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Review

Research progress on electrical signals in higher plants

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Abstract

This review introduces the characteristics of electrical signals in higher plants and their corresponding physiological significance, and describes in detail the impact of environmental factors (e.g. light and temperature) on the electrical potential of the plants. Also, we evaluate the measurement techniques used for electrical signals in plants, including intracellular measurement, extracellular measurement, measurement of the ion channel based on the patch-clamp technique and on the non-invasive microelectrode vibrating probe technique. We also give a brief review of the applications of these methods for investigating electrical signals in plants. The ionic mechanism of electrical activity in plants is then discussed in terms of environmental response in higher plants, and this is used to provide a theoretical basis for quantitative description of the electrical signals in plants. A model for interpretation of the electrical signal mechanisms in higher plants is discussed, but further experiments are required for the verification of this model.

Keywords: Ion channel; Temperature-induced; Light-induced; Ion mechanism; Measurement technology

1. Introduction

Bioelectrical activity due to stimulation in plants was first discovered by Burdon-Sanderson in 1873 [1]. Later, electrical signals in plant cells were discovered and studied in various plants [2–11]. Electrical signal is the most important physical signal in the organisms [12], and is capable of transmitting signals more quickly over long distances when compared with chemical signals (e.g. hormones). Recently, biologists have discovered that electrical signals are very important to many physiological activities [12–14]. In fact, the intracellular electrical signals serve as one of the basic modes of information transmission in plant cells [13]. Electrical signals have been shown to be involved in many processes in plant life, including respiration [15], water uptake [16], leaf movement [17] and biotic stress response [18]. Electrical signals may not only be induced by exterior stimulus in lower plants (e.g. *Characeae*) and in sensitive higher plants (e.g. *Mimosa* and *Flycatcher*), but they also play an important role in non-sensitive higher plants (e.g. *Vicia faba* L. and *Cucumber*) [3,5,6,8].

However, the ionic mechanism of the electrical signals in higher plants has not been fully determined [19,20]. Therefore, further investigation on the detailed properties of electrical signals in higher plants is necessary. In 1984, Schroeder et al. discovered that ion channels which normally exist in animals were also found in plant cells [21]. From then on, further studies were conducted to verify the kinds of ion channels which are essential to the generation of electrical signals in plants, and to determine whether these ion channels are the same as those in animal bodies. Experiments and analyses were conducted by means of the voltage clamp technique, the patch-clamp

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technique in combination with the ionic displacement method and by pharmacological approaches. These experiments and analyses showed that ion channels activated in the bioelectrical activity of plants in perceiving exterior stimulus are different from those in animals [22].

This review evaluates the measurement techniques for the electrical signals in plants, analyzes the light-induced and cold-induced bioelectrical activities of higher plants, and provides an overview of progress in the study of the mechanism for the generation of electrical signals in higher plants, in order to aid further study.

2. Electrical signals in plants

2.1. Types and features of electrical signals in plants

By considering the electrical excitation of plants, electrical signals in plants can be divided into three types: local electrical potential (LEP), action potential (AP) and variation potential (VP). LEP is a sub-threshold response induced by change in environmental factors (e.g. soil, water, fertility, light, air temperature and humidity). Although LEP is only locally generated and is not transferred to other parts of a plant, it has tremendous impact on the physiological status of the plant [23–26]. In contrast, both AP and VP can transmit from the stimulated site to other parts of the plant.

AP is induced by non-damaging stimuli (e.g. cold, mechanical and electrical stimuli), and is a widespread signaling phenomenon which can rapidly transmit information over long distances. AP has three major features that differ from the other electrical signals [12]: (1) AP transmits at constant velocity and maintains constant amplitude; (2) AP follows the all-or-none law, in that stimuli weaker than a certain threshold cannot trigger AP, but increases in the above-threshold stimulus strength do not change the amplitude and shape of AP; (3) after AP is generated, the cell membrane enters into absolute and relative refractory periods in succession. When the environmental stimulus reaches the threshold, AP is transmitted in all directions within the plant via the plasmodesma, and realizes the long-distance transmission of electrical signals via the phloem. AP can be gradually transmitted from the stimulation site to almost all living organs and tissues [27].

VP is induced by damaging stimuli (e.g. burning and cutting). Unlike AP, VP is characterized by a decrease in magnitude as it spreads away from the stimulated site [13]. Furthermore, VP does not follow the all-or-none law, as the magnitude and shape of VP vary with the intensity of the stimulus. VP depends mainly on the xylem for transmission, which can penetrate the dead tissues or organs inside the plants [13].

