Hydraulic and electrical transmission of wound-induced signals in Vitis vinifera

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Abstract. The nature and characteristics of the electrical response to wounding in the woody plant Vitis vinifera L. were examined. Following burning of a small area of a leaf, bioelectrical events spread throughout the shoot. The heat wound triggered stem deformations (widening–contraction), which preceded changes in biopotentials and that are interpreted as reflecting wound-induced hydraulic signals. It also caused marked decrease of extracellular resistance in stem tissues, starting about 15 s after stimulation, while intracellular resistance did not show any modification. Under an N₂ atmosphere (hypoxic conditions), action potentials disappeared and the amplitude of variation potentials decreased approximately 50%. At saturating humidity variation potentials were completely eliminated, while action potentials were evident. Taken together, the results demonstrate that action and variation potentials differ both in their mechanism of propagation and electrogenic nature. Action potentials are ‘genuine’ self-propagating electrical signals travelling at a velocity of about 10 cm s⁻¹, with a metabolic nature involving active components (electrogenic pumps). Variation potentials are a ‘local’ response to the passage of an hydraulic wave. Results support the hypothesis that both ion channels and pumps are involved in variation potential depolarisation.

Keywords: Action potential, cellular resistance, variation potential, Vitis vinifera, wounding.

Introduction

The wounding of a leaf or a part of the shoot is known to cause variations in the extracellular electrical potential measured with surface contact electrodes (Van Sambeek and Pickard 1976; Shiina and Tazawa 1986; Wildon et al. 1989) or with platinum or silver wires inserted directly into the tissues (Roblin 1985; Zawadzki et al. 1995). Wound- or stimulus-induced electrical phenomena in plants consist of (1) a so-called variation potential (VP) or ‘slow wave’, which appears as a wave of negativity with a variable length, shape and amplitude, capable of passing through dead tissues (Roblin and Bonnemain 1985; Malone 1996), and (2) briefer and faster signals, called action potentials (AP), considered to be real self-propagated electrical signals (Pickard 1973; Malone and Stankovic 1991; Stankovic et al. 1997). Though the pathway of APs is uncertain, intracellular recordings tend to locate the activity in the phloem parenchyma (Samejima and Sibaoka 1983) or in the phloem sieve tubes (Wildon et al. 1992).

Variation potentials have been reported following wounding (heating, burning, cutting), in Lycopersicon esculentum, Ipomoea hederacea, Cucurbita pepo, Xanthium strumarium, Gossypium hirsutum (Van Sambeek and Pickard 1976), Vicia faba (Roblin 1985; Robin and Bonnemain 1985), Helianthus annuus (Zawadzki et al. 1991, 1995), Luffa cylindrica (Shiina and Tazawa 1986). Comprehensive reviews on action potentials in higher plants have been written by Sibaoka (1966, 1969) and Pickard (1973).

The nature and transmission of these electrical events are a vexed question. Ricca (1916) showed that burning part of a leaf of Mimosa pudica or M. spegazzinii caused the release of a factor (‘Ricca’s factor’, Van Sambeek and Pickard 1976) which spread from the xylem to nearby leaves and caused them to droop immediately. Houwink (1935) showed that the passage of ‘Ricca’s factor’ resulted in an electrical response in the cells. Different interpretations on the nature of ‘Ricca’s factor’ have been proposed: a depolarising chemical substance (Pickard 1973; Van Sambeek and Pickard 1976) or a propagating electrical signal (Wildon et al. 1989). Recently, however, evidence was shown that ‘Ricca’s factor’ is an hydraulic signal in seedlings of wheat (Malone and Stankovic 1991; Malone 1992), tomato (Malone and Alarcon 1995) and pea (Stahlberg and Cosgrove 1992, 1997).

Numerous papers have been published on the study of variation and action potentials. However, only rarely have the researches focused on woody plants (Tilia cordata and Prunus avium, Boari and Malone 1993; Salix viminalis, Fromm and Spanwick 1993) although it is in such plants that the need for rapid and efficient signals other than chemicals (hormones) becomes more obvious. Instead, they have been mainly limited to study the electric signals in herbaceous or in sensitive plants like Mimosa pudica and related species because of their visible response to the stimuli (Haberlandt 1890 cited in Ricca, 1916; Ricca 1916; Houwink 1935; Weintraub 1952; Sibaoka 1962, 1969; Malone 1994).

