Relationship between Growth and Electric Oscillations in Bean Roots

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ABSTRACT

Extracellular and intracellular electric potentials in bean roots are known to show electric oscillations along the longitudinal axis with a period of several minutes. The relationship between growth and the electric oscillations was studied using roots of adzuki (Phaseolus chrysanthus). We measured surface electric potentials with a multielectrode apparatus while simultaneously measuring elongation using a CCD camera and monitor. Roots having an electric oscillation grew faster than roots with no oscillation. Furthermore, elongation rate was higher in roots with higher oscillation frequency. Oscillation frequency had a strong dependence on temperature; i.e., Q10 was estimated at 1.7. These results suggest a correlation between electric oscillation and elongation.

A periodic electrical pattern is formed near the surface of the internodal cell of Characean species (13). Electric current patterns appear along the surfaces of roots (1, 4, 20, 25). Oscillations of surface electric potential also occur (7, 8, 16, 19, 22). Bean roots show spontaneously electric oscillations without a stimulus along the root surface with about 5 min period, which continues constant for over a few hours (16, 19). The amplitude of the oscillation is maximal in the elongation region and the phase of the oscillation in that region differs by 180° from that in the mature region. It is noticeable that the oscillation appears consistent (or coherent) in the mature region over several centimeters, because the phase and also the period are the same at any position (16, 19, 22). Resonance of oscillation occurs in such a way that the phase of osmotic pressure variation sets the phase of electric oscillation when an oscillation of osmotic pressure is applied to a root (7, 8).

In a previous paper (22), the membrane potential within the root was measured with a microelectrode technique (2) while the surface electric potential was measured using a multielectrode measuring method (4, 19, 20). In the elongation region, the membrane potential of epidermal cells oscillated with the same period and the same phase as the surface potential. In the mature region, on the other hand, the membrane potential did not oscillate, although the surface potential oscillated. It was concluded from a theoretical analysis using an equivalent electrical circuit that the source of surface-potential oscillations existed in the membrane at the xylem/parenchyma interface in the elongation region and the oscillation was propagated to the mature region inside the parenchyma. A mechanism for the oscillations has not, however, been elucidated especially from the viewpoint of a relation to growth.

The purpose of the present paper is to study the relationship between growth and electric oscillation in roots. The surface electric potentials were measured together with a measurement of elongation.

MATERIALS AND METHODS

Plant Material

Experiments were done using roots of adzuki bean (Phaseolus chrysanthus) seedlings which were 4 to 5 d old. Seeds were soaked in water at 40 ± 1°C for 3 h and were placed on filter papers wetted with 0.1 mM KCl plus 0.05 mM CaCl2 solution in darkness at 30 ± 1°C.

Measurement System

Figure 1 shows the measurement system. An acrylic case was divided into two chambers by an acrylic sheet for measurements. A filter paper was laid on the bottom of the upper chamber and a seedling was laid horizontally on the filter paper. A seedling was submerged in 0.1 mM KCl and 0.05 mM CaCl2. A root was fixed on the filter papers by applying a 0.5% agar plate on the elongation zone, because the root tended to float on the aqueous solution 5 mm in depth. Three to seven pipette electrodes were arranged near the root surface at about 1 mm intervals along the tip and elongation zone; a reference electrode was placed in the medium. Each electrode has a tip diameter of approximately 300 μm and filled with 100 mM KCl and 1% agar, containing an Ag/AgCl wire. A waterproof transistor was placed in the solution to measure the temperature. It was connected to an amplifier, and hence the temperature was measured from a change of the electric current in the transistor.

A CCD camera mounted on a microscope was set above the case for tracking the movement of the root tip. We illuminated the case with a miniature bulb (4.8 V, 0.5 A) from the bottom for the purpose of saving the intensity (300 lux) of light and increasing the contrast. The image of the root was recorded using a video-tape recorder and also displayed on a monitor. To control the temperature of the solution bathing the root, the water temperature in the lower chamber was adjusted using a heater and a thermistor con-
Figure 1. Measurement system. The acrylic case was divided into two chambers. The temperature of solution in the upper chamber was controlled by changing the water temperature in the lower chamber. A CCD camera was set above the case to measure the elongation, and at the same time the extracellular surface potentials were measured with pipette electrodes.

connected to a temperature controller. The measured surface electric potentials were loaded into a personal computer after analog-digital conversion.

Measurement

After positioning the root and electrodes in the upper chamber, the system was allowed to equilibrate for 1 h. Then, short-term measurements of the surface electric potential were made for 30 min while displacement of the root tip was recorded using the CCD camera. The temperature, which was measured by transistor in solution, was controlled with the heater and the air conditioner in room. In this experiment, three electrodes were arranged near the root surface in the elongation region. Removing the root after the measurement, the offset potential caused by each electrode itself in the aqueous medium was input to the computer and extracellular surface potential was obtained by subtraction of offset potential (19, 20). Elongation was evaluated from the change of the position of the root tip on the monitor.