2.2. Physiological significance of electrical signals in plants

In addition to hydraulic and chemical signals, plant cells also produce and transfer bioelectrical signals as extracellular signals in response to changes in their environmental conditions [28]. Also, investigations confirmed that the neurotransmitters found in higher plants (e.g. acetylcholinergic) participated in the bioelectrical activity of higher plants to regulate the membrane permeability of plant cells and the physiological processes in higher plants [29,30]. Accordingly, electrical signals are probably the initial response of the plant to an exterior stimulus. This type of response may trigger physiological variation (e.g. elongation growth, respiration, moisture absorption, substance unloading at the phloem, reduction of the turgor pressure and variation of photosynthesis and transpiration, gas exchange, and activation and transcription of the protease inhibitor gene), and thus may mediate the interrelationships between each organ and tissue inside the plant as well as between itself and the external environment [31–33].

During plant growth, the electrical signals in plants may display different features due to weak light, high humidity and shortage of potassium [34]. This suggests the potential use of these electrical signals in applications indicating the

Table 1
Influence of electrical signals on the activities of higher plants and physiological significance.

Plant	Stimulus	Type of signal	Physiological response	Reference(s)
Dionaea muscipula	Mechanical	AP	Trap closure	[35]
Mimosa	Chilling	AP	Regulation of leaf movement	[36,37]
Lycopersicon	Electrical	AP	Evoking *pin2* gene expression	[33]
Hibiscus	Pollination	AP	Transient increase in the ovarian respiration rate	[38]
Salix viminalis L.	Chilling	AP	Effect on gas exchange	[39]
Zea	Chilling	AP	Reduction in phloem transport	[40]
Cucurbita pepo	Chilling	AP	Decrease in elongation growth of the stem	[41]
Cucumis sativus L.	Water stress	LEP	Affect stomatal opening and closing	[11]
Vicia faba	Light	LEP	Guard cell swelling and stomatal opening	[25]
Tomato	Wounding	VP	Enhancement of *pin* genes activity	[42]
Pisum	Wounding	VP	Inhibition of protein synthesis, formation of polysomes	[43]
Mimosa, *Populus*	Heating	VP	Transient reduction of photosynthesis	[44]
Helianthus annuus L.	Heating	VP	Decrease in elongation growth of the stem	[45]
Vicia faba	Heating	VP	Increase in respiration	[46]
Mimosa pudica	Heating	VP	Transient reduction in photosynthesis	[14]

physiological status of the plants for the adjustment and control of greenhouses.

Electrical signals in plants can bring local stimulation information to other cells, tissues and organs to make them respond appropriately. Closure of the trap in carnivorous plants (e.g. *Flycatcher*) represents the result of the transmission of electrical signals. AP can adjust the movement of leaves in some sensitive higher plants (e.g. *Mimosa* and *Loosestrife*). For non-sensitive higher plants, electrical signals also play an important role in physiological activities (e.g. gas exchange, pollination, fertilization and gene expression) [12]. Therefore, electrical signals are very important and have significant physiological effects on the plants (Table 1).

3. Approaches for the study of electrical signals in plants

Approaches to the study of electrical activities in plants include intracellular and extracellular measurements, the patch-clamp technique and the non-invasive microelectrode vibrating probe technique. Among these methods, the signal observed using extracellular measurement is due to the depolarization–repolarization process in a group of cells, while intracellular measurement can record the value of an individual cell membrane potential directly. The patch-clamp technique shows the characteristics of the ion channels, allowing *I–V* curves to be plotted, and makes it possible to understand the ion mechanism for change in membrane potential in a plant cell. Moreover, the non-invasive microelectrode vibrating probe technique is an excellent new electrophysiological approach which can measure the dynamic influxes and effluxes of ions and small molecules from cells and organs. It can show the channel activity of the membrane and link the molecular fluxes with the important physiological processes to which they are related. Different measurement techniques can show variation in the features of the electrical signals in plants at different levels. These approaches and their features are described in the following sections.

3.1. Extracellular measurement

Extracellular measurement can detect the electrical signals produced by multicells, and is applicable to the monitoring of an individual plant (Fig. 1). As electrical signals in plants are weak signals, they usually must be amplified and the recording device must have a high input impedance ($>10^9\ \Omega$) [11,19,31,47].

