Endogenous oscillations of electric potential difference in the cambial region of the pine stem. I. General characteristics of the oscillations

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Abstract

Extracellular measurements of electric potential difference (PD) in isolated tissue blocks from Scots pine were performed. Endogenous transient PD changes (oscillations) generated exclusively in the cambial region were found. They were not recorded in tissue blocks exposed to drastic thermal treatment. The oscillations were significantly damped by respiration inhibitors and stimulated by fusicoccin. The results point to the cambial region as the main source of electric activity in a woody stem.

Key words: cambium, electric potential difference (PD), electric oscillations, fusicoccin, Scots pine

INTRODUCTION

Electrophysiological investigations on plants dealing in most cases with action potentials (APs) events have been performed mainly on herbaceous genera. Electrical measurements on woody species are rather scarce in the literature. Various types of methodic approaches were used in those studies. Lund (1931, 1932) applied superficial contacts of electrodes with plant organs and investigated the effect of exogenous factors on the electric polarity of *Pseudotsuga* shoot segments. But most authors working with woody plants used

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electrodes penetrating into the tissue. Fensom (1955) investigated the effect of 2,4-dichlorophenoxyacetic acid on the electrophysiological behaviour of pine trees as well as seasonal changes of electric potential difference (PD) in tree trunks of growing trees (Fensom 1963). Results of the latter type of measurements on trees were also published by Burr (1947). Penetrating electrodes were employed by Levengood (1970, 1973) to look for involvement of endogenous redox currents in the physiological activity of cambium. The authors cited above pointed out the particular role of cambium in electrophysiological events, but direct evidence for this tissue being the main source of electric activity in the trunk/shoot is still absent. This study was undertaken to learn more details about the electrophysiological activity of cambium in a woody plant. Measurements were aimed at finding oscillatory activity of recorded PD.

MATERIAL AND METHODS

MATERIAL AND EXPERIMENTAL ARRANGEMENT

Electrophysiological measurements were performed on isolated tissue blocks ($20 \times 16 \times 4$ cm) taken at breast height (1.3 m) from trunks of 80-year-old Scots pines (*Pinus sylvestris* L.) grown in the Experimental Forest of the Warsaw Agricultural University in Rogów (Poland). After transportation to the laboratory in Warsaw the tissue blocks were kept at room temperature and high humidity. In such conditions electrophysiological activity was maintained for 3–4 days: tissues taken for measurements 1, 2 or 3 days after excision in the forest exhibited similar electrophysiological behaviour (frequency and amplitude of oscillations) to tissue examined on the day of collection (data not shown). Immediately before electrophysiological measurements the block of tissue was cut to the final dimensions: 15 cm (axially) $\times$ 3 cm (circumferentially) $\times$ 2 cm (radially). Such a block encompassed more than ten annual rings of mature xylem, cambial region, functional and nonfunctional phloem and a few millimetres of dead bark. Agar strips (1.5%) were placed on the cut surfaces of both apical and basal ends of the block to avoid drying out of the cambial region and neighbouring tissues during the course of measurements which usually lasted 6 hours after beginning the measurements.

In experiments with fusicoccin (FC) a block of tissue was soaked for 3 hours immediately before measurement in a FC solution adjusted to pH 5 by means of TRIS-HCl buffer. A control block of tissue was soaked in an analogous buffer solution without FC. Experiments with inhibitors of respiration (sodium azide, potassium ferricyanide and 2,4-dinitrophenol) were carried out in a similar way, with 3 hours of soaking in an appropriate solution or in distilled water (controls).

