BIOLOGIA PLANTARUM (PRAHA)

31 (5) : 327–335, 1989

The Comparison between Membrane and Transorgan Electric Potentials in *Chenopodium rubrum*: The Methods

L. ADAMEC

Institute of Experimental Botany, Czechoslovak Academy of Sciences,
Ke dvoru 15, 166 30 Praha 6, Czechoslovakia

Abstract. A new method of simultaneous measurement of membrane (E_m) and transorgan (E_{tr}) electric potential in intact *Chenopodium rubrum* plants and changes in E_m and E_{tr} under various experimental conditions are described. E_m was measured in mesophyll leaf cells, and E_{tr} in the same plant as a potential difference between a first pair leaf tip and roots. The two potentials differed distinctly, E_m averaging −156 mV and E_{tr} −5 mV. But their changes in E_m and E_{tr} had approximately the same magnitude and time-course after changing light and darkness or KCl concentration in solution flowing around the leaf or during the photoperiodic inductive cycle. In relation to the same electrode connection they had an opposite polarity. The nature of E_{tr} and its relation to E_m are discussed.

The measurement of E_{tr} is much more stable and simpler than that of E_m. The similarity of E_m and E_{tr} time-courses justifies the use of E_{tr} measurement in electrophysiological studies as a replacement for E_m measurements especially in intact plants.

This paper is the first of three papers dealing with the use of electrophysiological methods in the study of photoperiodic flower induction in the short-day plant *Chenopodium rubrum* L. (ADAMEC and KREKULE 1989a, b). The electrophysiological parameters most commonly measured are cell membrane potential and transorgan (surface) potential, the potential difference between various surface parts or between inner tissues in intact plants or excised organs.

Measurement of E_{tr} is much simpler and easier than that of E_m, but its principal disadvantage is its unclear relation to E_m. E_{tr} has been considered commonly to be a "reflection" of the cell E_m (*e.g.* OKAMOTO *et al.* 1978). It has repeatedly been found in simultaneous measurements of E_m and E_{tr} in the same object after various treatments that the character of changes in E_m and E_{tr} was more or less similar but the changes were of an opposite polarity (NOVAK and BENTRUP 1972, OKAMOTO *et al.* 1978, 1984, ZAWADSKI and TREBACZ 1985, POLEVOĬ 1986). The employment of

Received November 27, 1988; accepted January 26, 1989

Present address: Czechoslovak Academy of Sciences, Institute of Botany, Section of Plant Ecology, Dukelská 145, 379 82 Třeboň, Czechoslovakia.

leaves in intact higher plants for E_m measurement is still sporadic (NOVAK and GREPPIN 1979, MONTAVON *et al.* 1983, MONTAVON 1984, STARRACH *et al.* 1984).

The aim of the present paper is to test the degree of similarity of E_m and E_{tr} time-courses in intact *C. rubrum* plants, and thus to find whether it is possible to replace one parameter by the other.

MATERIALS AND METHODS

Cultivation of Plants

Chenopodium rubrum (selection 374) plants 14–17 days old were used in all E_m and E_{tr} measurements. The conditions of seed germination and plant cultivation are described by ULLMANN *et al.* (1985). The plantlets were cultivated in small plastic vessels in half-strength Knop's solution in perlite and to lesser extent also hydroponically. They were grown in small-volume growth chambers under non-inductive conditions of continuous illumination by fluorescent tubes at 20 ± 1 °C. The irradiance amounted to *ca.* 38 W m^{-2}

Measurement of E_m

E_m was measured in mesophyll cells of the first leaf pair according to the method described by NOVAK and GREPPIN (1979) and MONTAVON *et al.* (1983). The intact plants were fixed by a first leaf tip in a plexiglass flow chamber (volume 1 ml) by means of a perforated translucent plate *ca.* 3 h before measurement (see Fig. 1). The fixed part represented about one third of the leaf area. The flow chamber was perfused continuously by a solution containing 1 mM KCl and 0.1 mM $CaCl_2$ (DUNLOP and BOWLING 1971) with a flow rate of 0.3–0.5 ml min^{-1}. In investigating the dependence of E_m and E_{tr} on ion concentration, the KCl concentration or pH of the solution was changed. In these cases, the flow rate was 2–3 ml min^{-1}.

