J Wood Sci (1998) 44:249–254

ORIGINAL ARTICLE

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Measurements of the bioelectrical potential of a Japanese oak (*Quercus crispula* Blume) sapling: Effect of the radial distribution of inorganic ingredients within a tree stem on the diurnal change in resting potential

Received: September 22, 1997 / Accepted: February 10, 1998

Abstract This study investigated the relation between the resting potential of a 2-year-old Japanese oak (*Quercus crispula* Blume) sapling and its physiology, especially the radial transport of water containing inorganic ingredients in the stem using the scanning electron microscope-energy dispersive X-ray microanalyzer (SEM-EDXA) method. The resting potential of a sapling could be monitored continuously with our measuring apparatus. Changes in resting potential were due to the light. The hyperpolarization and depolarization peaks of the resting potential, whose absolute voltage was about 10 mV, occurred right after lights-off and lights-on, respectively. The resting potential was found to show periodic responses for each day unit. At night (lights-off), the resting potential tended to depolarize with an increase in tangential strain. On the other hand, during the daytime (lights-on) the resting potential tended to hyperpolarize, depolarize, or show a nearly constant value for the tangential strain. The water containing inorganic ingredients was transported, via transpiration, in both directions between the mature xylem zone and the phloem zone through differentiating xylem cells. This water transport within a tree stem had a significant effect on the diurnal changes in resting potential.

Key words Resting potential · Radial transport of water · Energy dispersive X-ray microanalyzer · Hyperpolarization · Depolarization

Introduction

A living plant cell contains water, which acts as a solvent in protoplasm consisting of protein. In this solution, inorganic ingredients such as potassium, calcium, chlorine, sodium, and phosphoric acid exist in an ionized state. An electromotive force is generated within a cell according to the ion-selective permeability of the plasmalemma. As a result, a plant also has an electromotive force because it is an aggregate of cells. A living plant has a negative bioelectrical potential called the resting potential. The resting potential can be classified into two main categories[1]: (1) the diffusion potential generated by the ion gradient between the inside and the outside of a cell; and (2) the electrogenic potential generated by a H^+-extrusion pump. This pump can generate H^+ transport between the inside and the outside of a cell; and in this case organic matter such as amino acids, sugars, and Cl^- are transported in the same direction as H^+. At the same time, K^+, an important cation for a cell, is absorbed in the cell by extruding H^+.[2–4]

Though the bioelectrical potential for living plants and plant cells is well understood, there are few published studies on living trees.[5–7] The main purposes of this study were to establish a method to measure diurnally the resting potential of a tree and to clarify experimentally the relation between the resting potential and physiological behavior of a tree, especially the radial transport of water in the stem. This radial transportation was estimated indirectly by observing the distribution of inorganic ingredients using the scanning electron microscope–energy dispersive X-ray microanalyzer (SEM-EDXA) method.

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Part of this paper was presented at the 47th annual meeting of the Japan Wood Research Society, Kochi, April 3–5, 1997

Materials and methods

Plant materials

Three sample saplings [2-year-old Japanese oak (*Quercus crispula* Blume)] with a height (H) of about 98 cm and a diameter at H = 20 cm of about 1.8 cm, at the beginning of the measurement were studied. The saplings were planted in moist vermiculite in wagnel pots (height 30 cm, diameter 20 cm) and were placed in a growth cabinet in late April 1996. The growth cabinet (temperature 20° ± 0.1°C, relative humidity 75% ± 1%) was set up to provide different light intensities using a combination of eight mercury lamps (1 kW, HRF-1000X; Matsushita Denki, Osaka, Japan) and 12 positive glow lamps (400W, D-400; Toshiba Denki, Tokyo, Japan). The lighting period, during which illumination was about 350 ± 10 μE/$m^{-2}s^{-1}$ (λ 400–700 nm) 145 cm from the light source, was controlled to 14 h of light and 10 h of darkness (lights-on at 6:00 a.m. and lights-off at 8:00 p.m.). Saplings were irrigated and fertilized with 1 l of a nutrient solution[8] (in ppm: N 50.0, P_2O_5 25.0, K_2O 30.0, CaO 20.0, MgO 10.0, Fe_2O_3 2.0, Cu 0.1, Mn 0.1, Zn 0.1, B 0.2, and Mo 0.1; initial pH 5.2) every 2–3 days.