Extracellular measurement is mainly performed with two different types of measuring electrodes: surface contact electrodes and metal electrodes [13,19]. The surface contact electrode mainly refers to the calomel electrode, which adopts a suitable ion solution (or nutrient solution) to connect a salt bridge between the electrode and the plant. This type of measuring approach is not harmful to the plants, but the testing electrode medium (cotton thread or agar) may tend to dry out, which changes the ionic status of the region being measured, and thereby affects the test results eventually. Therefore, this approach is only applicable to short-term measurement of the electrical potential

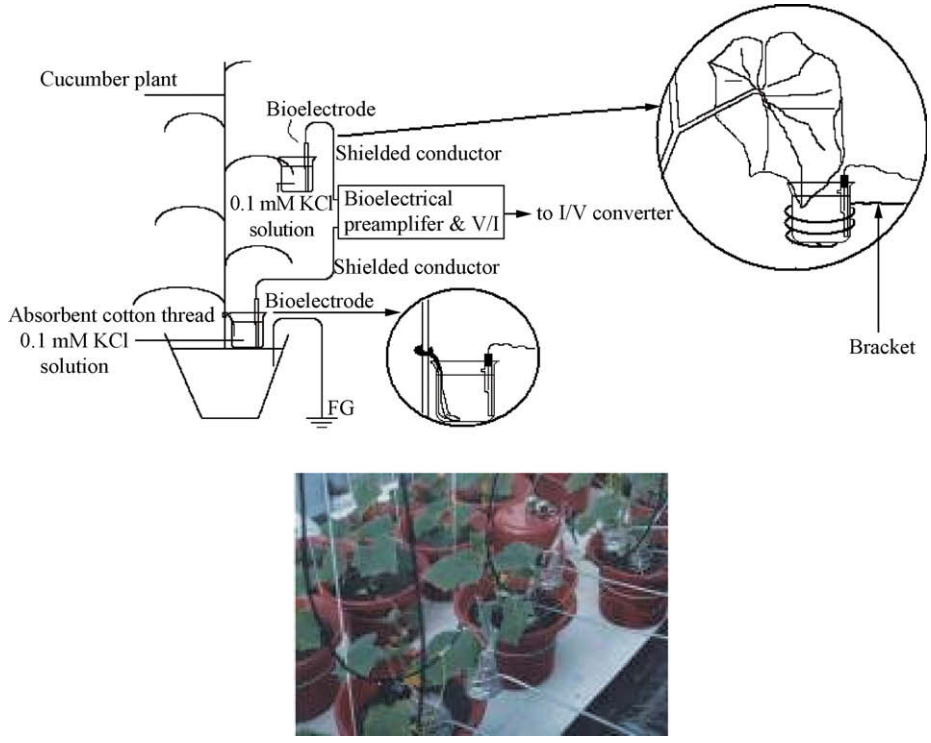

Fig. 1. Experimental set-up for detecting electrical signals in plants [11].

(<12 h). The metal electrode mainly refers to Ag/AgCl, platinum and silver electrodes, which are normally inserted into the plants to measure the membrane potential. The disadvantage of this approach is that it may cause slight mechanical damage to the plants. Nevertheless, it is applicable to long-term testing for the purpose of observing the long-term variation (>24 h) of plant electrical potential [47].

3.2. Intracellular measurement

The intracellular measurement is applicable to the observation and study of bioelectricity at the cell level, and normally uses a glass microelectrode with a tip diameter lower than 1 μm [13,31] inserted into the periphery of the living cell or into the cell body to observe the electrical activity of several cells or even a single cell. The intracellular measurement is performed with one electrode placed inside a cell while the reference electrode is situated in the bath solution surrounding the cell.

A glass microelectrode filled with a 0.3–3 M KCl solution is used as the measuring electrode, and the Ag/AgCl electrode can usually be used as the reference electrode; the glass microelectrode is then connected to an amplifier with high input resistance. The plant material must be immersed in the bath solution. The ingredients of the bath solution and their concentrations are determined by the various conditions of the plant cells to be tested. For instance, the ingredients of the bath solutions for measuring the epidermis and mesophyll cells of *Pisum sativum*, the mesophyll cells of *Arabidopsis* and the cells of *Conocephalum conicum* are different [3,48,49]. The microelectrode is inserted into the cell by means of a micromanipulator with a stereomicroscope, whereas the reference electrode is placed in the bath solution.

3.3. Patch-clamp recording technique

The patch-clamp recording technique was developed by Neher and Sakmann in 1976. From a whole cell to isolated membrane patches, the current of a single or multi-ion channel can be recorded [50]. Due to the use of the patch-clamp technique, the knowledge of ion channels in plants has been growing rapidly over the last twenty years. Studies of different plant species and various cell types have revealed that all subcellular membranes investigated so far (plasma membrane, tonoplast, plastidial and mitochondrial membranes) are equipped with a variety of channels exhibiting different ion selectivities and specific regulation mechanisms [51].