Microelectrodes pulled out from thick-walled glass capillary tubing (Simax) had outer tip diameter 0.4–0.6 μm. They were filled first by methanol using underpressure, then by distilled water and finally by 1 M KCl. Filled microelectrodes had an electrical resistance of 15–35 MΩ and a tip potential within ± 5 mV in most cases. An agar salt bridge connected the microelectrode with a measuring calomel electrode. The earthed reference calomel electrode was connected with the flow chamber by a tube (Fig. 1). E_m was measured by digital mV-meters with high input resistance (10^{12} Ω) and recorded by means of pen recorder. The whole equipment was located in a Faraday's cage.

The microelectrodes were inserted into the leaf vertically through the holes in the

---

Abbreviations used: E_m = cell membrane electric potential; E_{tr} = transorgan electric potential; APW = artificial pond water; MES = morpholinoethanesulphonic acid.

fixing plate by means of a micromanipulator (Zeiss, Jena, GDR) with two mobile holders. Optical check of microelectrode insertion was ensured by stereo-microscope (magnification 50). The position of the microelectrode tip in tissue was estimated according to the degree of microelectrode shift and the level of measured potential (NOVAK and GREPPIN 1979). It is assumed that the tip was located in the vacuole. E_m was measured in mesophyll cells only. The flow chamber together with the fixed plants were illuminated using a halogen lamp and light-fibre cable (*ca.* $20\,W\,m^{-2}$). The neutral safety green light was used for the measurements in darkness. The measurements of E_m and E_{tr} in relation to ion concentration were carried out in continuous light. When comparing E_m and E_{tr} time-courses during the photoperiodic flower induction the plants were exposed to 13 h of darkness and 11 h of light. All measurements were performed at 21.5 ± 1.5 °C. E_m was measured either in two intact plants independently or both E_m and E_{tr} were measured in the same plant simultaneously.

Measurement of E_{tr}

The method of E_{tr} measurement consisted in connecting a calomel electrode *via* flexible tubing to a plexiglass vessel containing the roots of fixed plants immersed in

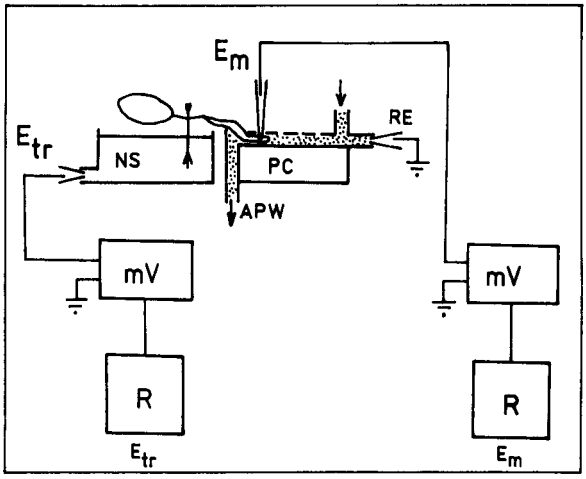

Fig. 1. Scheme of simultaneous E_m and E_{tr} measurement in intact *C. rubrum* plant. First leaf tip was fixed in the plexi-glass flow chamber (PC) washed by solution (APW, artificial pond water) whereas the rest of plant was grown in a vessel in nutrient solution (NS) with or without perlite. E_m was measured in mesophyll cells by means of microelectrode. Earthed reference electrode (RE) was connected to APW. E_{tr} was measured as a potential difference between roots (by means of calomel electrode) and the leaf tip (the reference electrode is common for E_m and E_{tr}). The measuring electrodes were connected to mV-meters (mV). R: recorders. As in the E_{tr} measurements was an opposite polarity of the electrode connection than in E_m ones for comparing both values it is necessary to reverse the polarity of E_{tr} measured.