The aim of the present paper is to describe the nature and characteristics of the electrical response to wounding in Vitis vinifera, with the purpose of distinguishing the mechanism of propagation and the possible involvement of passive
(ionic diffusion) and/or active components (electrogenic pumps) in the generation of the different electrical events: APs and VPs, contributing to the identification of these signals that are frequently a matter of controversy.

Materials and methods

Plant material and growth conditions

Vitis vinifera L. cv. Sangiovese (clone SSF9A548) plants, 2 years old, grafted onto Kober SBB, and grown in pots containing a 50:50 (v/v) mixture of sandy gravel and peat, were used. Plants were grown in a greenhouse at 15–22°C, in normal humidity and without artificial light. Plants of similar appearance with two or three shoots 70–120 cm long were selected for the experiments.

Plants were brought into a laboratory 4 days before the experiment, and additional light was furnished to the plants (from fluorescent tubes; 110 µmol m⁻² s⁻¹ at canopy height); temperature was between 20 and 26°C and the relative humidity ranged from 40 to 55%.

Surface potential measurements

Plants selected for the experiments were placed in a Faraday cage and illuminated by four 36 W fluorescent tubes Gro-Lux, 120 cm long, placed around the cage at about 70 cm from the plants. Non-polarisable Ag–AgCl pelletted electrodes were routinely connected to the shoots of the plants by means of a conductive aqueous gel of the type commonly used in ECG (electrocardiograms). The employment of a conductive gel was more comfortable and less time-consuming, in comparison with the elaborate experimental setups normally used to maintain contact between electrodes and plant. Furthermore, a minimum of conductive gel was sufficient to maintain contact for over 24 h without any further treatment.

Some experiments were duplicated using silver electrodes (wires 0.4 mm in diameter) inserted into the shoot or using a salt-bridge consisting of a agar-coated cotton thread protruding from a glass pipette containing 0.4 mm in diameter) inserted into the shoot or using a salt-bridge consisting of a agar-coated cotton thread protruding from a glass pipette containing 1 M KCl / 1% agar as in Van Sambeek and Pickard (1976). Results were always very similar to those achieved with the conductive gel, although often with a smaller amplitude of the signals. An Ag–AgCl pelletted electrode (identical to the working electrodes) in a plastic tube containing 3 M KCl / 2% agar was impaled in the wet soil and was grounded. After electrode connection, a settling time of 1 h was allowed prior to the start of the experiment. Localised scorching of leaves was applied by passing a flaming match for 3 s under an area of about 1 cm².

The electrodes were connected by screened cables to a four-way high input impedance (10¹⁴ Ω) electrometer (homebuilt based on an AD 645 JN operational amplifier). The output signals from the electrometer were low-pass filtered, amplified and connected via a multichannel A–D convertor card (Lab-PC-1200 National Instrument, USA) to a P133 personal computer.

Impedance measurements

For the impedance measurements two Ag–AgCl pelletted electrodes were put in contact with the shoot using a conductive paste (of the type commonly used for ECG) in order to keep the electrode/tissue interface polarisation to a minimum (Mancuso 1999). The absolute impedance was then measured at two frequency points (20 Hz and 1 MHz) using an impedance meter (LX 1192, Nuova Elettronica, Italy). The input voltage level of the signal was 30 mV (rms).

Strain measurements

Changes in stem deformation (widening–contraction) were measured using miniature strain gauges (length 2 mm; width 1.6 mm; RS 632-124; RS Components, UK). Four miniature strain gauges, placed on the opposite sides of the shoot, and connected in a Wheatstone bridge, to increase the effective gauge factor and thus the sensitivity to about 1 microstrain (1 microstrain is equivalent to an extension of the gauge of 0.0001%), were used for each measurement. Two full bridge circuits, with active gauges in all four arms, were fixed on the shoot with a short length of adhesive tape, to measure simultaneously the deformation in two zones, 25 cm apart. Strain gauges were run from a homebuilt strain gauge amplifier based on the chip RS 846-171. Voltage outputs were low-pass filtered and fed to a personal computer via a multichannel A–D convertor card.