MEASURING CIRCUIT

The measuring circuit comprised a DC (direct current) amplifier of input resistance $10^{13} \Omega$ and a pair of electrodes connected with the block of tissue. A pen recorder connected with the amplifier was used to plot PD as a function of time which resulted in a diagram called after Gensler (1974) an 'electrophytogram'. Measuring electrode: a sharpened 3–4 mm long platinum wire 0.3 mm in diameter was inserted tangentially (from the radial side) into the cambial region of the block of tissue. The electrode was placed at a distance of ca. 4 cm from the apical end. Microscopical observations of transverse sections of the tissue block at the site of electrode location made after electrophysiological measurements revealed that the platinum wire contacted the cambium, xylem cells belonging to the zone of radial cell enlargement (terminology after Wilson et al. 1966) and the youngest phloem cells. A calomel reference electrode was connected through an agar salt bridge (20 mM KCl) with the mature xylem at the basal end of the tissue block. This was achieved by removing bark, phloem, cambium and xylem down to the 6th–10th annual ring counting from the cambial side in the 4 cm long basal part of the block. Exposure of cambial cells to open air and healing processes isolating the measuring electrode from undamaged, physiologically active cells caused a decrease in the frequency and amplitude of oscillations which started to be visible 8–9 hours after inserting the electrode into the tissue (preliminary experiments). Thus, the electrophysiological recordings were carried out immediately after cutting the block of tissue to the final dimensions.

All measurements were performed in a Faraday cage to eliminate external electromagnetic disturbances. The cage was additionally covered with polyethylene foil, creating a chamber with an internal relative humidity close to 100%. Registered oscillations were counted in each electrophytogram and divided into five classes of amplitudes: $<0.1, 1$ mV [i.e. oscillations not less than 0.1 mV but less than 1 mV]; $<1, 3$ mV; $<3, 5$ mV; $<5, 10$ mV and all transient PD changes greater than 10 mV. The mean number of oscillations as well as standard error in each amplitude class are shown in histogram plots.

RESULTS AND DISCUSSION

Extracellular measurements of PD performed on pine tissue blocks resulted in electrophytograms which are typically a curve with a logarithmic-like outline with PD values rising in the positive direction. A specific feature of the PD time course plots were transient monophasic changes of PD to the negative direction occurring against a drifting background (Fig. 1A). They were irregularly spaced in time with intervals between consecutive transient changes varying from a fraction of a minute to a few tens of minutes. These changes termed here 'PD oscillations' were needle-shaped or were more extended in time, sometimes

overlapping each other. The amplitude of the oscillations varied from 0.1 mV (sensitivity threshold of the measuring set) up to more than 20 mV. The duration of such a PD change ranged from a fraction of a minute for oscillations with minor amplitudes (0.1 mV-0.5 mV) to over 10 min. for oscillations greater than 10 mV.

Fig. 1. Plots of PD as function of time. First 3 hours of measurements are shown. A — a typical fragment of an electrophytogram obtained from the cambial region of the pine stem. Material was collected in June. B — a typical plot obtained for a pair of electrodes positioned in a 20 mM potassium chloride solution. C — an exemplary fragment of an electrophytogram from the tissue block subject to freezing procedure (see text for details). Material was collected in October. D — an exemplary fragment of an electrophytogram from the tissue block soaked before the measurement in a 20 mM sodium azide solution. Material was collected in March

The drift reported in this study was also observed by Gensler (1978a, b) who used similar redox electrodes made of palladium. The rate of the drift and the overall shape of the electrophytograms varied quite significantly in the present study (average PD increment — ca. 200 mV/6h) but no seasonal trends in

the summated PD increment during 6 hours of measurement were registered.

Separate experiments carried out in the present work revealed that electrodes "short circuited" by dipping them into a 20 mM solution of potassium chloride (concentration close to that naturally occurring in plant tissue) drifted within a smaller range than those positioned in a block of tissue and in a larger range than electrodes "short circuited" by a solution of redox buffer (Fig. 2). One can conclude from these results that an important part of PD drift seen on the electrophytogram comes from localization of the measuring electrode in the unbuffered redox solution of the plant tissue and the observed PD drift is a manifestation of stepwisely attained electrochemical balance between the platinum electrode and the redox system in tissue.