nutrient solution (with or without perlite; Fig. 1). This electrode measured the potential difference between the roots and the first leaf tip of an intact plant (E_{tr}). It enabled us to record both potential types, E_m and E_{tr}, in the same intact plant simultaneously. The reference calomel electrode connected to the flow chamber was common for both E_m and E_{tr} measurements. The E_{tr} measurement did not in any way disturb that of E_m in the same plant and *vice versa*. It was also possible to record E_{tr} in two plants independently after the roots of these plants had been placed in two isolated vessels. E_{tr} measurements had an opposite polarity of the electrode connection than E_m measurements (*i.e.* the measuring electrode around roots and the reference electrode around leaf). In order to compare the two values it is necessary to reverse the polarity of E_{tr} measured. However, the E_{tr} records are presented in Results without correction. In addition, investigating the influence of pH on E_{tr} the flowing solution contained 2 mM buffer ·MES (sodium salt; $pK_A = 6.15$). Solutions of pH of 5.0; 6.0 and 7.0 were prepared by a titration by HCl.

RESULTS

After a microelectrode had been inserted into the mesophyll cell E_m was fairly stable after 3–20 min and usually remained stable for only 0.5–2 h. It was restored by moving the microelectrode. E_{tr} was not stable until three hours but remained much more stable than E_m. The comparison of E_m measured in leaf mesophyll cells and E_{tr} measured in intact *C. rubrum* plants showed a marked difference: E_m reached a mean value of −156 mV under continuous light before photoperiodic induction and −171 mV at the end of a 13 h inductive dark period, while the corresponding values of E_{tr} were only −5 and −20 mV (see Table 1). Because of the low value and wide variation of E_{tr}, its polarity was not clearly apparent. The mean shifts of both values were identical during 13 h of inductive dark period but respecting the same electrode connection their polarity was opposite. The switching of light and darkness triggered marked transient changes in E_m and E_{tr}. Their magnitude and time-course were very similar but not identical (Fig. 2). The amplitude ratio of the transient changes in both E_m and E_{tr} ranged from 0.8 to 1.5 when they were measured simultaneously in the same plant or independently at the beginning and end of the inductive dark period (Table 1). E_m and E_{tr} were also similar in simultaneous measurements during an inductive cycle (Fig. 3). In most cases, the curves of E_m and E_{tr} measured in the same plant closely resembled each other even in small oscillations; resemblance in the more distinct oscillations was occasional (Fig. 3A, B).

E_m of mesophyll cells of first leaf segments reached approximately the same value as in the leaf cells of intact plants in solutions with various KCl concentrations (Table 2). Tenfold increase of KCl concentration led to a similar E_m depolarization by 13–44 mV in the material of both types. The main changes of E_m occurred in both

types in 2–3 min after beginning the solution exchange. The dependence of E_{tr} on KCl concentration in flowing solution in intact plants was very close to that of E_m in mesophyll cells (Table 3). Tenfold increase of KCl concentration led to E_{tr} depolarization by 25–30 mV. The rate of E_{tr} changes after solution exchange was the same as that of E_m. pH drop from 7.0 to 5.0 depolarized E_{tr} only slightly. The pH drop per one pH unit represented 1–7 mV. Large individual differences were found between both sets of E_m and E_{tr} measurements.

