

# Variation potential in higher plants: Mechanisms of generation and propagation

Vladimir Vodeneev\*, Elena Akinchits, and Vladimir Sukhov

Department of Biophysics Lobachevsky State University of Nizhni Novgorod; Nizhni Novgorod, Russia

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**Abbreviations:** AP, action potential; ES, electrical signals; VP, variation potential.

Long-distance intercellular electrical signals, including variation potential (VP) in higher plants, are a potential mechanism of coordinate functional responses in different plant cells under action of stressors. VP, which is caused by damaging factors (e.g., heating, crushing), is transient depolarization with an irregular shape. It can include a long-term depolarization and fast impulse depolarization ('AP-like' spikes). Mechanisms of VP generation and propagation are still under investigation. It is probable that VP is a local electrical response induced by propagation of hydraulic wave and (or) chemical agent. Both hypotheses are based on numerous experimental results but they predict VP velocities which are not in a good accordance with speed of variation potential propagation. Thus combination of hydraulic and chemical signals is the probable mechanism of VP propagation. VP generation is traditionally connected with transient H<sup>+</sup>-ATPase inactivation, but AP-like spikes are also connected with passive ions fluxes. Ca<sup>2+</sup> influx is a probable mechanism which triggers H<sup>+</sup>-ATPase inactivation and ions channels activation at VP.

## Introduction

Plants live under variable environmental conditions, but they are immovable organisms and cannot escape from stressors. As a result, adaptive physiological responses are very important for plant life. Development of these responses requires intracellular and intercellular signals that coordinate functional responses in different plant cells. In particular, long-distance intercellular signals are necessary under local action of stressors because they induce functional changes in nonstimulated organs and tissues, i.e., the systemic plant response.<sup>1,2</sup>

Chemical, hydraulic and electrical signals are considered to be potential long-distance intercellular signals in plants.<sup>2-10</sup> Electrical signals (ESs) traditionally include action potential (AP), which is induced by non-damaging stimuli (e.g., cooling, touching), and variation potential (VP), which is caused by damaging factors (e.g., heating, crushing) (Fig. 1).<sup>8,11-14</sup> Both signals are affected via transient depolarization, although the dynamics of

membrane potential changes at AP and VP are different.<sup>7,11,12</sup> Recently, an ES, termed 'system potential' (SP), has been shown in higher plants.<sup>15</sup> In contrast to AP and VP, SP is transient hyperpolarization that can be induced by different stimuli and is likely connected with H<sup>+</sup>-ATPase activation.<sup>15</sup>

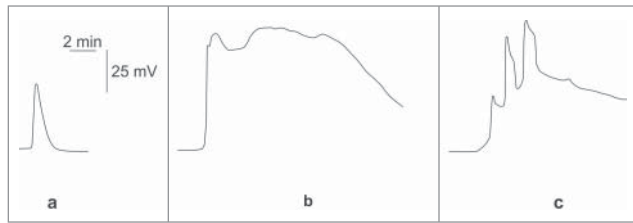
Propagation of AP and VP affects numerous physiological processes in plants. For example, ESs induce gene expression,<sup>16-18</sup> phytohormone synthesis,<sup>17,19,20</sup> phloem transport decrease,<sup>21</sup> changes in root absorption,<sup>11</sup> activation of respiration,<sup>22-26</sup> and inactivation of photosynthesis.<sup>23,24,26-31</sup> Retivin et al.<sup>32</sup> hypothesized that the ultimate role of these physiological changes is the increase of plant resistance to stressors. Indeed, it has been shown that ESs can have a positive influence on resistance to stressors at the whole plant level,<sup>32</sup> including increasing photosynthetic machinery resistance,<sup>30,33</sup> which is strongly connected with inactivation of photosynthesis. The precise mechanisms of ESs' influence on physiological processes are under investigation. It has been hypothesized<sup>34</sup> that changes in ion concentrations could be the initial mechanism that leads to a functional response. Investigation of ESs-induced photosynthetic response in *Chara* algae and higher plants showed that Ca<sup>2+</sup><sup>27,28</sup> and (or) H<sup>+</sup><sup>26,29</sup> influxes, which participate in development of electrical reactions, can be initiators of the functional response.