When the effect of temperature on the oscillation of surface potential was studied, water at 19°C was poured into the lower chamber before placing the seedling in the upper chamber. Potentials near the elongation region were measured after 1 h at a low temperature (~20°C) for 1.5 h at a middle temperature (~23°C) for 2 h, and then at a high temperature (~27°C) for 30 min. The temperature of lower chamber reached the desired one in 1 min and then the temperature of upper chamber reached the same in several minutes. In these long-term experiments, the elongation region was displaced more than 3 mm during measurements; hence, seven electrodes were arranged in a straight line ahead of the root tip and near the root-tip side, including the elongation region. The electrodes were placed about 400 μm distant from the root surface; it is a distance enough for electrodes not to contact with the root if root extension occurs. The electric oscillations could be detected safely by this arrangement. Elongation per 10 min was calculated using the monitor, which could distinguish 5 μm. The power spectra of electric potentials were calculated by means of a maximum entropy method in order to estimate the frequency of oscillations. This method (23) is useful and accurate in the case of not so many data points like this experiment. Because the root elongated substantially on the filter paper during measurements, the positions of electrodes changed relative to the position of root tip. However the oscillations near the elongation region, when they occurred, had similar wave forms at all electrode locations. Therefore, only representative data from one electrode will be shown in “Results.”

RESULTS

Relation between Elongation and Oscillation

Figure 2 shows examples of extracellular electric potentials in a root exhibiting an oscillation (A) and a root with no oscillation (B). The oscillation period is about 7.5 min and the oscillations were observed at all three electrode positions (A). In (B) very small fluctuations appear at all the electrodes. The elongation speeds were estimated at 667 and 429 μm/30 min for (A) and (B), respectively.

Figure 2. Extracellular electric potentials at three points in elongation region of a root showing oscillations (A) and a root with no oscillations (B).
The oscillation period for a given temperature or narrow temperature range (also see Fig. 4). However, the period has a tendency to become shorter at higher temperatures as shown by a line to fit the data using the least squares method. The temperature coefficient, $Q_{10}$, of the period can be calculated to be 1.7.

Figure 6 shows the relationship between the logarithm of the frequency and the inverse of the absolute temperature. The data are from Figure 5, the line being obtained with the method of least squares. The slope of the line is $-2031$. Regarding the frequency as a measure of the reaction velocity, an activation energy can be calculated to be about 9249 (cal/mol), which is close to the activation energy of typical enzymic reactions (26). This result along with the above $Q_{10}$ suggests that the oscillation has some relation with enzymic reactions.

**Real-Time Correlation between Elongation and Oscillation**

Statistical treatments were made in Figures 3 to 6 for about 100 roots. The behavior of electric oscillations was studied below for one root in Figures 7 and 8. Figure 7 shows one example of the change in period in a root when the temperature was changed. The data indicates the potential at one point in the elongation region. At 90 min, the temperature was changed from 20.5°C to 23°C. During the first 30 min of the experiment, the oscillation gradually became distinct with a period of 14.8 min. When the temperature was changed at 90 min, the oscillation became obscure. About 20 min later, however, the oscillation gradually reappeared with a period of 8.9 min, which is different from the period at 20.5°C. The period of oscillation was usually shorter at higher temperatures for a given root, as found in Figure 5 using many roots.

Figure 8 indicates the variation of elongation rate and electric potential in the elongation region measured for over 5 h in one root. The temperature was changed at 90 min from 20 to 22.5°C, and then at 210 min to 27°C. Initially, the oscillations were obscure. About 50 min later, an oscillation

**Effect of Temperature on Oscillation**

Figure 5 shows a relationship between temperature and the oscillation period. The data are from the 87 roots showing oscillations from Figure 3A. There is a wide variation of

**Figure 3.** Difference of the elongation per 30 min between oscillating (A) and nonoscillating roots (B). The total number of used roots was 118. Among them, 87 roots showed the electric oscillations.

Figure 3 shows the distribution of elongation rates for roots showing electric oscillations (A) and roots showing no oscillations (B) at temperatures between 18 and 30°C. Of the 118 roots examined, 87 showed oscillations. The elongation rate of oscillating roots extends over a wide range between 300 and 1000 $\mu$m/30 min and has two peaks around 500 and 700 $\mu$m/30 min. The average elongation rate was 668 $\mu$m/30 min. The roots without oscillations, on the other hand, tended to elongate more slowly (the average, 501 $\mu$m/30 min).