Fig. 2. Time course of PD drift as dependent on medium connecting a measuring electrode to a reference one. A — electrodes in a redox buffer (50 mM potassium ferro-ferricyanide); mean of 7 replicates. B — electrodes in 20 mM potassium chloride; mean of 31 replicates. C — electrodes positioned in the cambial region of pine tissue block (transient PD changes not included); mean of 41 replicates from measurements throughout the season 1984. Standard errors are marked on plots

When a pair of electrodes is positioned in redox buffer or in a potassium chloride solution no transient PD changes are registered (Fig. 1B). This suggests that the PD oscillations are not artifacts arising in the measuring circuit, but come from the living plant tissue. This supposition was confirmed by parallel recordings in living and dead pine tissues. In one type of these experiments one block of tissue was rapidly frozen 4-5 times to the temperature of solid carbon dioxide (ca. -79°C) and rapidly thawed to room temperature to destroy the cytoplasmic membranes. A control block of tissue was kept throughout this time at room temperature. In another series of experiments one tissue block was

dipped immediately before measurements into boiling distilled water for 2 min. which caused denaturation of the tissue proteins. A control block of tissue was dipped into distilled water of room temperature for the same period of time. It is clearly visible from Fig. 3 and Fig. 1C that after drastic thermal treatment tissues hardly generate PD oscillations. Thus, it may be assumed that the transient PD changes are attributes of living tissue. This was confirmed by an additional experiment which used tissue blocks not protected against drying, left for 12 days at room temperature. Electrophysiological measurements on two different tissue blocks revealed no PD oscillations over a period of 6 hours.

Fig. 3. Effect of drastic thermal treatment on the number of PD oscillations in the cambial region of the pine stem. A — tissue frozen 3-4 times to the temperature of solid CO₂ and thawed to room temperature; means of 5 replicates obtained in October and November. B — tissue dipped into boiling water or into water at room temperature; means of 2 replicates obtained in November. The diagrams are drawn for 5 different classes of amplitudes from the least one: <0.1, 1) mV [i.e. oscillations not less than 0.1 mV but less than 1 mV] to the biggest ones: ≥ 10 mV

Since PD changes appeared to be an attribute of living tissue, a possible relation between endogenous PD oscillations and respiration was tested. For this purpose the following substances influencing cell respiration were tested: sodium azide (20 mM), potassium ferricyanide (50 mM) and 2,4-dinitrophenol (DNP, 50 mM). Application of the three chemicals resulted in a significant decrease of the number of recorded oscillations in all amplitude classes (Fig. 4 and Fig. 1D). The strongest effect was that of ferricyanide which damped endogenous oscillations almost completely. The less pronounced effect of DNP in comparison with the two other inhibitors might result from different modes of action of the three chemicals since DNP is an uncoupler of oxidative phosphorylation, while azide and ferricyanide affect electron transport in the respiratory chain.

In order to obtain some additional details on the electrogenic background of the PD oscillations fusicoccin (FC) was used as a standard experimental tool, causing rapid plasmalemma hyperpolarization in plant cells (Marrè 1979).

Fig. 4. The effect of inhibitors of respiration applied by means of soaking on PD oscillations in the cambial region of the pine stem. A — NaN_3 in 20 mM concentration; means of 5 measurements performed in March, April and September. B — $\text{K}_3\text{Fe}(\text{CN})_6$ in 50 mM concentration; means of 3 measurements from tissue blocks collected in October. C — DNP in 50 mM concentration; means of 3 measurements from tissue blocks collected in September and October. All numbers used for averaging come from series of parallel, synchronous measurements on two separate, neighbouring tissue blocks: one — soaked in distilled water (control), and second — in a solution of an inhibitor.