DISCUSSION

Approximately the same pattern of E_m and E_{tr} changes was found under treatments of switching light and darkness and KCl concentration changes, as well as during spontaneous long-term course (*cf.* Figs. 2 and 3. Tables 1–3). It shows that the correlation between E_m and E_{tr} is generally valid. E_m in mesophyll cells of intact plants reflected KCl concentration changes in the surrounding solution as sensitively and quickly as in leaf segment cells (Table 2). However, as the number of

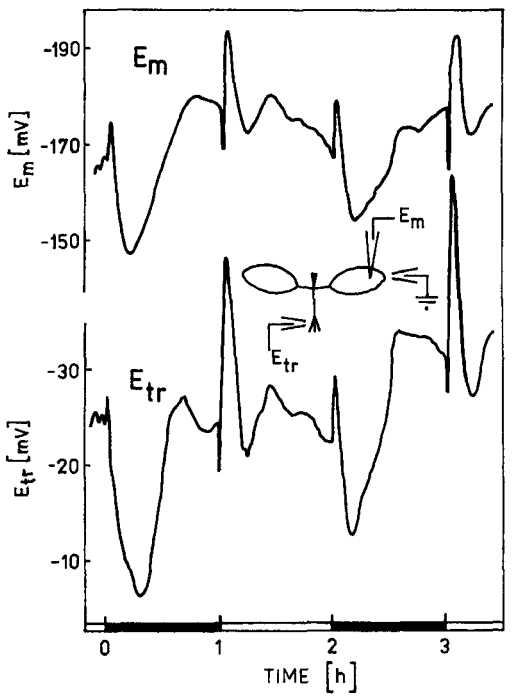

Fig. 2. Comparison of E_m and E_{tr} time-courses in intact *C. rubrum* plant after changing light and darkness. E_m was measured in the first leaf mesophyll cells and E_{tr} between roots and the leaf (see Fig. 1). The presented values of E_{tr} should have an opposite polarity for comparison with E_m. A typical example is presented. The E_m scale is densed twofold than E_{tr} one. – Full line: darkness; double line: light.

TABLE 1

Comparison of mean values of E_m and E_{tr} in intact *C. rubrum* plants in continuous light immediately before induction and in the end of 13-h inductive dark period and comparison of mean amplitudes of E_m (ΔE_m) and E_{tr} (ΔE_{tr}) after the changes in light régime at the beginning and end of the inductive dark period. The presented values of E_{tr} and E_{tr} should have opposite sign for comparison with E_m and E_m (see Fig. 1). The changes of E_m and E_{tr} were obtained as a difference of the highest amplitude and resting value. S.E.M., standard error of mean; *n*, number of measurements

Measured object	E_m			
	E_m [mV]		ΔE_m [mV]	
	Continuous light	13 h of darkness	Continuous light-darkness	13 h of darkness-light
First leaf mesophyll cells of intact plants	−156 S.E.M. = 3.0 *n* = 19	−171 S.E.M. = 3.6 *n* = 10	+31.3 S.E.M. = 2.6 *n* = 13	−25.1 S.E.M. = 2.7 *n* = 8
	E_{tr}			
	E_{tr} [mV]		ΔE_{tr} [mV]	
Intact plants (roots — first leaf tip)	−5 S.E.M. = 4.0 *n* = 15	−20 S.E.M. = 3.1 *n* = 11	+22.9 S.E.M. = 1.8 *n* = 17	−23.4 S.E.M. = 2.3 *n* = 13

measurements was small, the dependence of E_m and E_{tr} on ion concentration needs to be confirmed (Tables 2 and 3). STARRACH *et al.* (1984) found marked differences of electrophysiological characteristics between freshly cut off *Phaseolus* pulvini and the intact ones.