Electrodes for detecting resting potential

Electrodes for detecting the resting potential consisted of a 0.50 mm diameter platinum electrode and a 0.50 mm electrode composed of an 87% platinum/13% rhodium alloy. These electrodes are generally used for measuring bioelectrical potential, as they are inert and have low polarization resistance. The platinum electrode was attached to the surface of inner bark with electrode starch (OJ-O9; Fukuda Denshi, Tokyo, Japan) after removing the outer bark with a steel knife; the platinum/rhodium electrode was inserted in the stem as shown in Fig. 1. In addition, shielding wires 1.0 cm in diameter (Nihon Denkei, Tokyo, Japan) were connected to each of the electrodes.

Measurements of resting potential

A block diagram of the measurement system of resting potential/tangential strain/temperature in the stem used in this experiment is shown in Fig. 2. The resting potential generated within the sapling was detected with the electrodes and amplified after noise removal through a bandpass filter whose impedance of input was 10 $M\Omega$ (Nihon Denkei, Tokyo, Japan). The tangential strain and temperature were measured by a strain gauge (gauge length 1 cm; Tokyo Sokki, Tokyo, Japan) and a thermocouple (K-type, 0.30 mm in diameter; Ninomiya, Tokyo, Japan), respectively. Like the platinum electrode, the strain gauge and thermocouple were attached to the surface of the inner bark near the platinum electrode after removing the outer bark with a steel knife. The output values of these instruments were stored simultaneously in a data acquisition controller (NEC Sanei, Tokyo, Japan), whose measurement speed was 20 ms integral time; they were input and saved in a personal computer (PC-9821Ap/U2; NEC, Tokyo, Japan).

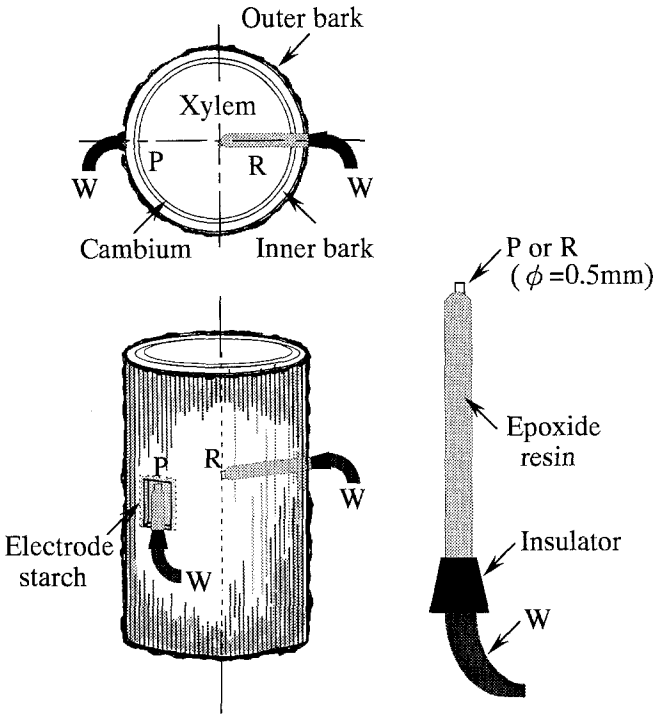


Fig. 1. Procedure for measuring resting potential. *P*, platinum electrode; *R*, rhodium-platinum electrode; *W*, shielding wire. The platinum electrode was pasted on the inner bark by electrode starch after removing the outer bark, and the rhodium-platinum electrode was inserted in the stem