Saturating humidity and hypoxia

Saturating humidity conditions were obtained by enclosing well-watered plants in polyethylene bags for 6 h before the starting of the experiment. The heat wound was applied without opening the bag, using a metal block, heated for 10 min in boiling water and set for 10 s on the leaf (through the thin layer of polyethylene). Hypoxia was obtained by enclosing the plants in polyethylene bags. Oxygen was eliminated by blowing N₂ into the bag. The time necessary to take the O₂ content to zero was determined as 30 min using a Clark type electrode for pO₂ (E.C.D. model 0225, Italy), connected to a monitor. The heat wound was applied in the same way as for saturating humidity experiments. Experiments were begun only after the end of the hypoxia-induced depolarisation (elimination of the active component of the transmembrane potential), when a stable surface potential had been reached.

Results

Surface potentials associated with wounding

When a grapevine leaf is damaged with a flaming match there is a rapid fluctuating electrical response in the shoot. Fig. 1 shows a typical recording. A few seconds after the stimulus, the first electrode placed on the shoot below the damaged leaf, records a rapid negative-going shift of about 40 mV. This is followed by a relatively slower recovery back to the initial values and then by fluctuations with rapid spikes superimposed. The pattern described above was remarkably
similar in 24 of the 30 plants tested. The others presented more varying responses, and in one case, no signal. The velocity of propagation of the front of the main negative-going shift signal (variation potential) was $2.7 \pm 0.8 \text{ mm s}^{-1}$.

**Effect of wounding on the shoot diameter**

Changes in shoot diameter, after localised scorching of one leaf, were recorded to determine if a hydraulic component was involved in the generation of the variation potential. Results represented in Fig. 2a show a small transient decrease in diameter starting a few seconds after the remote burning, followed by a major increase, and developing with a half-time of about 1 min with a rate of propagation of the front of the signal of $15 \pm 3.7 \text{ mm s}^{-1}$.

Fig. 2b, shows the recording of the surface potential simultaneously to the change in shoot diameter. The two phenomena were not simultaneous: the change in the variation potential started only when the increase in diameter had already reached its maximum.

**Transmission of wounding signals through the heat-killed petiole**

Transmission across the dead zones of tissue provides a method for distinguishing between hydraulic and electrical transmission of wound signals (Malone 1996). A region 25 mm long in the centre of the petiole in one leaf was solder-girdled applying a hot soldering iron (100°C) for 15 s from the two opposing sides of the petiole (Malone and Alarcon 1995). Microscopic examination (not shown), revealed the complete collapse of the phloem whereas the xylem apparently remained undamaged. When the leaf distal to the solder-girdled petiole was wounded, the variation potential passed throughout the heat-killed zone whereas the action potentials were completely suppressed (Fig. 3).

**Transmission of wounding signals at saturating humidity**

To eliminate the hydraulic component of the wound signals, well-watered plants were enclosed for 6 h before experiment in polyethylene bags at saturating humidity conditions. Fig. 4a shows a representative recording: the main negative-going shift signal (VP) was completely eliminated but, at the same time, the presence of action potentials propagating along the shoot with a velocity of about 100 mm s$^{-1}$ was evident. In addition, at saturating humidity, no variation of the diameter of shoots appeared following wounding (Fig. 4b).

**Transmission of wounding signals under hypoxia**

To test the existence of ATP-dependent mechanisms involved in the transmission of wound-induced signals, plants were enclosed and wounded under a N$_2$ atmosphere. In these conditions action potentials were not apparent (Fig. 5), and the average amplitude of the VP decreased approximately 50% (Fig. 5; Table 1).
Effect of wounding on the extra- and intracellular resistance

Impedance variations of the shoots following the burning of a leaf were recorded to test the effects of wounding on extra- and intracellular resistances. When a low frequency alternating current is applied to plant tissue it flows through extracellular spaces, its passage through the symplast being limited by the high impedance of the membrane. With increased frequency, the amount of current that passes through the symplast increases consequentially with the decrease of membrane impedance (Cole 1968). Impedance measurements made at low (20 Hz) and high (1 MHz) frequencies, therefore, reveal information about extracellular fluids.