Thus ESs-induced physiological changes associated with AP and VP generation depend on ion mechanisms. AP generation is mainly based on passive Ca<sup>2+</sup>, Cl<sup>-</sup> and K<sup>+</sup> fluxes,<sup>35</sup> but transient H<sup>+</sup>-ATPase inactivation also participates in this process.<sup>36</sup> VP generation is still under investigation<sup>7,11-13</sup> and it has been difficult to determine its precise functional role. AP is subjected to the 'all-or-none law'; i.e., propagation of this signal isn't directly related to its functional role. In contrast, VP propagation isn't subjected to the 'all-or-none law', and propagation parameters can directly influence plant physiological responses. As a result, a better understanding of VP generation and its propagation principles is important for future investigations of its functional role. The aim of our review is to provide an analysis of modern conceptions about mechanisms of VP generation and propagation.

## General information about variation potential

In higher plants, local damage, including burning and other mechanical injuries<sup>7,11,13,15,37</sup>, induces a unique ES referred to as variation potential (VP), which is defined as a transient depolarization with an irregular shape. Burning is the most commonly

\*Correspondence to: Vladimir Vodeneev; Email: v.vodeneev@mail.ru  
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**Figure 1.** Electrical signals in hypocotyl of pumpkin seedling induced by different stimuli. (a) action potential induced by ice water;<sup>11</sup> (b) variation potential without spikes induced by leaf burning;<sup>46</sup> (c) variation potential with AP-like spikes induced by leaf burning.<sup>56</sup> Electrical signals were registered in 7 cm from zone of stimulation. Redrawn from works.<sup>11,46,56</sup>

used stressor for inducing VP in a wide variety of plants, including barley,<sup>37</sup> faba bean,<sup>37</sup> soybean,<sup>38</sup> tobacco,<sup>39</sup> sunflower,<sup>40</sup> mimosa,<sup>11,41,42</sup> tomato,<sup>6,43</sup> *Bidens pilosa*,<sup>44,45</sup> pumpkin,<sup>11,46</sup> wheat,<sup>47</sup> and pea.<sup>26,30,31</sup> Mechanical injury also can induce VP. It was shown that pricking sunflower hypocotyls induced VP,<sup>7</sup> and cutting stems and roots induced VP in sunflower,<sup>7</sup> maize,<sup>48</sup> and pea.<sup>49</sup> However, similar mechanical injuries did not induce VP in tomato and wheat.<sup>47,50</sup> Because the presence/absence of VP induction depends on the type of damage, burning has become the preferred inductor of VP.

VP has an irregular shape that can include 2 components. The first is long-term depolarization over the course of a minute to several minutes,<sup>5,7,12</sup> and which is an absolutely necessary component of VP. It has wide-range variable amplitude ( $\sim$  tens mV) and low velocity propagation ( $\sim$  mm s<sup>-1</sup>).<sup>46,47</sup> However, VP may also include fast impulse depolarization, a similarity it shares with AP.

These ‘AP-like’ spikes can take the lead over the long-term depolarization in mimosa, sunflower and bean,<sup>42,51,52</sup> join the depolarization and form the first fast depolarization in pumpkin, geranium and pea,<sup>24,30,46</sup> and be generated during the depolarization in mimosa, sunflower, tomato, pumpkin and cucumber.<sup>11,43,47,51,53</sup> However, VP can occur without ‘AP-like’ spikes, as well. Only long-term depolarization was observed in pea epicotyls,<sup>49,53,54</sup> wheat<sup>47</sup> and spiderwort.<sup>55</sup> It should be noted that presence and absence of ‘AP-like’ spikes may be observed in the same plants, i.e., its generation is determined by damage parameters and distance from the zone of stimulation (Fig. 1).<sup>46,56</sup>