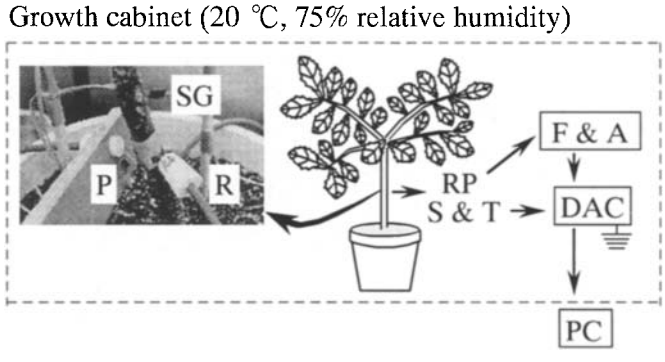


Fig. 2. System used for measuring the resting potential (*RP*), tangential strain (*S*), and temperature (*T*) of a 2-year-old Japanese oak (*Quercus crispula* Blume) sapling in a growth cabinet. *SG*, strain gauge; *P*, *R*, see legend to Fig. 1; F, filter; *A*, amplifer; *DAC*, data acquisition controller; *PC*, personal computer

SEM-EDXA method

Samples for the SEM-EDXA method were collected from intact living sapling stems frozen with liquid nitrogen (LN_2). A watertight collar composed of a plastic funnel was fitted around the stem near the electrode of a sample sapling, and the collar was filled with LN_2.[9] The stem was completely frozen within approximately 30 s. A disk about 5 cm in

length was immediately cut from the frozen area of stem with a hand saw. These samples were stored in a container filled with LN_2 and were transferred to a refrigerator kept at −30°C. Samples were equilibrated at exactly −30°C to prevent contamination by frost during subsequent treatments. After that, samples covered with outer bark were cut into disks about 5mm in thickness with the saw, and the cross-sectional surfaces of these disks were cleanly cut with a freezing microtome (Miles-Sankyo, IN, USA) (temperature −30°C). These disks were freeze-dried for 24h in a freeze-drying device (Virtis, NY, USA) and were coated with carbon in a vacuum evaporator (Akashi, Tokyo, Japan). They were then conditioned in a desiccator with P_2O_5.

After this treatment each disk was observed with the SEM (JXA-840A; Jeol, Tokyo, Japan) and the distribution of their elemental composition was analyzed with an EDXA system (JED-2110; Jeol).

Results and discussion

Responses to light and diurnal changes in resting potential

Figure 3 shows an example of the experimental results and a comparison of the diurnal changes of resting potential (E) at H = 10cm with those of tangential strain (ε_t) for 5 days from September 5 to 9. Changes in E due to light (i.e., the depolarization and hyperpolarization peaks of E, whose absolute voltage was about 10mV) occurred right after lights-on at 6:00 a.m. and lights-off at 8:00 p.m., respectively. The anomalous value measured near noon on September 6 was generated by the addition of nutrient solutions, and the continual drop of the absolute value of E between September 5 and 9 can be considered the result of declining electrode response caused by the formation of traumatic tissue around the electrodes.

The autocorrelation function of E (Fig. 4) was used to investigate the periodicity of E. That of ε_t is also shown. In this case the autocorrelation functions were considered for the period after lights-off at 8:00 p.m. on September 6

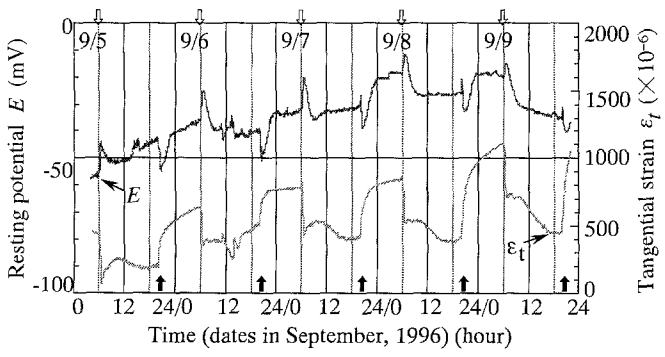


Fig. 3. Examples of diurnal changes in the resting potential and tangential strain. *Down arrows*, lights-on at 6:00 a.m.; *up arrows*, lights-off at 8:00 p.m.