Fig. 6 represents the effect of wounding on the extracellular \( R_e \) and intracellular \( R_i \) resistances (impedance at 20 Hz and 1 MHz, respectively): \( R_e \) showed a rapid diminution followed by a more gradual recovery, while \( R_i \) did not suffer any modification. Repeating the experiment at high humidity conditions (no hydraulic signals), the passage of the action potentials did not show any effect on the extracellular and intracellular resistances (Table 1). Under hypoxic conditions, the effect of wounding on \( R_e \) and \( R_i \) was similar to the one recorded in normal conditions (Table 1).

Discussion

Research during the last few years has showed that electrical events are the early responses elicited in the plants by wounding (Roblin and Bonnemain 1985; Malone and Stankovic 1991) or by environmental phenomena (Gunar

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>VP</th>
<th>VP amplitude (mV)</th>
<th>AP</th>
<th>AP amplitude (mV)</th>
<th>( R_e )</th>
<th>( R_i )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Yes</td>
<td>48.4 ± 6.0</td>
<td>Yes</td>
<td>46.5 ± 9.2</td>
<td>Decrease</td>
<td>Stable</td>
</tr>
<tr>
<td>Under ( N_2 )</td>
<td>Yes</td>
<td>23.9 ± 4.8</td>
<td>No</td>
<td>—</td>
<td>Decrease</td>
<td>Stable</td>
</tr>
<tr>
<td>At saturating humidity</td>
<td>No</td>
<td>—</td>
<td>Yes</td>
<td>42.3 ± 3.4</td>
<td>Stable</td>
<td>Stable</td>
</tr>
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Fig. 4. (a) Kinetics of wound-induced change in surface potential in grapevine plants at saturating humidity status, showing the elimination of the variation potential. At the time indicated by the arrow, a small area of one leaf was wounded without opening the bag (through the thin layer of polyethylene) using a metal block, heated for 10 min in boiling water. The first (more apical) and second (more basal) electrodes were separated by 20 cm. The distance from the injured site to the first electrode was about 20 cm. The inset expands a portion of the two traces showing an action potential propagating through the shoot from the first to the second second electrode at a velocity of about 100 mm s\(^{-1}\). (b) Kinetics of wound-induced increase in shoot diameter at saturating humidity status. At the time indicated by the arrow, a small area of one leaf was scorched with a flaming match for 3 s.

Fig. 5. Kinetics of wound-induced change in surface potential in grapevine plants under \( N_2 \) atmosphere showing the elimination of the action potentials. The two traces show a variation potential travelling through the shoot. At the time indicated by the arrow, a small area of one leaf was wounded without opening the bag (through the thin layer of polyethylene), using a metal block heated for 10 min in boiling water. The first (more apical) and second (more basal) electrodes were separated by 20 cm. The distance from the injured site to the first electrode was about 10 cm.
and Sinyukhin 1963; Stolarek et al. 1980; Minorsky 1985). The most important classes of electrical phenomena in non-sensitive plants are the APs and the VPs. The differences between these two phenomena are fundamental and frequently confused or not emphasised by many authors.

**Nature and characteristics of the VP**

As in many species, localised burning in grapevine leaves causes variation potentials propagating through the shoots (Fig. 1) with amplitudes and propagation velocities decreasing with increasing distance from the injured site (Table 2).

![Fig. 6. Kinetics of wound-induced change in extracellular (R_e) and intracellular (R_i) resistance in grapevine. At the time indicated by the arrow, a small area of one leaf was scorched with a flaming match for 3 s. The first (more apical) and second (more basal) electrodes were separated by 20 cm. The distance from the injured site to the first electrode was about 20 cm.](image)

Immediately before the appearance of the VP we see a very rapid, transient decrease in shoot diameter, followed by a massive increase (Fig. 2). This behaviour matches very well with the small, transient increase in the length of shoot followed by a major contraction showed by Stankovic et al. (1997). Taken together, the results presented here support the hypothesis that VP results from an hydraulic wave transmitted through the xylem, since: (a) VPs are associated with the systemic changes of the diameter of shoots (Fig. 2); (b) VPs are not stopped at dead regions of tissue (Fig. 3); (c) when xylem tension becomes negligible (as with plants at saturating humidity), VPs are not generated (Fig. 4) and (d) Re decrease (Fig. 6) shows the same temporal evolution of the transient decrease in shoot diameter and precedes the electrical signal. Therefore, as the hydraulic surge precedes the electrical changes, VP seems to be a local response to the passage of an hydraulic wave.