Propagation of VP has a number of specific properties. It is known that VP amplitude is proportional to the intensity of the damaging stimulus.<sup>11</sup> Amplitude and propagation velocity of VP decreases with increasing distance from the damage zone.<sup>46,47,56</sup> This decrement was about 10% cm<sup>-1</sup> in pumpkin<sup>46</sup> and wheat.<sup>47</sup> Our theoretical investigation<sup>56</sup> showed that this decrement depends on initial damage intensity. Another interesting property of VP propagation is its transmission through inactive or dead plant parts.<sup>11</sup>

Thus, parameters of VP are essentially distinguished from AP in major 3 ways:<sup>7,11,13,15</sup> (i) AP is induced by undamaging stimuli and VP is caused by damage; (ii) AP is a spike whereas VP has an irregular shape, including long-term depolarization and ‘AP-like’ spikes; and (iii) AP is subjected to the ‘all-or-none law’

and VP depends on stimulus type, distance from zone of damage, and plant species. As a result, generation and propagation mechanisms of VP are considered to be distinguishable from those for AP.

### Mechanism of variation potential propagation

There are 3 basic hypotheses that describe potential mechanisms of VP propagation in plants. The first hypothesis<sup>55</sup> supposes that VP propagation is a self-propagating ES, which is similar with AP transmission in principle. It was shown that VP propagation velocity is well described by a cable equation,<sup>55</sup> i.e., its process may be based on active ES transmission. However, this hypothesis doesn’t explain several important properties of VP transmission. In particular, VP can propagate through zones where its generation was suppressed by low and high temperatures,<sup>42,57,58</sup> potassium cyanide,<sup>53</sup> sodium azide,<sup>46</sup> and EGTA.<sup>46</sup> Moreover, it was shown that VP could propagate between cut stems, if plant parts were joined by solution.<sup>11</sup> These results are contrary to a hypothesis regarding self-propagating VP and show that xylem is likely to be the main pathway of variation potential transmission. Data of Tzaplev and Zatzepina<sup>55</sup> can be connected with measurement of propagation velocity of ‘AP-like’ spike, which may take the lead over long-term depolarization and is similar to AP.

According to the other 2 hypotheses, VP (or only long-term depolarization) is a local electrical response induced by propagation of a specific factor. This factor may be a hydraulic wave<sup>4,7</sup> or chemical agent (‘wound substance’, ‘Ricca’s factor’).<sup>3,11,13</sup> A combination of hydraulic and chemical signals is also proposed in some studies.<sup>59</sup>

The hydraulic hypothesis maintains<sup>4,7</sup> that damage increases hydraulic pressure in the stimulated zone that induces a hydraulic wave that is propagated through the plant body. A number of experimental data support this hypothesis. First, it was shown in several studies that local damage induced changes in stem or leaf thickness that reflected hydraulic wave propagation through the plant.<sup>4,47,53,60,61</sup> Changes in thickness started before the electrical reaction was initiated<sup>4,40,47,51</sup> thus supporting their key role in VP induction. Additionally, artificially increasing xylem hydraulic pressure in the positive direction<sup>7,49,53</sup> induces electrical reactions that are similar to VP.

Thus, participation of a hydraulic wave in VP propagation is likely. However, the velocity of hydraulic wave propagation (up to the speed of sound in water) is essentially greater than the velocity of VP transmission ( $\sim$ mm s<sup>-1</sup>). Stahlberg and Cosgrove<sup>53</sup> supposed that low VP velocity was connected with a lag-phase prior to the start of VP generation. This lag-phase was extended as hydraulic wave amplitude decreased; in turn, the amplitude was reduced with distance from the damage zone.