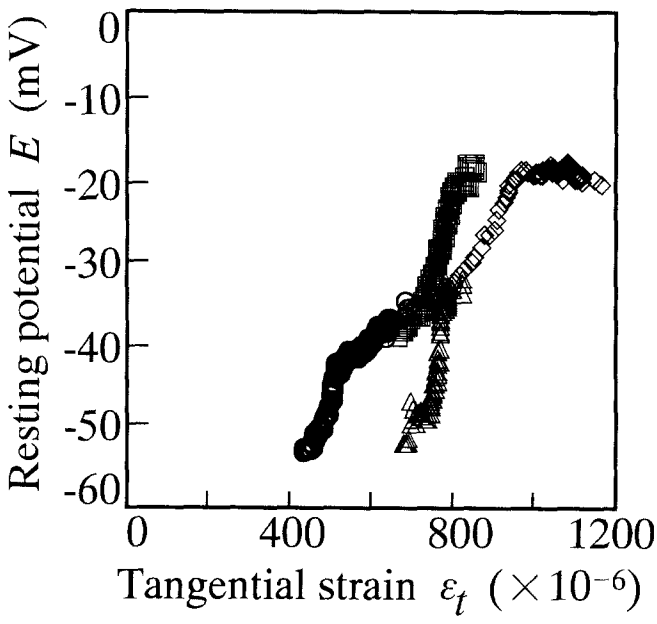


Fig. 4. Periodicity of the resting potential

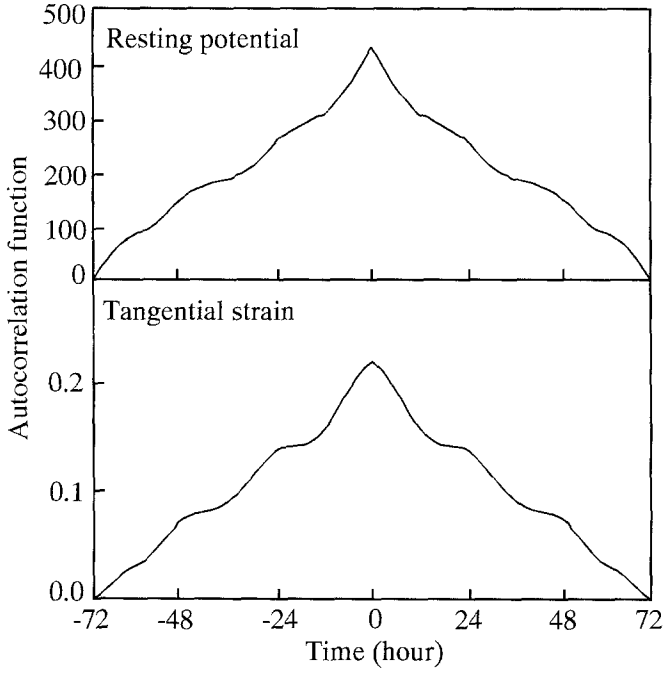


Fig. 5. Relation between the resting potential and tangential strain at lights-off. *Circles*, 9/5; *triangles*, 9/6; *squares*, 9/7; *diamonds*, 9/8

to eliminate the anomaly mentioned previously. As a result, although the peak of the autocorrelation function of E is less distinct than that of ε_t, the peak clearly appeared at T = 24 (−24), 48 (−48), as was the case for ε_t. Hence E (ε_t) was found to show periodic responses for each day unit.