Little is known about the mechanism by which the hydraulic wave could cause such an electrical response. One major hypothesis (Stankovic et al. 1997) in explaining this connection holds that a large influx of water could be accompanied by the stretching of the membranes, so affecting mechanosensitive ion channels (Cosgrove and Hedrich 1991; Ding and Pickard 1993; Ramahaleo et al. 1996). The present results do not, however, indicate any large ion flux into the cells (Fig. 6). The stability of the intracellular resistance during depolarisation is more in agreement with a change in pump activity, the unit conductance of which is much smaller than that of channels (Bush 1993). However, under an N₂ atmosphere, VPs reduce their amplitude, but do not disappear (Fig. 5; Table 1), supporting both channel and pump involvement in the generation of the VP depolarisation.

A well documented response to injury in plant tissue is the opening of ion channels and the efflux of potassium (Spyropoulos et al. 1961; Oda 1976; Hanson et al. 1986; Davies 1987; Ries et al. 1994) and chloride (Gaffey and Mullins 1958; Oda 1976). On the other hand, the increase in intracellular calcium (a second messenger in the transduction

<table>
<thead>
<tr>
<th></th>
<th>Amplitude 1 (mV)</th>
<th>Amplitude 2 (mV)</th>
<th>Propagation velocity 1 (mm s⁻¹)</th>
<th>Propagation velocity 2 (mm s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variation Potentials</td>
<td>46.3 ± 8.3</td>
<td>35.3 ± 6.8</td>
<td>3.7 ± 1.2</td>
<td>2.3 ± 1.0</td>
</tr>
<tr>
<td>Action Potentials</td>
<td>39 ± 7.3</td>
<td>38.5 ± 8.3</td>
<td>96.6 ± 9.3</td>
<td>98.3 ± 7.1</td>
</tr>
</tbody>
</table>
Nature and characteristics of the AP

The present results indicate that AP differs from VP in its mechanism of propagation: (a) APs cannot pass through dead regions of tissue (Fig. 3); (b) APs are present in the plant at saturating humidity (Fig. 4); (c) the APs are not associated with changes in diameter of the shoots (Fig. 4b) and (d) the amplitude and propagation velocities of the APs are fairly constant through the shoot (Table 2). Therefore, APs are propagating electrical signals and not merely a local response to a hydraulic dispersal. In addition, they propagate at a velocity of about 100 mm s⁻¹ from one electrode to another, in a stable manner, well in harmony with a physiological signal. Differences between AP and VP are not limited to the mechanism of propagation but also include the electrogenic nature of the signal. APs disappear under an N₂ atmosphere showing a ‘metabolic’ nature in agreement with a physiological signal. The involvement of ATP-dependent pumps in the generation of APs is further supported as the results of the impedance measurements show no change in intracellular resistances during the passage of APs at saturating humidity (Table 1).

With a few exceptions (Gradmann and Mumert 1980; Weldon et al. 1992), the present findings are in contrast with the results of previous works, showing APs based upon activity changes of chloride, potassium and calcium ion channels. It is not clear if the differences in results are to be attributed to the different species used by other researchers, frequently green algae (Tazawa et al. 1987; Kourie 1994; Wayne 1994) or, in higher-plant systems, to a mis-interpretation of the electrical phenomena. APs are frequently confused in several published examples, so that phenomena referred to as AP by the authors, could also be interpreted as VP by other researchers (see e.g. Malone 1996, discussion on Zawadzki and Trebacz 1985 and Zawadzki et al. 1991). APs are self-propagating signals and should be brief, rapid, fully reversible events conforming to an all-or-none rule (Malone 1996). Following these criteria, most, if not all, of the published examples are not definable as APs.

In conclusion, grapevine plants exhibit different forms of rapid communication after a wound stimulus. Following perception of environmental stimuli, hydraulic and electrical signals, travelling for long distances in the plant, are early events in the coordination of the whole plant or some of its organs. Further studies are necessary, especially in woody plants, to clarify the ionic basis of the AP propagation (if specific ions, such as calcium, are involved in the process) and the mechanism of transformation of hydraulic signal into electrical one. These studies may improve understanding of a phenomenon that seems to be ubiquitous in all plants and could represent the main plant inter-organ signalling system.

References


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