These low pressure steps are unlikely to involve wounding (Stahlberg and Cosgrove, 1997; Stahlberg et al., 2006).
The hydraulic hypothesis of VP propagation is not unique. A chemical hypothesis has also been proposed, according to which an electrical reaction is induced by migration of a wound chemical substance from the zone of damage. The chemical hypothesis of VP transmission was suggested by Ricca (1916), who showed that VP could be propagated through a solution that connects two parts of a severed stem. Subsequently, it was demonstrated that wound extracts can induce VP-like electrical reactions (Umbrath, 1959; Sibaoka, 1997). The results of different studies indicate that the role of a wound chemical substance may be played by certain oligosaccharides or plant hormones such as systemin (Hlaváčková et al., 2006; Peña-Cortés et al., 1995).

However, neither the hydraulic nor chemical hypotheses can explain the mechanism of VP propagation. In particular, the velocity of VP propagation is not comparable to the speed of hydraulic or chemical signals. A hydraulic wave is transmitted at a speed that significantly exceeds VP velocity, and typical values for the velocities of long-distance transport of substances by the phloem and xylem are lower than VP velocity.

Thus, the nature of VP propagation requires further investigation. Examination of the mechanism of VP transmission by analysis of the propagation speeds of hydraulic and chemical signals and VP propagation velocity was the aim of the present work.

Materials and methods

Plant material

Seedlings of wheat (Triticum aestivum L.) were cultivated hydroponically in a Binder KBW 240 (Germany) plant growth chamber at 24 °C under a 16/8-h (light/dark) photoperiod. Seedlings that were 14–21 days old with a leaf blade length of 15–20 cm were used in experiments.

Electrical measurements

Surface electrical potential (V5) was measured using Ag/AgCl electrodes (Belarus) as described previously (Vodeneev et al., 2011). The electrodes were connected to a high-impedance three-channel amplifier IPL-113 (Russia) and PC. The measurement electrodes contacted the leaf through ‘Unigal’ conductive gel (Russia). The reference electrode was placed in the standard solution (1 mM KCl, 0.5 mM CaCl2 and 0.1 mM NaCl) surrounding the roots. Electrical activity was monitored by three electrodes, and the distance between the wounding zone and each electrode was from 3 to 15 cm.

The VP was induced by open-fire burning of the leaf tip (apical 2–3 mm) for 2 s. In the experiments, the leaf was severed between the burned zone and the zone of electrical activity measurement. The line of excision was 3 cm from the leaf tip and 1 cm from the first measuring electrode.

Optical coherence microscopy

The dynamics of wound-induced leaf deformation were examined by means of optical coherence microscopy (OCM). The OCM technique provides real-time imaging for visualization of the internal structure of plants. The method is intended for image acquisition with submicron resolution at depths up to a few millimeters, which is sufficient to visualize the whole cross-section of a leaf (Reeves et al., 2002; Sapozhnikova et al., 2004). Wound-induced changes in the leaf blade were assessed using the Thorlabs’ OCM1300SS Swept Source OCT Microscope System (USA). Two-dimensional OCM images of the leaves were acquired every 3 s. A series of observations at various distances r between the zone of stimulation and the point of observation were conducted. The distance r varied within the range of 5–15 cm. Concurrent with OCM examination, the electrical activity of the leaf was recorded. A measurement electrode contacted the leaf through conductive gel in the zone of OCM examination, and two additional electrodes were placed acropetally and basipetally in relation to this zone.

Radioactive marker measurement

14C-sucrose was used as a model substance (Malone et al., 1994). Before the experiment, the apical portion (~0.3 cm long) of leaves was excised and the plant was adapted for at least 2 h. Immediately after burning, the leaf tips (1 cm long) were immersed in 14C-sucrose solution for 100 or 200 s. Subsequently, the leaf was cut into 20-mm-long sections. The first of these sections was not investigated because it had been in direct contact with 14C-sucrose solution. The leaf slices were dried, weighed and used for fixed fraction preparation for measurement of radioactivity. Activities of preparations were recorded with a MST 17 end-window counter (Russia) and B-2 radiometer (Russia).

Statistical analysis

Each series of experiments comprised five to eight repetitions, and every measurement was performed on a separate plant. Representative records obtained in individual measurements, mean values and standard errors are presented.