The chemical hypothesis proposes that damage induces synthesis and (or) excretion of a wound substance that propagates through xylem vessels and induces a local electrical reaction.<sup>3,13</sup> This wound substance can be contained in plant homogenates because homogenization breaks cells in a manner similar to a damage stimulus. It was shown that homogenates can induce an electrical reaction in mimosa,<sup>11</sup> biophytum,<sup>62</sup> and tomato.<sup>63-65</sup>

In contrast, the homogenate didn't change VP, induced by cut, in pea.<sup>54</sup> The exact nature of the wound substance isn't known. This substance must: (i) be synthesized quickly in response to damage, (ii) induce electrical reactions, and (iii) propagate easily in plants. Oligosaccharides of broken cell walls,<sup>66</sup> systemin,<sup>67</sup> jasmonate,<sup>18,68</sup> salicylic acid,<sup>69</sup> ethylene<sup>19,70</sup> and abscisic acid<sup>1,2</sup> are considered to be potential wound substances. In recent studies,<sup>71</sup> hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) has been shown to propagate from the damage zone and induce an electrical reaction. It is known that H<sub>2</sub>O<sub>2</sub> can activate Ca<sup>2+</sup> channels<sup>72-74</sup> that can trigger VP development (see below), and hydrogen peroxide molecules can be quickly synthesized and propagated when a plant is under stress.<sup>75-78</sup> These properties support the hypothesis of H<sub>2</sub>O<sub>2</sub> participation in VP transmission.

The mechanism of wound substance propagation through plants is not well known. The first modification of the chemical hypothesis supposed that wound substances propagate through the plant body with water flow in the xylem.<sup>11</sup> However, this supposition cannot explain basipetal VP transmission, which has been shown in a number of studies.<sup>12,26,30,31</sup> Molecular diffusion in water solutions is a relatively slow process,<sup>79</sup> and it is unlikely that this mechanism is the basis for wound substance propagation. Alternative possible mechanism of electrical signal propagation may be connected with diffusion of volatile wound substance (e.g., ethylene) in air spaces of plant. This mechanism has not been shown for VP; however, propagation of light-induced electrical signals through *Coleus* leaves on 12–20 mm is possible to be caused by CO<sub>2</sub> diffusion in leaf air spaces.<sup>80</sup> It shows potential possibility of this mechanism of VP propagation in plants.

It was also shown that water-soluble chemical agents (fluorescent dyes, radioactive isotopes) can propagate throughout the plant body with a velocity that was similar to the velocity of VP transmission.<sup>6,47,50,81</sup> As a result, an alternative mechanism of wound substance transmission was suggested. According to Malone,<sup>59</sup> local damage increases hydraulic pressure in the stimulated zone; in turn, this pressure growth induces acropetal and basipetal water flows in xylem, which transfers wound substances ('hydraulic dispersion'). Another possible way of wound substance propagation was suggested previously.<sup>47,56</sup> It is known that water flows in xylem are rather turbulent,<sup>82</sup> and diffusion in turbulent flow is 3–4 orders of magnitude faster than molecular diffusion,<sup>79</sup> and velocity of turbulent diffusion is similar to the speed of VP propagation.<sup>47</sup> Therefore, we proposed that wound substance propagation is based on turbulent diffusion.<sup>47,56</sup>

Both mechanisms of wound substance propagation (hydraulic dispersion and turbulent diffusion) suppose that this propagation can be intensified by damage-induced changes in hydraulic pressure (hydraulic wave transmission), i.e., the chemical and hydraulic mechanisms cooperate during VP propagation.<sup>47,59</sup>

### Mechanisms of variation potential generation

Both potential mechanisms of VP propagation rely on damage-induced signals that influence ion transporters in plant cell plasma membranes, which are ligand-dependent (chemical mechanism) or mechano-sensitive (hydraulic mechanism). Therefore,