Since the gigaohm seal ($10^9\ \Omega$) technique in several recording modes was established in 1982, it has been applied to life sciences especially for plant physiology. The patch-clamp technique normally adopts the following four recording modes (Fig. 2): cell-attached or on cell mode, inside-out mode, outside-out mode and whole-cell recording.

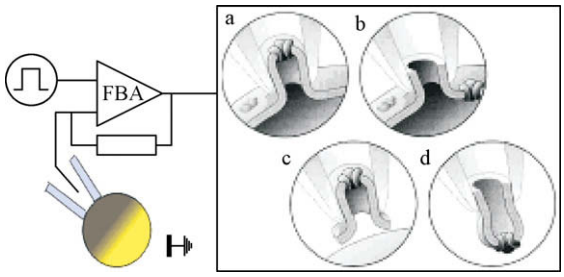

Fig. 2. Four recording modes of patch clamp. (a) Cell-attached or on cell mode; (b) whole-cell recording; (c) inside-out mode; (d) outside-out mode.

This simple picture of ions diffusing through a pore must be refined by the consideration of two properties common to all ion channels in higher plants. The first is selectivity. Selectivity implies the presence of binding sites for recognition of the ion during permeation, and the channels are often named in terms of the most prevalent permeation or after the ions of proposed physiological significance. However, it is important to recognize that selectivities are not absolute, and that many channels will conduct a range of ions to some extent. This property is reflected in the so-called ionic selectivity sequence for the channel, which can have great physiological significance. The selectivity of ion channels can be derived either by measuring the conductance of different ions through the channel or by determining the reversal voltage of the current through the channel (This is the membrane voltage where the net current is zero and reverses its sign). The second universal property of ion channels is their ability to reside in "open" or "closed" conformational states, which, respectively, either permit or do not permit ion permeation. This conformational switching can occur in response to ligands or to a change in the membrane voltage, after which the channels are activated (open) or deactivated (close).

Currently, the patch-clamp recording technique is used to record the ion channel current (Table 2) with regard to the cell membrane activity of plants and provides a basis for the study of the ionic mechanism of the electrical signals. In contrast to animal cells, plant cells have evolved unique electrical properties, mostly based on the transport of H^+, K^+, and anions. It is affirmed that fast and slow speed anion channels, as well as inward or outward K^+ channels are in existence on the cell membrane of plants. The relationship between the current and the voltage of the proton pump has also been discovered using this technique. This indicates that the proton pump is active over the whole process of the electrical activity of plants, and that the current increases with the elevation of membrane potential [52,53]. However, further research is required to determine whether the existence of the voltage-dependent Ca^{2+} selective channel in the membranes of higher plants predominates the depolarization [48,54–58].

The variation of cell membrane potential reflects the changes of ion channel conductance and the activity of the proton pump in a plant cell. Therefore, we can gain a

Table 2
Ion channels recorded by patch-clamp technique, which are activated in electrical activity of higher plants.

Channel	Physiological role during AP	Plant	Tissue	Reference(s)
Outward rectifying K^+ channel	Repolarization	*Vicia faba*	Guard cell	[59]
		Nicotiana tabacum L.	Suspension cell	[60]
		Vicia faba	Mesophyll cell	[61]
		Nicotiana tabacum L.	Mesophyll cell	[62]
		Arabidopsis thaliana	Guard cell	[63]
Inward rectifying K^+ channel	Hyperpolarization	*Vicia faba*	Guard cell	[64,65]
		Arabidopsis	Guard cell	[66]
Fast anion channel	Depolarization	*Vicia faba*	Guard cell	[67,68]
		Arabidopsis thaliana	Epidermal cell	[69]
Slow anion channel	Whole phase	*Vicia faba*	Guard cell	[70,71]
		Arabidopsis thaliana	Guard cell	[72]

better understanding of the electrical activity of plants and its physiological significance based on the knowledge of ion channel and the proton pump on the protoplasm membrane. The patch-clamp recording technique can be used directly to measure the single ion channel of a whole cell, which is favorable for the investigation of the ionic mechanism of plant cell response to external stimulation.