Relation between E and ε_t

As shown in Fig. 3, changes in E correlated with ε_t in an extreme fashion. The relation between E and ε_t at night after lights-off at 8:00 p.m. is shown in Fig. 5. E caused

depolarization with an increase of ε_t in most of the plot on September 9. On the other hand, during the daytime after lights-on at 6:00 a.m., E tended to hyperpolarize, depolarize, or show a nearly constant value for ε_t, although these changes were not as distinct as those at night. A similar phenomenon was reported with the membrane potential of a guard cell and an epidermal cell adjacent to a guard cell of *Vicia faba* in response to light–dark cycles, interpreted to be a phenomenon caused by K^+ fluxes.[10]

Distributions of inorganic ingredients during daytime and nighttime

Distributions of inorganic ingredients right before lights-on and lights-off were investigated with the SEM-EDXA method. Potassium, calcium, chlorine, magnesium, phosphoric acid, and sulfur were detected from sample disks, especially potassium and calcium. Our study therefore focused on potassium and calcium, as they are important cations for tree physiology and are the most abundant components of trees after carbon, hydrogen, and oxygen.

Figure 6 shows the distribution of potassium and calcium in the region from the inner bark to the pith right before lights-on and lights-off. In this case, sample disks were easily cracked near the cambial zone, as they were severely damaged by freezing and drying.

There is a difference in the distribution of the two cations in the xylem for right before lights-on and lights-off. Right before lights-off the intensity of each cation showed a nearly constant low value throughout the xylem zone. On the other hand, right before lights-on the intensity of each cation increased gradually toward the outside of the xylem. Figure 6b shows potassium to be distributed from the outer xylem to the phloem right before lights-on and lights-off. Potassium distribution at both times was maximum around the cambium, but the maximum intensity right before lights-on was about twice that right before lights-off. The