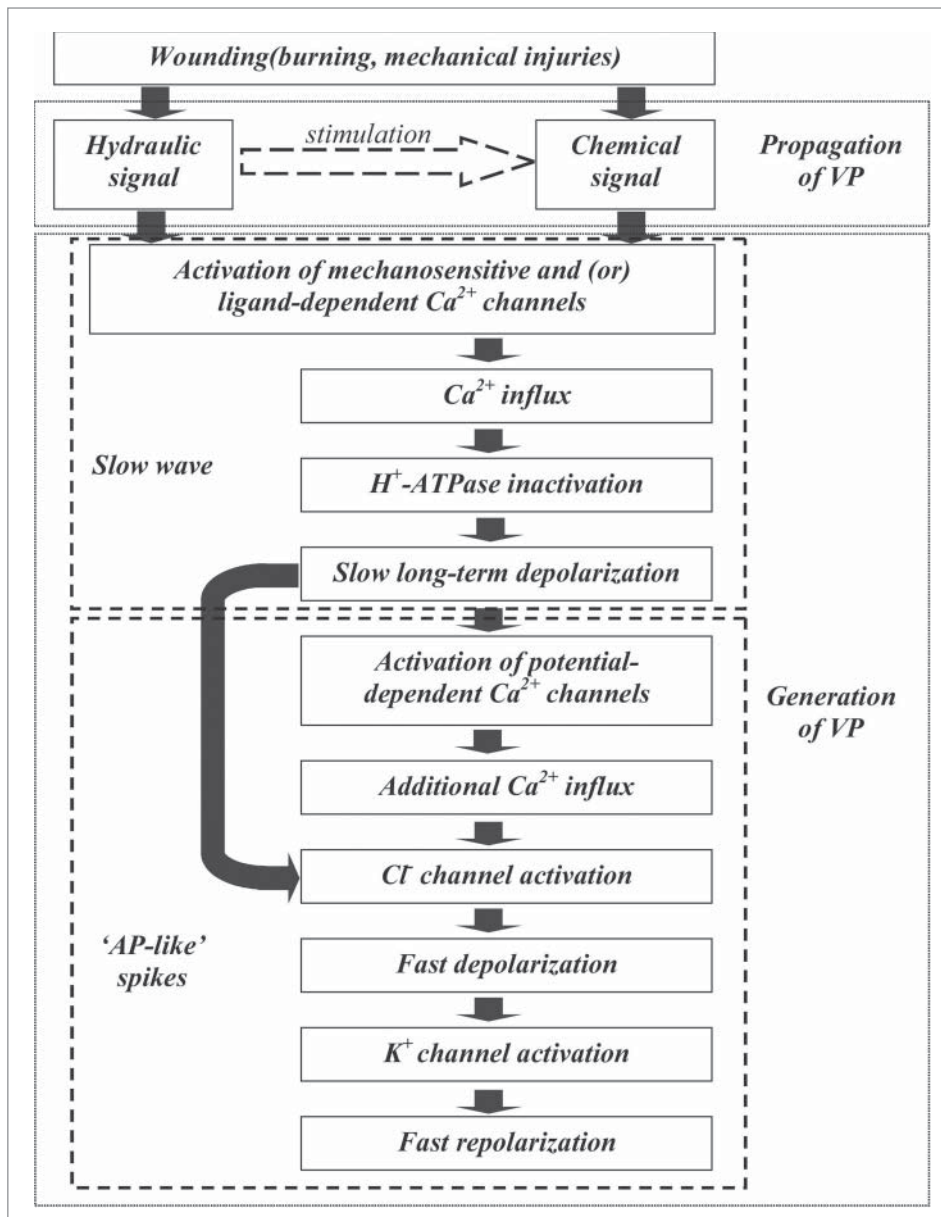
discerning the nature of these transporters is key to understanding VP generation mechanisms.

In contrast to AP, which is mainly based on passive ions fluxes, VP generation is traditionally connected with transient H<sup>+</sup>-ATPase inactivation.<sup>7,13,14</sup> This hypothesis was initially supported by inhibitor analyses. It was shown that metabolic inhibitors (CN<sup>-</sup>, NaN<sub>3</sub>) decreased VP amplitude and velocity of depolarization, or suppressed generation of variation potential in pea, pumpkin, tomato and *Bidens pilosa*.<sup>43-46,54</sup> Katicheva et al.<sup>83</sup> showed that amplitude of VP and its depolarization and repolarization velocities were essentially reduced in wheat after treatment by sodium orthovanadate, a specific inhibitor of H<sup>+</sup>-ATPase. It should be noted that the repolarization phase of VP was more suppressed by inhibitor treatment than depolarization.<sup>44-47,54,83</sup> Activation of H<sup>+</sup>-ATPase by fusicoccin induced the opposite effect and VP amplitude increased under the treatment.<sup>49</sup>