3.4. Non-invasive microelectrode vibrating probe technique

The non-invasive microelectrode vibrating probe technique was developed by Kühtreiber and Jaffe based on a computer-controlled measuring system with automatic positioning function designed in 1990 [73], and is the most advanced selective ion/molecule measuring technique used to link genetic/genomic data to cellular physiological behavior. Some of its key features (e.g. non-invasiveness, high spatial and temporal resolution) allow us to establish and quantify the causal links between membrane-transport processes and metabolic or other physiological processes in the cell under almost natural conditions. In this context, this technique may be considered as a connection between molecular biologists and whole plant physiologists or agronomists.

The method makes use of the special selective electrode to perform the measurement without contact with the sample under the automatic control of the computer, in order to measure the concentration (mM) of various small molecules and ions, and especially for the direct measurement of influx/efflux of ions in cell membranes in three dimensions. Currently, the non-invasive microelectrode measuring system (Fig. 3) can detect various ions and relevant parameters (e.g. H^+, Ca^{2+}, K^+, NH_4^+, Al^{3+}, Na^+, Cd^{2+}, NO_3^-, Cl^-, O_2, NO, CO_2, amino acids and temperature) [74].

The electrodes used in the non-invasive microelectrode vibrating probe technique include a reference electrode and an ion selectivity microelectrode. The microelectrode is composed of a glass pipette, an Ag/AgCl wire, an electrolyte and a liquid ion exchanger (LIX), as indicated in Fig. 4. This microelectrode vibrates between two positions (distance in terms of μm) at a specific distance dx, and the concentration gradient of the ion to be tested determines

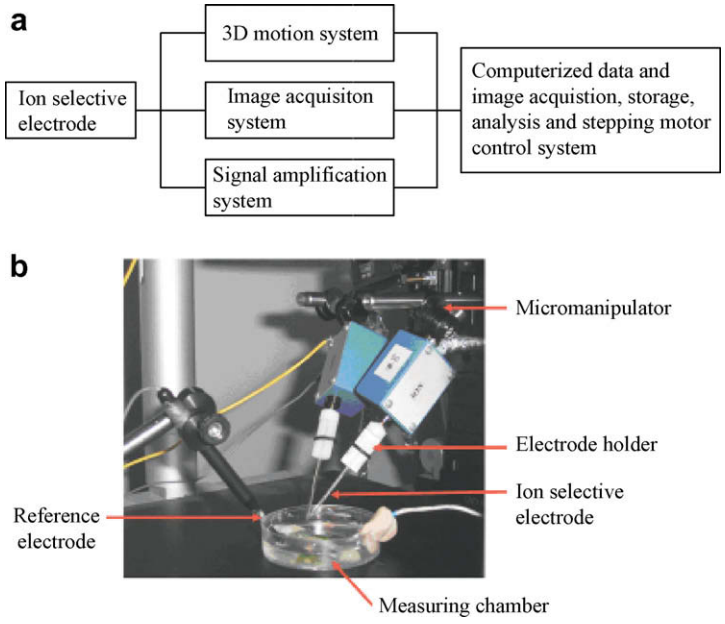

Fig. 3. Non-invasive microelectrode measuring system. (a) Diagram for system structure; (b) electrode position in experimental set-up (dual channel test).

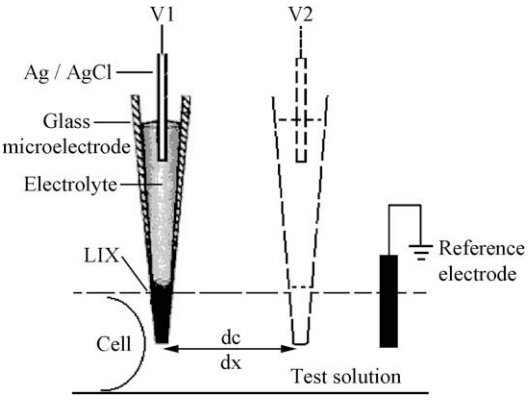

Fig. 4. Physical and mathematical principles of non-invasive microelectrode vibrating probe technique (mono-channel).

the voltages V_1 and V_2 between the reference electrode and the two points of the microelectrode [75]. The concentration difference between the two points can be calculated from the voltage gradient near the surface (V_1 and V_2) using the known voltage/concentration calibration curve of this microelectrode. It is possible to obtain the moving velocity (unit: pmol $cm^{-2}\,s^{-1}$) of this ion through the substitution of these diffusion constants in Fick's first law of diffusion formula: $J_0 = -D^{*}\,dc/dx$, where D represents the differential diffusion constants of the ion/molecule (unit: $cm^{-2}\,s^{-1}$). The flux rate is also known as the mole value of the ion/molecule passing through one square centimeter per second [74].