Results and discussion

Electrical reaction induced by leaf burning

The burning of a wheat leaf tip caused an electrical response in the form of a transient depolarization of the plasma membrane. Development of this response was monitored by three electrodes, as shown in Fig. 1. The propagation velocity of the electric signal decreased from 0.40 cm s⁻¹ at a distance of 3 cm from the burn site to 0.07 cm s⁻¹ at a distance of 15 cm from the burn site. The amplitude of reaction also decreased with increasing distance from the burn site. Such features of the electrical reaction as long, delayed repolarization, decrement of amplitude and propagation velocity differed from AP properties and allowed us to characterize the reaction as VP (Stankovic et al., 1998; Stahlfberg et al., 2006; Vodeneev et al., 2011). Characteristics of the VP registered in the present study are consistent with previous findings on other plant objects such as tomato, tobacco, pea (Rhodes et al., 1999; Hlaváčková et al., 2006; Stahlfberg et al., 2006), and wheat in particular (Malone, 1992). In addition, unlike AP, which is transmitted as an electric signal throughout the plant body, VP is a local electrical response to the signal propagated from the zone of wounding (Stahlfberg et al., 2006; Trebacz et al., 2006). Such a wound-induced signal could be represented either by a hydraulic wave or by a chemical agent. Investigation of these two possible mechanisms of VP transmission induced by burning of wheat leaf tips was carried out.

OCM investigation of hydraulic signal propagation

According to the hydraulic hypothesis, VP transmission is associated with a rapidly propagated hydraulic pressure wave that is usually observed by changes in leaf thickness (Malone and Stankovic, 1991; Stahlfberg and Cosgrove, 1992; Mancuso, 1999). In the present study, OCM was used to observe wound-induced changes in the leaf blade (Sapozhnikova et al., 2004). A bright-field microscopic image of a wheat leaf in cross-section is shown in Fig. 2a and a typical OCM image of a wheat leaf is presented.
The propagation of variation potential (VP) induced by the burning of a leaf tip. The arrangement of electrode positions is shown in the lower figure. E1, E2, and E3 are electrodes; E0 is the reference electrode. The distance from the zone of burning to E1, E2, and E3 was 3, 5, and 7 cm, respectively. The arrow indicates the burning site.

Fig. 1. Propagation of variation potential (VP) induced by the burning of a leaf tip. The arrangement of electrode positions is shown in the lower figure. E1, E2, and E3 are electrodes; E0 is the reference electrode. The distance from the zone of burning to E1, E2, and E3 was 3, 5, and 7 cm, respectively. The arrow indicates the burning site.

Distance A was measured by OCM and shows the degree of flexing in the wheat leaf (Fig. 2). The arrow indicates the timing of leaf burning. Measurement of OCM and electrical activity was carried out at the point located at the distance r = 7 cm from the burning site.

Fig. 2. Light-microscopic image (a) and OCM image (b) of leaf blade in cross-section. Diagrammatic illustration of wheat leaf deformation induced by burning of the leaf tip (c) A – is the distance between two opposite symmetrically located points of the leaf blade.

Fig. 3. Time dependence of burning-induced changes in electric potential (VP) and in the distance A between two symmetric points on the opposite leaf halves. Distance A was measured by OCM and shows the degree of flexing in the wheat leaf (see Fig. 2). The arrow indicates the timing of leaf burning. Measurement of OCM and electrical activity was carried out at the point located at the distance r = 7 cm from the burning site.

in Fig. 2b. The superficial single-cell layer in the OCM image corresponds to the epidermis, the dark circular areas interspersed along the leaf blade are vascular bundles, and the remaining cells comprise the leaf mesophyll. By means of OCM we recorded burning-induced rapid reversible leaf deformation in the form of leaf flexion along the midrib (Fig. 2c). The distance A between two opposite symmetrically located points (Fig. 2b and c) rapidly decreased during flexion. The value of A changed by about 15 μm. Such a change may be associated with propagation of a hydraulic signal in plant tissues in response to wounding (Malone and Stankovic, 1991; Stahlberg and Cosgrove, 1992; Mancuso, 1999). It should be noted that the previously demonstrated changes in wheat leaf thickness that accompanied the hydraulic wave were detected using displacement transducers located on the leaf surface (Malone and Stankovic, 1991; Malone, 1992). It cannot be discounted that such changes are connected with the deformation of the organ, which may be apparent as the increase in leaf thickness.

Dynamics of the leaf flexion caused by burning (dotted line) and the change in electrical activity observed in the same zone (solid line) are shown in Fig. 3. It is clear from Fig. 3 that the leaf movement precedes the electrical response. These data may indicate that a hydraulic signal induces generation of VP. However, considerable disparity in the propagation of electrical and hydraulic signals was detected. The time interval between wounding and the beginning of the electrical reaction as well as the beginning of leaf deformation was measured. The time interval between wounding and the beginning of the electrical reaction (curve 2, Fig. 4) corresponds to the previously reported decrease of VP velocity with increasing r (Stankovic et al., 1998; Vodeneev et al., 2011). As shown in Fig. 4, the difference in the...
time interval between leaf deformation and the electrical reaction increased considerably with the increase of distance \( r \).