Participation of H<sup>+</sup>-ATPase inactivation in VP generation has also been shown by using different external pH conditions. Decrease in pH from 7 to 4 induced depolarization that was caused by lowering of H<sup>+</sup>-ATPase activity<sup>44,45</sup>. VP amplitude was low under these conditions. Application of the protonophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), which increases permeability of the plasma membrane for H<sup>+</sup>, decreased VP amplitude.<sup>44,45</sup>

Changes in extracellular and intracellular pH, which were observed during VP development, also supported an important role of H<sup>+</sup>-ATPase inactivation in generating variation potentials. Using potentiometric and fluorescence methods, it was shown that VP generation was accompanied with apoplast alkalization<sup>26,29,37,46,49,84</sup> and that its magnitude varied from about 0.2<sup>23</sup> to about 0.7<sup>26,34</sup> pH units. pH in cytoplasm was also affected by VP and decreased in the range of 0.3 to 0.6 pH units.<sup>23,26</sup> Dynamics of pH changes were similar to the dynamics of the electrical response,<sup>23,46</sup> VP amplitude was correlated with magnitude of pH change in the apoplast and cytoplasm.<sup>23</sup>

These results show that changes in H<sup>+</sup>-ATPase activity are an important mechanism of VP generation: depolarization caused its inactivation and repolarization was associated with its reactivation. However, the question 'Is H<sup>+</sup>-ATPase inactivation the sole mechanism of variation potential?' is still under investigation. Presence/absence of changes in conductivity of the plasma membrane is one potential argument that supports/rebutts the participation of ion channels in VP generation. According to Stahlberg et al.,<sup>54</sup> VP development did not induce changes in plasma membrane conductivity in pea, and that was considered as an argument supporting absence of ion channel participation in VP generation. However, investigation of wheat leaves<sup>83</sup> showed that VP generation was accompanied with increased conductivity of plasma membranes, and that observation supports activation of ion channels during VP development. These contradictory data can be explained by the complicated nature of total plasma membrane conductivity, which includes ion channel conductivities as well as H<sup>+</sup>-ATPase conductivity. As a result, total conductivity can be weakly changed with increase of ion channel conductivities (ions channel activation) and decrease of H<sup>+</sup>-ATPase conductivity (H<sup>+</sup>-ATPase inactivation) taken together. Therefore,



**Figure 2.** Hypothetical mechanism of VP generation and propagation. Propagation of chemical signal, hydraulic signal or combination of these signals (hydraulic dispersion and turbulent diffusion) activated ligand-dependent or mechano-sensitive  $\text{Ca}^{2+}$  channels.  $\text{Ca}^{2+}$  influx inactivates  $\text{H}^{+}$ -ATPase and induces long-term depolarization. Depolarization to AP threshold activates potential-dependent  $\text{Ca}^{2+}$  channels. Additional  $\text{Ca}^{2+}$  influx and plasma membrane depolarization activate potential-dependent  $\text{Cl}^{-}$  channels, and, later,  $\text{K}^{+}$ -channels. As a result AP-like spike is formed.

participation of passive ion channels in VP generation requires more detailed analysis.

Participation of passive  $\text{Ca}^{2+}$  influx is the most thoroughly investigated VP generation mechanism. It was shown that inhibition of  $\text{Ca}^{2+}$  channels and (or) lowering of external concentrations of calcium ions essentially decreased VP amplitude or suppressed this electrical reaction in pumpkin, wheat, barley, tomato and *Bidens pilosa*.<sup>37,44,46,83</sup> Removal of  $\text{Ca}^{2+}$  can also decrease number of AP-like spikes during VP development<sup>43</sup>. Moreover, VP generation was accompanied with decreased

concentration of calcium ions in the apoplast<sup>34</sup>. These results show that  $\text{Ca}^{2+}$  influx is needed for development of VP. Also, it should be noted that strong local stressor can induce propagation of long-distance calcium signals in plant leaves, and parameters of these signals are similar with parameters of VP propagation.<sup>85</sup>