The non-invasive microelectrode is used to measure the ion/molecule activity around the materials to be tested, which has the following unique features when compared with the patch-clamp technique [74]: (1) the non-invasive microelectrode vibrating probe technique can detect the flux rate of the ion/molecule in differential directions, whereas the patch-clamp technique mainly aims to detect the ion channel; (2) the non-invasive microelectrode vibrating probe technique is not in contact with the materials tested, whereas the patch-clamp technique must be in contact with the tested materials using a high resistance seal; (3) the non-invasive microelectrode vibrating probe technique can be used to measure an individual cell, a group of cells, tissue, a separated organ and even small plants, whereas the patch-clamp technique can only be used to measure the cell membrane or plasma membrane patch of plants; (4) the temporal resolution of the non-invasive microelectrode vibrating probe technique is of second level with spatial resolution between 2 and 5 μm; whereas that of the patch clamp is of millisecond level with spatial resolution at approximately 1 μm.

Also, the patch clamp is a sophisticated method which requires high level technical and data interpretation skills, and the cell wall must be removed to establish the so-called "giga seal", enabling measurements of very low (pA range) currents through the prepared isolated plasma membrane patch in response to a series of voltage clamps. This may increase the difficulty of the experiments. In contrast, the non-invasive microelectrode vibrating probe technique has no need to remove the plant cell wall because it is capable of performing measurements without contact with the samples to be tested. It can be used to measure the ion/molecule transportation without causing any damage to the cells, tissues or organs. As an important supplement to the patch-clamp recording technique, the non-invasive microelectrode vibrating probe technique has provided a useful tool for the identification or verification of the functions of the conveying systems of biomembranes. Using the non-invasive ion-specific microelectrode ion-flux measurement technique, Shabala and Newman determined the kinetics of H^+, Ca^{2+}, K^+, and Cl^- fluxes and the changes in their concentrations near bean (*V. faba*) mesophyll and attached epidermis due to illumination. Also, they investigated on which ion acted as the depolarizing agent in the initial phases of plasma membrane depolarization [76].

4. The action of environmental factors on electrical signals in plants

4.1. Light-induced electrical activity of plants

4.1.1. Response of guard cell to light stimulus

One hundred years ago, Haake showed for the first time that light could trigger the bioelectrical activity of plants [77]. Changes in the light conditions may trigger variation in the potential of the guard cell membrane. The transition of the guard cell of a broad bean cultivated in a normal environment from light to dark (light-off) meant that the membrane potential would transfer from a hyperpolarized state to a depolarized state. Conversely, a transition from dark to light (light-on) would lead to guard cell membrane hyperpolarization.

Upon a dark/light transition, proton extrusion via the H^+-ATPase can result in the hyperpolarization which accompanies stoma opening [25,53,78,79]. Under the light condition, the average resting potential of the guard cell membrane of a broad bean is approximately −112 mV [25,80], which is slightly negative compared with the activation potential of the inward K^+ channel. Therefore, the inward rectifying K^+ channel is activated and then K^+ influx evokes an increase in the turgor pressure of the guard cell, which makes the stoma open. Upon a light/dark transition, the plasma membrane of the guard cell changes from the hyperpolarization state to the depolarization state, which leads to the inward rectifying K^+ channel becoming inactive. However, the anion channel and the outward K^+ channel would be activated at the time. K^+ and Cl^- effluxes would make the turgor pressure of the guard cell decrease and then the stoma would close.

4.1.2. Response of epidermal and mesophyll cells to light stimulus

Transition from dark to light can evoke a transient membrane depolarization in the epidermal cells and mesophyll cells of many kinds of plant [81–83]. Despite the same

light stimulus (dark to light), the changes of membrane potential in a mesophyll cell, an epidermal cell in contact with mesophyll, and an epidermal cell isolated from mesophyll are different. In the mesophyll cell, light could induce a large transient depolarization which is similar to that in the epidermal cell in contact with mesophyll. However, in the isolated epidermal cell, light could induce a small and variable transient depolarization. The light-off response resembles the inverse of the light-on reaction: an initial transient hyperpolarization followed by a depolarization [3].

Despite the fact that many different plants and different experimental techniques have been used to investigate the ionic mechanism of the light-induced depolarization in epidermal and mesophyll cells, the mechanism remains obscure. Some researchers insist that the influx of Ca^{2+} is the main depolarizing agent in light-induced electrical signals in mesophyll cells [82,84,85] and that K^+ and Cl^- are not required as depolarizing agents [86,87]. However, others insist that Cl^- efflux involves light-induced membrane depolarization [62,82]. Elzenga et al. concluded that the ionic mechanism differs between the epidermal and the mesophyll cells. They suggest that under the light stimulus, Cl^- efflux triggers the plasmalemma depolarization in mesophyll cells, whereas Ca^{2+} influx and the activity of the H^+ pump would trigger the plasmalemma depolarization in epidermal cells [3].