Our results are in agreement with those of Stahlberg and Cosgrove (1997), who showed that the velocity of hydraulic signal propagation exceeds the velocity of VP. The increase of the lag period between the hydraulic signal and the beginning of the electrical reaction was explained by the authors as pressure dissipation with increasing distance from the stimulus (Stahlberg and Cosgrove, 1997). However, the difference in the velocities of hydraulic and electrical responses allows us to infer that the inducer of VP is not the hydraulic wave. An alternative mechanism of VP transmission is propagation of a wound substance throughout the xylem (Ricca, 1916; Umrath, 1959; Rhodes et al., 1999). Therefore, analysis of the propagation of a wound substance was examined theoretically by means of a diffusion equation.

**Analysis of propagation of wound substance with a diffusion equation**

Diffusion in the xylem vessels is considered to be one-dimensional, subject to significant excess of their length compared with the transverse dimension. One-dimensional diffusion is described by Eq. (1) (Rubin, 1987; Codling et al., 2008):

\[
\frac{\partial c(r, t)}{\partial t} = D \frac{\partial^2 c(r, t)}{\partial r^2}
\]  

where \( c(r, t) \) is concentration of the wound substance, \( r \) is the distance from the zone of damage, \( t \) is the time after wounding, and \( D \) is the coefficient of diffusion of the wound substance.

The solution of Eq. (1) is Eq. (2) (Rubin, 1987; Codling et al., 2008):

\[
c(r, t) = \frac{C_0}{2\sqrt{\pi D t}} \exp \left( - \frac{r^2}{4Dt} \right)
\]  

where \( C_0 \) is the initial content of the wound substance in the zone of damage.

It has been assumed that an electrical reaction at distance \( r \) from the zone of wounding evolves at the moment the concentration of the wound substance in this region reaches the critical value \( C_{\text{th}} \). The dependence of the distance \( r \) where the concentration of the wound substance reaches the critical value and, consequently, evolution of the electrical reaction, on time, is described by Eq. (3):

\[
r = \sqrt{-4Dt \ln \left( \frac{2C_{\text{th}}\sqrt{\pi D t}}{C_0} \right)}
\]  

The theoretical dependence (the relation between the time interval after stimulation and the distance of VP propagation) was deduced from Eq. (3) (curve 3, Fig. 4). This dependence (for \( C_{\text{th}}/C_0 = 10^{-4} \text{L}^{-1} \) and \( D = 0.045 \text{cm}^2 \text{s}^{-1} \)) was similar to the experimental dependence for the electrical response. However, the calculated value \( D \) is 2000 times larger than the coefficient of diffusion of small molecules in a water solution (Levich, 1962).

It is well known (Levich, 1962) that convection may cause considerable growth of diffusion velocity. In this instance (turbulent flow), the total coefficient of diffusion equals the sum of the coefficients of molecule diffusion \( (D_{\text{mol}}) \) and of turbulent diffusion \( (D_{\text{tur}}) \):

\[ D = D_{\text{mol}} + D_{\text{tur}} \]

\( D_{\text{tur}} \) may be thousands of times larger than \( D_{\text{mol}} \) (Levich, 1962). Turbulent convection in the xylem may be because of the composite trajectory of the water flow, which is caused by the complicated geometry of xylem vessels (Roth, 1996) and by wound-induced hydraulic wave propagation. The theoretically estimated high velocity of chemical substance transmission was analyzed experimentally by means of a radiotracer method.

**Radioactive marker transmission from a wounding zone**

For analysis of the velocity of any chemical agent distribution from a zone of local wounding, \( ^{14} \text{C}-\text{sucrose} \) is used as a model substance (Malone et al., 1994). The dependence of \( ^{14} \text{C} \) activity on distance from the zone of burning at different times after stimulation is shown in Fig. 5. Counts of radioactivity of the leaf sections after wounding showed that the radioactive marker spread to ~4 cm from the burn site after 100 s and to ~10 cm after 200 s.

A control experiment, which was carried out on non-wounded leaves, clearly showed that the propagation of radioactive sucrose was much less than after the burning of the leaf tip. The radioactive marker moved only 6 cm per 200 s, while after burning it covered 10 cm in the same time.

Based on the experimental data presented in Fig. 5, the diffusion coefficient was calculated for \( ^{14} \text{C}-\text{sucrose} \) in each plant. The estimate was calculated with the one-dimensional diffusion equation as for theoretical curve 3 plotted in Fig. 4. For the diffusion coefficient calculation the distance at which the counted activity (i.e. chemical substance concentration) was reduced by \( e \) times was defined. Eq. (2) then takes the form:

\[
D = \frac{r_2^2 - r_1^2}{4t}
\]  

where \( t \) is the time after wounding, \( r_1 \) is the distance from the wounding zone to the first point at which radioactivity was measured, \( r_2 \) is the distance at which \( l(r_2)/l(r_1) = e^{-1} \) is true, and \( l(r_1) \) and \( l(r_2) \) are activity at distances \( r_1 \) and \( r_2 \), respectively. The calculated coefficients of diffusion were \( 0.06 \pm 0.01 \text{cm}^2 \text{s}^{-1} \) after wounding and \( 0.018 \pm 0.002 \text{cm}^2 \text{s}^{-1} \) without wounding. The significant increase in the diffusion coefficient in response to wounding could be connected with spread of the hydraulic wave induced by damage. Wave propagation leads to changes in water flows, because turbulent convection parameters depend on flow velocity (Levich, 1962).