According to several studies,<sup>43,45,46,56,83</sup>  $\text{Ca}^{2+}$  influx initiates VP generation, inducing  $\text{H}^{+}$ -ATPase inactivation and, possibly,  $\text{Cl}^{-}$  channel activation;<sup>56</sup> i.e.,  $\text{Ca}^{2+}$  plays a regulator role in VP development. However, other studies<sup>49</sup> have shown that inhibition of  $\text{Ca}^{2+}$  channels only weakly influenced VP parameters in pea that could be connected with  $\text{Ca}^{2+}$  flux from internal sources during VP development.

Possible participation of anion and potassium channels on generation of VP has received significant attention. Several works<sup>44</sup> showed that  $\text{Cl}^{-}$  and  $\text{K}^{+}$  permeability of the plasma membrane was not changed during the repolarization phase of VP in *Bidens pilosa*. Moreover, inhibitor analysis did not show the influence of anion and potassium channels on slow wave parameters in pea and tomato.<sup>43,49</sup> However, we have shown that VP generation was accompanied with a decrease in plasma membrane electrical resistance,<sup>83</sup> and that this resistance change was affected by anion and potassium channel blockers. VP amplitude decrease or full suppression of these electrical signals under the action of ion channel blockers were also shown.<sup>46,86</sup> Potentiometric analysis showed that VP generation was accompanied by an increase in external concentrations of  $\text{Cl}^{-}$ .<sup>37,46</sup> What seems apparently contradictory in

these different studies may be explained by differences in ion mechanisms among different VP components. We hypothesized<sup>56</sup> that activation of anion and potassium channels is the main mechanism of AP-like spikes and initial fast depolarization, and transient inactivation of  $\text{H}^{+}$ -ATPase is the key mechanism for long-term depolarization.  $\text{Ca}^{2+}$  influx triggered both  $\text{H}^{+}$ -ATPase inactivation and anion channel activation.

**Figure 2** shows hypothetical mechanisms of VP generation and propagation. Accordingly, it shows propagation of a chemical or hydraulic signal or combination of these signals (hydraulic



dispersion and turbulent diffusion) activated via ligand-dependent or mechano-sensitive  $\text{Ca}^{2+}$  channels. Influx of calcium ions inactivates  $\text{H}^+$ -ATPase and induces development of long-term depolarization. Depolarization to the AP threshold activates potential-dependent  $\text{Ca}^{2+}$  channels. Additional  $\text{Ca}^{2+}$  influx and plasma membrane depolarization activates potential-dependent  $\text{Cl}^-$  channels, and, later,  $\text{K}^+$ -channels. As a result, an AP-like spike develops. Thus, according to the scheme, VP generation is connected with long-term  $\text{H}^+$ -ATPase inactivation (mainly long-term depolarization) and with anion and potassium channel activation (mainly AP-like spikes); and  $\text{Ca}^{2+}$  influx is possible to trigger both mechanisms.

Mechanisms of VP and AP generation are very similar. Depolarization of both electrical responses is connected with passive ion fluxes as well as with  $\text{H}^+$ -ATPase inactivation. Differences of VP and AP properties are possibly caused by different initiation mechanisms for these signals.<sup>14</sup> Activation of potential-dependent  $\text{Ca}^{2+}$  channels is the first stage of AP generation; whereas VP initiation is connected with activation of ligand-dependent (chemical mechanism) or mechano-sensitive (hydraulic mechanism) calcium channels. These differences allow for different participation of active and passive mechanisms in VP and AP generation.

### Simulation of variation potential

VP simulation can be an effective method for the theoretical analysis of mechanisms of variation potential generation and propagation.<sup>47,56</sup> There are several interconnected problems for simulation of VP: (i) modeling of VP propagation, (ii) modeling of VP generation, and (iii) description of irregular dynamics of the electrical potential, which can reflect VP propagation and VP generation.