Light is an essential factor in the photosynthesis of plants. The capability of perceiving variation of light is of significant physiological importance for plants. This shows that light can induce electrical signals on the leaf tissue of plants. However, the responses of guard cells, epidermal cells and mesophyll cells to light are all different. In fact, the extracellular recording by surface contact electrode used to measure the membrane potential on a plant leaf can detect a mixture of the signals of the three types of cells. The use of advanced signal processing algorithms (blind signal separation, e.g. independent component analysis, ICA) may help to obtain each trial signal from the three types of cells, respectively. Modern signal processing methods were used in our previous work (e.g. wavelet transform) [88,89]. Further study of the ion mechanism of the light-induced electrical signals in plants is still necessary.

4.2. Cold-induced electrical activity in plants

Cooling of plant cells at a rapid rate would result in transient plasma membrane depolarization. According to the corresponding studies, cold stimulation can induce plasmalemma potential changes in many higher plants, such as *Glycine* roots, *Avena* and *Hordeum* coleoptiles, *Arabidopsis* mesophyll and *Allium* epidermal cells [48,90]. Krol et al. have recorded the transferable AP evoked by a cold stimulus in Liverwort *C. conicum* [49]. However, local potential induced by chilling but without AP has been recorded in the mesophyll cells of higher plants (e.g. *Arabidopsis thaliana*, *Helianthus annuus* and *V. faba*) [91].

Chilling could lead to an increase in cytoplasmic calcium concentration, and then result in plasmalemma depolarization [48,92–95]. The rise in cytosolic calcium has been proved to originate by Ca^{2+} influx through the plasma membrane and Ca^{2+} release from internal stores [12,93,96]. Some studies indicated that the Ca^{2+} influx is mediated by the calcium-permeable channels in the plasma membrane and that Ca^{2+} efflux is mediated by calcium pumps [94]. The activity of calcium-permeable channels is only dependent on the cooling rate and not on absolute temperature. That is to say, as long as there is a sufficient cooling rate, Ca^{2+} would flow into the cell through the calcium-permeable channels. However, the activity of the calcium pumps is dependent on absolute temperature, which increases with rise in temperature. On this basis, Plieth has established a mathematical model for the properties of temperature sensing in plants and has used it to simulate the changes of Ca^{2+} concentration in response to different temperature cooling rates in *Arabidopsis* roots.

5. Ionic mechanism of electrical signal in plant

AP in animals relies on the Na^+ and K^+ channels. In response to the stimulus, the Na^+ channel is activated first, and a massive Na^+ influx depolarizes the plasma membrane. The depolarization results in the opening of the outward rectifying K^+ channel with the Na^+ channel inactivated gradually. Massive K^+ efflux causes the repolarization of the membrane, and finally the membrane potential returns to the steady state. Also, the activity of the sodium pump can balance the K^+ and Na^+ concentrations inside and outside the cells. This is important for the plasma membrane to retain resting potential. Biologists have not yet discovered Na^+ channels and sodium pumps [26] in plants when probing the AP ionic mechanism. This suggests that the ionic mechanism of electrical signals in plants is different from that in animals.

Considering the numerous and different levels of investigations of the electrical activity in plants, it is necessary to make a qualitative and quantitative description of the ionic mechanism for the electrical activity of plants. The knowledge of electrical signals in lower plants as represented in *Chara corallina* is relatively clear. After the external stimulus is perceived, the Ca^{2+} would flow into the cell via the non-selective cation channel on the membrane. The elevation of Ca^{2+} concentration in the cytoplasm can activate the anion channel, and then Cl^- efflux depolarizes the plasma membrane. Afterwards, the outward rectifying voltage-dependent K^+ channel would be activated with the anion channel inactivated gradually. Meanwhile, outflow of K^+ would lead to the repolarization of the membrane. Based on the aforesaid mechanism, Beilby and Coster have established a modified Hodgkin-Huxley model for the electrical activity of *C. corallina* [2,97,98]. However, this model is not suitable to describe the electrical activity