Figure 4 shows the relationship between elongation rate and the frequency of oscillation at nearly a constant temperature (28-29°C). The data are from 13 roots from Figure 3. The line is drawn by means of a least squares method. Similar results were obtained for other temperatures. Whereas the data are widely scattered, we can see a tendency of the larger elongation with the higher oscillation frequency.

**Figure 4.** Relation between the elongation per 30 min and the frequency of oscillations at a nearly constant temperature (28-29°C). The data are from Figure 3.
with a period of 11.1 min appeared. When the temperature was increased to 22.5°C, elongation was promoted. Although the period of oscillation did not change, the amplitude increased. For a while this period of oscillation continued. At 115 min its amplitude reached a maximum and then damped. A new period of oscillation appeared at 140 min. This oscillation had a period of 8.9 min, which was shorter than before. At the same time, the elongation was promoted further; this fact agrees with the result in Figure 4 obtained for the averaged behavior of 13 roots.

The present results, therefore, show that elongation is related to the period of oscillation in a given root. Such a correlation, however, was not always obtained. For example, at 210 min (Fig. 8), the oscillation disappeared when the temperature was raised from 22.5°C to 27°C. Elongation first increased and then gradually decreased. Whereas the elongation at 27°C was of the same order as that at 22.5°C, no oscillation was seen at 27°C.

**DISCUSSION**

The electric oscillations studied here depend strongly on the temperature. This property is different from various plant circadian rhythms, where the $Q_{10}$ is around 1 (18). The value of $Q_{10}$ ($\approx 1.7$) obtained for the oscillations observed in this study is close to those in a rhythm of protoplasmic streaming (9, 12). Elongation is also known to show oscillations or nutations (6, 11, 16, 18). The coleoptiles of the white mustard show an oscillation of elongation rate with a period of a few minutes. This period depends on the temperature; generally, at high temperatures the period is shorter than that at low temperatures (6, 11, 18). The $Q_{10}$ is estimated at about 2.2 and 2.4. These facts suggest a close correlation between oscillation of electric potential and the elongation. Recently, biophoton emission, which may reflect some biochemical steps, has been shown to be associated with the electric oscillation (24).

The electric oscillation is clearly related to the elongation rate measured as mm per 30 min. Figure 3 shows that roots generating oscillations have higher elongation speed than roots with no oscillations. This seems to agree with the results of Scott (16), who showed that concentrations of indoleacetic acid which inhibit root elongation also damp electric oscillation in the root. Nevertheless, Figure 8 shows that the electric oscillation does not always appear when the elongation is large (e.g. 200–300 min values) whereas a close connection between elongation and electric oscillation is often found, e.g. for the 50 to 200 min interval in Figure 8. It was reported (16) that the surface electric potential of roots does not oscillate when elongation oscillates. These results may imply that the electric oscillation is not directly related to the elongation in a real, short-time scale. At the present stage, there-

**Figure 5.** Relation between the temperature and the oscillation period. The period has a tendency to become shorter at higher temperatures.

**Figure 6.** Relationship between the logarithm of the oscillation frequency, $f$, and the inverse of the absolute temperature, $T$. The data are from Figure 5. The line was obtained by means of the method of least squares and expressed by the analytic function: $\log f = -2031/T + 4.17$.

**Figure 7.** Change in the oscillation period in a root when the temperature was changed. At 90 min the temperature in the solution bathing the root was changed from 20.5°C to 23°C, as shown by a dashed line.
fore, we cannot suggest a definite relation between the electric oscillations and elongation in short-term measurements. However, a clear connection appears in long-term measurements.

Regarding a relation between membrane potential and elongation, an acid growth hypothesis has been proposed (3, 5, 15). The electric current loops formed around the organ of a stem and a root may play a key role in accumulation of protons (21, 27). The resultant acidification of the cell wall leads to wall loosening and acceleration of growth. Since the oscillations arise from the membrane at the xylem/parenchyma interface (22), direct acidification of the epidermal cell wall may not be expected. It may be one of the reasons why a real-time correlation did not exist between the elongation and the electric oscillations. Whereas the electric oscillation can be considered to reflect or play an important role on processes of elongation (e.g., wall loosening), there is also a possibility that oscillation and growth are independent in the cause/effect sense.

Roots show the largest elongation around 28 to 30°C (14, 17). The present result implies that the frequency of electric oscillations increases on the whole with the larger elongation speed at higher temperatures (Fig. 4). Figure 8, however, shows that when the temperature was increased from 22.5 to 27°C, there was only a transient increase in the elongation followed by decrease. This result may be suggestive of a transient increase in H⁺ efflux found in excised roots (10).

The present study showed that the roots exhibiting electric oscillations had the larger elongation speed. Explication of underlying biochemical steps is a future task.

LITERATURE CITED

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