The diffusion coefficient for transmission of the radioactive marker after burning as determined experimentally is close to what has been calculated theoretically on the basis of parameters of propagation of an electrical reaction (Fig. 4). Rhodes et al. (1999) showed that the velocity of fluorescent dye (Lucifer Yellow) propagation from the zone of damage was 0.5 cm s\(^{-1} \) in tomato leaves.
The propagation was via the xylem vessels. The velocities of fluorescent dye diffusion and electrical signal propagation were similar. High similarity in velocities of chemical substance transmission and VP propagation were also found here. We therefore conclude that our data support the chemical hypothesis of VP propagation.

**Propagation of VP when transmission of wound chemical is blocked**

We examined whether disruption of chemical signal transmission from the zone of local wounding influenced VP propagation. In this experiment, the leaf blade was severed between the zone of wounding and the electrical activity measurement zone. It should be noted that leaf excision alone did not induce any distinct changes in electrical activity or induce a low-amplitude impulse depolarization that rapidly decreased with distance (Fig. 6a). The absence of an electrical response to leaf excision was observed, which agreed with the findings of other work (Malone et al., 1994; Rhodes et al., 1999). Mechanical wounding of a small area of a tomato leaf did not induce VP formation, whereas burning of the leaf led to VP propagation (Rhodes et al., 1999). However, in contrast, excision of roots of sunflower and pea seedlings induced VP generation (Stahlberg and Cosgrove, 1992; Stahlberg et al., 2005). However, it was also shown that VP propagation was suppressed when a plant was cut in air (Stahlberg et al., 2005), as was observed in the present study. Electrical responses to burning after leaf severance are presented in

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**Fig. 5.** Dependence of radioactive marker (\(^{14}\)C-sucrose) activity on the distance from the burning site \((r)\) in wheat leaf. The leaf tip was immersed in \(^{14}\)C-sucrose solution after burning (solid line) or without burning (dotted line), and incubated for either 100 s \((a)\) or 200 s \((b)\). Immediately after incubation, the leaf was cut into 20-mm long sections and radioactivity of each section was recorded. The apical-most section was not investigated because it was in direct contact with the \(^{14}\)C-sucrose solution. A control experiment – carried out without leaf burning – showed that the treatment increased the transport rate of radioactive sucrose for a distance of at least 10 cm.

**Fig. 6.** Changes in electrical activity induced by leaf excision without treatment \((a)\) and at different intervals after the burning of the leaf tip \((b = 1 \text{ s}, c = 3 \text{ s}, d = 6 \text{ s})\). The arrow indicates the timing of leaf burning. The vertical dotted line indicates the moment of leaf severance. Leaves were cut in 20-mm long sections from the burning point. The distance from the point of burning to \(E_1\), \(E_2\) and \(E_3\) was 3, 5 and 7 cm, respectively. Note that the rapid severance of the burn site prevents the generation of a variation potential at all electrode locations.
Fig. 6b–d. If the leaf was cut 1 s after burning (Fig. 6b), no changes in electrical activity were observed. Increase in the interval between burning and excision of the leaf from 1 to 3 or 6 s caused marked changes in electrical activity with distance from the zone of wounding (Fig. 6b–d).

Given that a hydraulic wave is transmitted very rapidly (up to 1500 m/s), leaf excision 1 s after burning could not block its propagation.

Consequently, the failure of the excised leaf segment to generate an electric response must have been connected with the slower transport of a depolarizing chemical substance from the burn site. This scenario is supported by the prompt appearance of a variation potential after the excision of the leaf was delayed. The speed of the transport of this substance should therefore be approximately 0.6 cm/s. This agrees with our calculations from Fig. 4. In summary, our data support the possibility of a chemical propagation mechanism for the VP.

Conclusion

On the basis of the present experimental and theoretical analysis, it is hypothesized that the propagation of a wound substance induces VP generation. The mechanism of wound substance transmission appears to be turbulent diffusion in the xylem vessels. Substance transmission by diffusion does not require physical displacement of water and is not strongly connected with water flow direction in the xylem. It should be noted that the significant increase in diffusion velocity after wounding may be caused by hydraulic wave propagation, which can intensify turbulent flow in vascular bundles. Thus, it is proposed that a wound substance induces VP generation in wheat leaves, at the same time a hydraulic wave is necessary for acceleration of the wound substance propagation.

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