of higher plants. At present, there is a generally accepted model of the ionic mechanism of electrical signals in higher plants. An AP is originated by calcium influx. The Ca^{2+} concentration in cytosol is then elevated, which leads to the activation of voltage-dependent anion channel, and Cl^- efflux depolarizes the plasma membrane. As the depolarization is sufficient to activate the outward K^+ channel, K^+ efflux would repolarize the membrane with the Cl^- channel gradually inactivating. A possible role in the conduction of an outward current in the repolarization phase of AP was attributed to the 40 pS potassium channel in cells of *V. faba* [22]. Anion channels have been characterized in the plasma membrane of mesophyll cells with unitary conductance of 32 pS [99], and 33 pS channels [100] have been examined in the plasma membranes of guard cells. Generally, K^+ efflux and the proton pump activity could lead to plasma membrane hyperpolarization. At this point, the voltage-dependent inward rectifying K^+ channel would be activated. Then K^+ influx and the proton pump activity would make the membrane return to the resting state [12,101,102]. Wang et al. have established a circuit model of the electrical signals of plants, and tried to build a modified H–H formula to describe it [103]. The model is shown in Fig. 5. In this model, the V_{K^+}, $V_{Ca^{2+}}$, V_{Cl^-} and V_{H^+} are ionic reversal potentials. G_{K^+}, $G_{Ca^{2+}}$ and G_{Cl^-} denote ionic maximum conductances. V and Cm denote the membrane potential and membrane capacitance, respectively. Istim is the stimulus current. In the depolarization phase of electrical signals, Ca^{2+} influx and Cl^- efflux take place. K^+ efflux and the activity of the H^+ pump induce the repolarization of the transmembrane potential.

Some issues still require further study. There is strong evidence that the influent Ca^{2+} originates from both the extra- and intracellular compartments during the excitation of higher plants, in the initial Ca^{2+} influx from the external solution through the plasmalemma, and the slower Ca^{2+} release from the internal stores [12,93,96,104]. The way in which Ca^{2+} passes through the plasma membrane requires further study. Some studies insist that Ca^{2+} traverses the plasmalemma via the Ca^{2+} channel, which is similar to the Cl^- and K^+ channels [54–56]. However, others contend that the selective Ca^{2+} channel does not exist on the plasmalemma, because no Ca^{2+} channel current has been recorded so far in higher plants. They propose that Ca^{2+} enters the cell through non-selective cation channels [48,57,58].

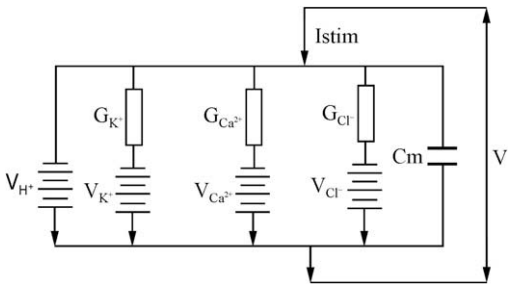

Fig. 5. A scheme illustrating the ion mechanism for membrane potential. V and Cm: the membrane potential and capacitance, respectively; Istim: the stimulus current; V_{H^+}, V_{K^+}, $V_{Ca^{2+}}$ and V_{Cl^-}: ionic reversal potentials; G_{K^+}, $G_{Ca^{2+}}$ and G_{Cl^-}: ionic maximum conductance.

6. Conclusions and future perspectives

In summary, the study of the mechanisms of electrical signals in higher plants in dynamic response to environmental changes during growth and development is inherently problematic. The kinetics and shape of AP differ strongly between animals and higher plants [22,67,105]. Despite the large number of reports describing electrical signals in higher plants, there are few quantitative mechanism models that yield reliable prediction of the measured shapes of electrical signals in higher plants [67], as the Hodgkin-Huxley equation does in animals. It is necessary to provide a mathematical model to further promote investigations of the electrical phenomena in plants. In order to develop a better quantitative prediction of the time-course of the electrical signals in higher plants, a more complete knowledge is required of the electrogenic ion transport systems involving their density, and the corresponding voltage-dependent kinetics. New measuring methods (e.g. the non-invasive microelectrode vibrating probe technique, the patch-clamp technique, as well as modern modeling and stimulation methods) can provide support for the development of an accurate kinetic model of the electrical signals in plants.

Due to the potential use of bioelectrical phenomena for indicating the physiological condition of plants in agricultural fields, there have been several attempts to analyze these signals and extract their features using statistical and signal processing methods; however, the results were unsatisfactory due to the lack of the biological and physiological data [106]. Combination of different electrophysiological techniques with modern signal processing methods may lead to the determination of the physiological responses that are yet not fully understood, and to interesting new discoveries.

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