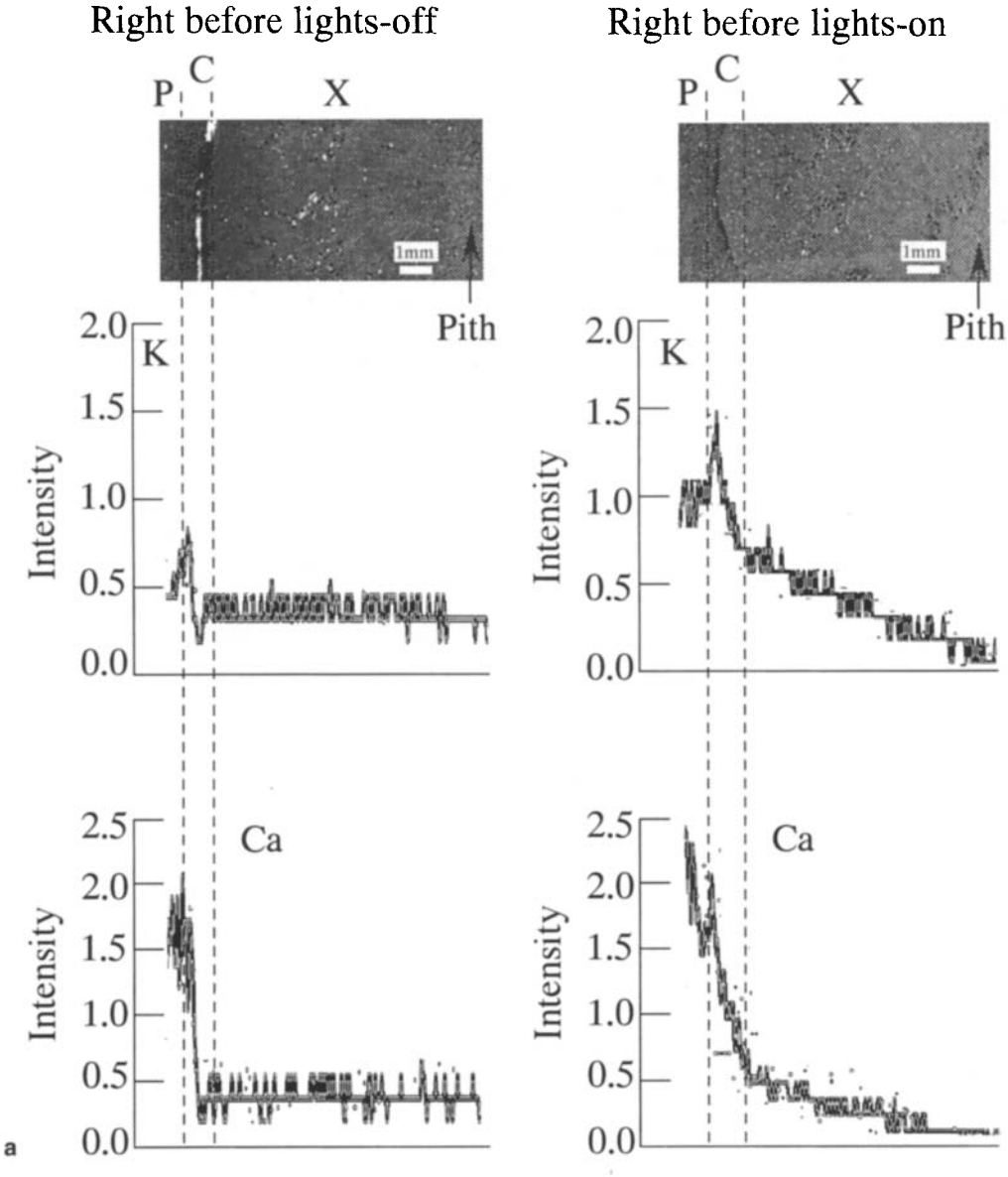


Fig. 6. Examples of the distribution of potassium and calcium from xylem to phloem of sample disks right before lights-off/lights-on. *P*, phloem; *C*, cambium; *X*, xylem. **a** Region containing phloem, cambium, and xylem. **b** Region from outer xylem to phloem