The diagrams are drawn for 5 different classes of amplitudes as in Fig. 3

Preliminary experiments comparing electrophysiological activity of soaked controls used in this series with fresh control tissue blocks revealed similar electrophysiological behaviour in respect to frequency and amplitude of registered PD oscillations. The experiments in this series showed a marked enhancement of tissue electrophysiological activity as expressed in the number of oscillations generated in tissue blocks treated with FC in concentrations of 10^{-3} – 10^{-2} mM (Fig. 5). This may suggest that the recorded transient PD changes are related to the proton pump located in the plasmalemma, according to known mode of action of fusicoccin as a promoter of that H^+ pump (Marré et al. 1974).

Fig. 5. Effect of fusicoccin (FC) in 10^{-3} - 10^{-2} mM concentrations applied to tissue by means of soaking on endogenous PD oscillations in the pine stem; means of 3 measurements from tissue collected in March, June and July. All numbers used for averaging come from series of parallel synchronous measurements on two separate, neighbouring tissue blocks: one soaked in a solution of fusicoccin (FC) and second — in a buffer without FC (control). The diagrams are drawn for 5 different classes of amplitudes as in Fig. 3.

The location of the measuring electrode in the cambial region suggests that the recorded PD oscillations are generated in these tissues. For more precise tissue localization of these transient PD changes a separate experiment was carried out which consisted of two parallel measurements on two tissue blocks. In one of them the measuring electrode was positioned in the cambial region (as in previous experiments) and in the second the electrode was located in phloem tissue, at a distance of ca. 2 mm from the cambium. It is shown in Fig. 6, the

Fig. 6. Comparison of electrophysiological activity recorded in the pine cambial region and in phloem. Means of 3 measurements from tissue collected in March and September. All numbers used for averaging come from series of parallel, synchronous measurements on two separate, neighbouring tissue blocks: one with the measuring electrode inserted into the cambial region and second — with the electrode in phloem. The diagram are drawn for 5 different classes of amplitudes as in Fig. 3

electrode positioned in phloem registered PD oscillations only occasionally and those PD changes belonged exclusively to the class of smallest amplitudes.

Endogenous, spontaneous PD oscillations have been reported in the literature in several plant genera: in roots of *Vicia* (Scott 1957), in the coleoptile of *Zea* (Sinyukhin and Stolarek 1961), in trunks of *Ulmus*, *Pinus* and *Acer* (Fensom 1963), in a stem of *Coleus* (Karlsson 1972, Gensler 1974), in shoots of *Ipomoea*, *Pisum* and *Xanthium* (Pickard 1972), in shoots of *Lupinus* and *Phaseolus* (Głębicki et al. 1986). In these studies the measurements were carried out by means of electrodes with surface contact to a plant organ (e.g. Pickard 1972) or electrodes penetrating a shoot in the radial direction (e.g. Gensler 1974), which resulted in recording of the mean electrophysiological behaviour of different tissues contacting an electrode. Thus, the more precise tissue localization of PD fluctuations was not discussed. Due to the tangential positioning of the electrode used in the present study it was possible to ascertain that PD oscillations are generated in the cambial zone and, conceivably, in the youngest derivatives of the lateral meristem. This is consistent with Fensom's (1963) suggestions for growing trees investigated by means of Ag/AgCl electrodes.

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Endogenne oscylacje różnicy potencjałów elektrycznych w regionie kambialnym pnia sosny. I. Charakterystyka ogólna oscylacji

Streszczenie

Przeprowadzono zewnątrzkomórkowe pomiary różnicy potencjałów elektrycznych (PD) w izolowanych wycinkach pnia sosny zwyczajnej. Stwierdzono występowanie chwilowych wahań PD (oscylacji), które były generowane wyłącznie w regionie kambialnym. Wahań tych nie rejestrowano w wycinkach pnia poddanych działaniu skrajnie wysokich lub skrajnie niskich temperatur. Oscylacje te były silnie tłumione przez inhibitory oddychania, a stymulowane — przez fuzykocynę (FC). Wyniki te wskazują, że region kambialny jest głównym źródłem aktywności elektrycznej pnia rośliny drzewiastej.