One-dimensional systems can be suitable models for theoretical investigations of VP propagation,<sup>44</sup> subject to significant excess of stem length compared with the transverse dimension. The chemical mechanism of VP propagation has been theoretically tested in our previous work<sup>47,56</sup> using the diffusion equation. It was assumed that a wound substance was instantly generated at wounding, and then it diffused along xylem vessels and induced VP generation when its concentration reached the threshold mean. Under this set of assumptions, the diffusion equation described well VP propagation at the diffusion coefficient (D), equaling about  $0.05 \text{ cm}^2 \text{ s}^{-1}$  in wheat.<sup>44</sup> This D magnitude was 2000 times larger than the coefficient of diffusion of small molecules in a water solution (molecular diffusion).<sup>79</sup> This difference has been explained using turbulent diffusion,<sup>44</sup> which can develop in turbulent flows (e.g., in xylem flow)<sup>82</sup> and may be thousands of times larger than coefficients of molecular diffusion.<sup>79</sup> Experimental analysis using a radioactive marker showed that D equaled  $0.06 \text{ cm}^2 \text{ s}^{-1}$  after wounding in wheat.<sup>44</sup> Theoretical analysis of VP propagation in pumpkin showed that D was about  $0.12 \text{ cm}^2 \text{ s}^{-1}$ .<sup>56</sup> Thus simulation of VP propagation supports participation of turbulent diffusion in the transmission of chemical signals under wounding.

Modeling VP generation requires a detailed electrophysiological model of the plant cell. These models vary widely in range

and scope; there are models that were based on Hodgkin-Huxley model,<sup>87,88</sup> and models that were based on detailed descriptions of ion transport in plant plasma membranes.<sup>56,89-92</sup> An electrophysiological model that was based on our previous simulation of VP generation<sup>56</sup> takes into account  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  channels,  $\text{H}^+$ - and  $\text{Ca}^{2+}$ -ATPase,  $2\text{H}^+/\text{Cl}^-$ -symporter and  $\text{H}^+/\text{K}^+$ -antiporter, changes of ion concentrations in the cell and in the extracellular space, and buffers in the cytoplasm and apoplast. Analysis of the VP generation model<sup>56</sup> supports the necessity of  $\text{Ca}^{2+}$  influx for VP generation, the key role of  $\text{H}^+$ -ATPase inactivation in long-term depolarization, and participation of  $\text{Cl}^-$  channel activation in the generation of AP-like spikes. Intensities of ions fluxes during VP generation and their dynamics are also calculated using the model. Thus, simulation of VP generation supports mechanisms of VP that have been shown in experimental investigations.

An additional important result of theoretical analyses of VP generation and propagation is the simulation of irregular dynamics of electrical potential during VP. Investigations using the VP model show<sup>56</sup> that imitation of damage with different intensity (different extraction of wound substance) induces different electrical potential dynamics during VP. This result is in good accord with data on variability of VP shape<sup>7,46</sup> and about dependence of VP parameters on wounding intensity. Also, the electrical potential dynamics depend on distance from the wounding zone; and that the distance dependence is in good accord with experimental data.<sup>46,56</sup> The VP model has taken into account VP generation as well as VP propagation, and can describe the irregular shape of variation potential.

## Conclusions

There are 2 main questions that are connected with generation and propagation of variation potential. First, is the wound-induced signal chemical, hydraulic, or a combination of the 2? Second, are the passive ion fluxes that participate in VP generation or  $\text{H}^+$ -ATPase inactivation the sole mechanism of variation potential development? We propose that the solution to both questions involves a combination of mechanisms. There is good evidence to connect VP propagation with an interaction between chemical signals and a hydraulic wave. VP generation is a combined process, including long-term  $\text{H}^+$ -ATPase inactivation (long-term depolarization) and short-term activation of anion and potassium channels (AP-like spikes). Testing these hypotheses should form the basis for future VP investigations.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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