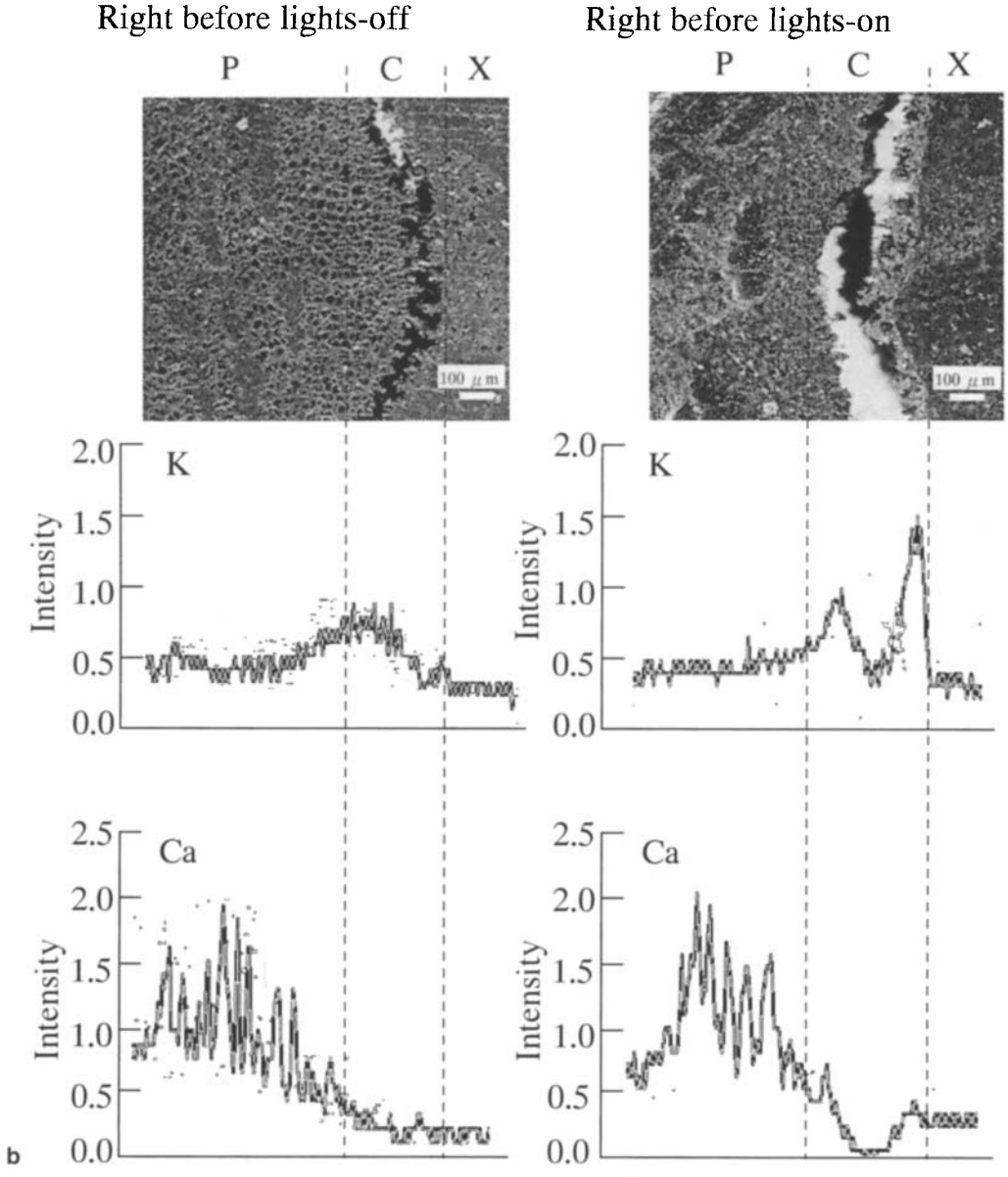
calcium distribution was similar, but the two intensities were almost the same value. Calcium was plentiful in the phloem, probably as a result of the effect of calcium salts stored in the phloem parenchymal cells.[11] That is, there was a less striking difference between the times right after lights-on and lights-off in the phloem than in the xylem.

These results indicate that some cations move in both directions between the mature xylem zone and the cambinal zone through differentiating xylem cells. In other words, there is transpiration of water in the radial direction of the stem.

Relation between changes of E and the radial transport of inorganic ingredient ions

Water is transported to the upper part of a tree through vessels by transpiration. After transpiration stops (i.e., after lights-off at 8:00 p.m.) ε_i increased (Fig. 3). At the same time, E tended to depolarize, as shown in Fig. 5. Figure 6 indicates that the water containing the inorganic ingredients was transported radially through the xylem. From this information, a model showing water transport and cell expansion was proposed (Fig. 7). This model suggests that xylem is divided into four parts: the cambial and phloem zone (zone A), expanding zone (zone B), developing zone (zone C), and mature xylem zone (zone D). The state of water stored in the stem estimated with this model indicates that the water was transported through the some apoplastic passageways and diffused in the tissue from zone D to zone A when transpiraton was restricted at night, as shown in Fig. 7. As a result, cations such as K^+ and Ca^{2+} in the inorganic ingredients in the water were distributed densely in zone A and gradually decreased toward the inner part of the xylem through zones B and C. Thus E eventually caused depolarization. In contrast,

Fig. 6. (cont.)



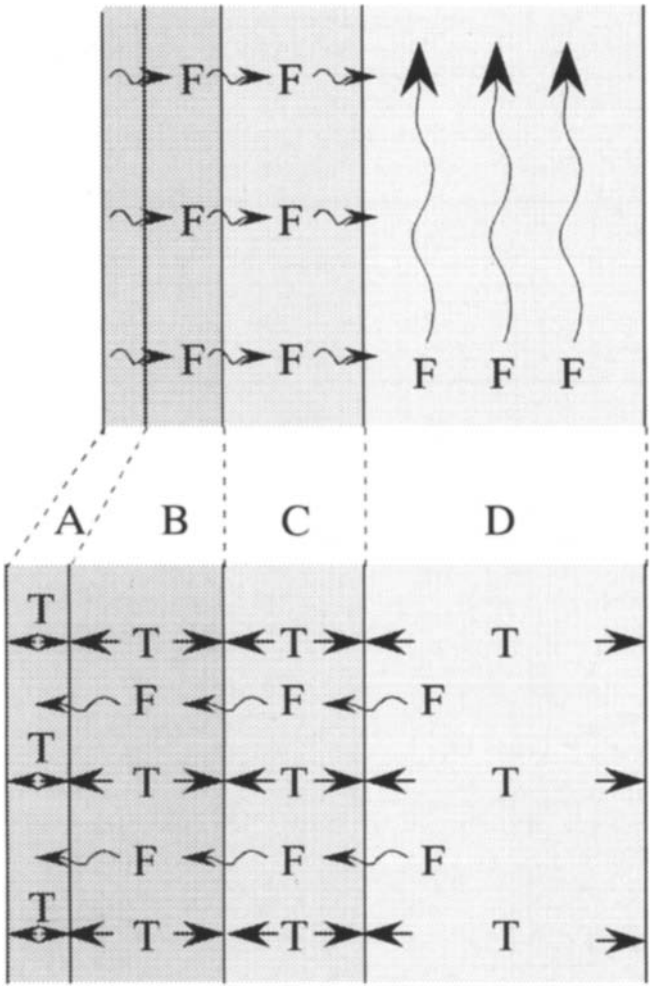


Fig. 7. Water transport and cell expansion. **A** Cambial and inner bark zone. **B** Expanding zone. **C** Developing zone. **D** Mature xylem zone. **F**, water flow; **T**, Pressure potential

when evaporation was accelerated during daytime, water mainly flowed through vessels in zone D and was transported to the upper part of the tree. Then, water containing inorganic ingredients in zones A, B, and C was drawn toward zone D.

As mentioned before, Ishikawa et al.[10] reported that changes in membrane potential of a guard cell are caused by K^+ fluxes. Although our study focused on sapling organs, the following is presumed: Changes in E are a result of cations such as K^+ being transported with water at an organic level similar to the transport at a cellular level.

Conclusions

This study was designed to investigate the relation between the resting potential of saplings and tree physiology, especially radial transportation of water in the stem, using the SEM-EDXA method. The resting potential, monitored continuously using our measuring apparatus, showed periodic daily responses to lights-on and lights-off. Changes in resting potential were due to the light; at night the resting potential tended to depolarize as the tangential strain increased, and during daytime the resting potential tended to hyperpolarize, depolarize, or showed a nearly constant value for the tangential strain. As expected, the results obtained by the SEM-EDXA method showed that water containing inorganic ingredients was transported via transpiration in both directions between the mature xylem zone and the cambial zone through differentiating xylem cells.

We plan to monitor the long-term resting potential for the life of a tree. At the same time, the radial transport of inorganic ingredients, particularly cations, in the stem must to be investigated further using an ion-selective electrode to clarify the relation between the resting potential and the continuous movement of each cation.

Acknowledgments We thank Professor Takashi Okuyama of the School of Agricultural Science, Nagoya University, and Dr. Akinori Nagao and Dr. Toshiro Sumizono of the Forestry and Forest Products Research Institute, for their valuable advice about conducting the experiment.

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