E_m and E_{tr} values in intact plants differ markedly in magnitude, but the pattern of changes is approximately the same with an opposite polarity. Thus, the results

TABLE 2

E_m dependence of mesophyll cells in leaf segments or in intact plant leaves of *C. rubrum* on KCl concentration in flowing solution in light. Results of two measurements (A, B) are presented for both types of material

Composition of flowing solution [M]	E_m of first leaf mesophyll cells [mV]			
	Leaf segments		Intact plants	
	A	B	A	B
— — — 10^{-4} $CaCl_2$	−151	−119	−161	−176
10^{-4} KCl + 10^{-4} $CaCl_2$	−159	−126	−153	−175
10^{-3} KCl + 10^{-4} $CaCl_2$	−146	−110	−132	−152
10^{-2} KCl + 10^{-4} $CaCl_2$	−116	−66	−104	−113

Table 3

E_{tr} dependence on KCl concentration and on pH of the solution flowing around first leaf in intact *C. rubrum* plants in light. The presented values of E_{tr} should have opposite sign for comparison with E_m. Results of two measurements (A, B) are presented. APW, artificial pond water

Composition of flowing solution	E_{tr} of intact plants [mV]	
	A	B
— — — 10^{-4} M $CaCl_2$	−12.2	−15.5
10^{-3} M KCl + 10^{-4} M $CaCl_2$	+7.5	+12.5
10^{-2} M KCl + 10^{-4} M $CaCl_2$	+37.2	+37.5
APW (10^{-3} M KCl + 10^{-4} M $CaCl_2$)	+3.6	+9.5
APW + 2 mM Na-MES, pH = 7.0	+11.2	+18.2
APW + 2 mM Na-MES, pH = 6.0	+15.0	+19.0
APW + 2 mM Na-MES, pH = 5.0	+22.0	+23.8

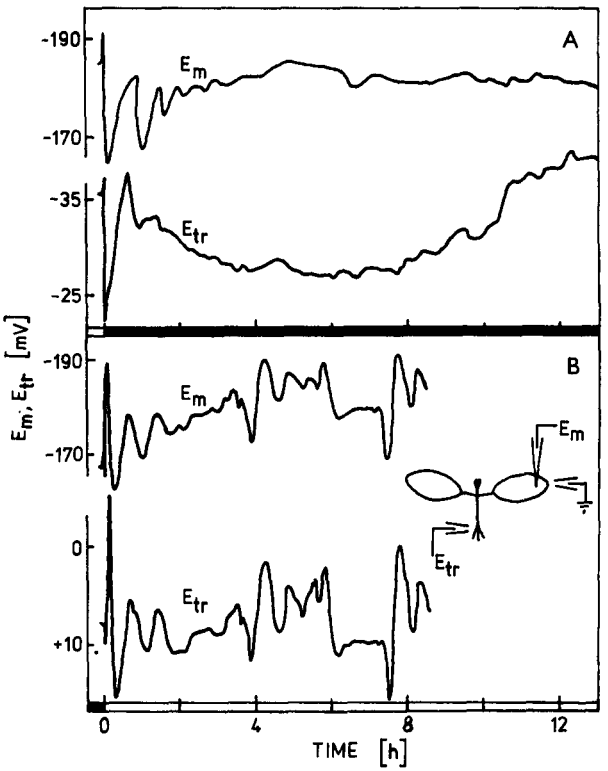

Fig. 3. Comparison of E_m and E_{tr} time-course during 13-h inductive dark period (A) or 9-h light period after induction (B) in intact *C. rubrum* plants. – For the other see Fig. 2.

hitherto obtained either in lower plants (NOVAK and BENTRUP 1972, ZAWADSKI and TREBACZ 1985) or in excised parts of higher plants (OKAMOTO *et al.* 1978, 1984, POLEVOÏ 1986) have also been confirmed in intact plants.

The interpretation of E_{tr} in relation to E_m is not yet clear, but some suggestions about the nature of E_{tr} can be made. E_{tr} in intact plants includes the sum of E_{tr} of organs (tissues) situated between the places of electrode contact (*e.g.* OKAMOTO *et al.* 1978). E_{tr} in a single organ depends on E_m differences of cells along the organs (*i.e.* on electric heterogeneity of symplast; NEWMAN and BRIGGS 1972, OKAMOTO *et al.* 1978, ZAWADSKI and TREBACZ 1985). This means that E_{tr} of single organs depends on the ratio of electrogenic symplastic cell activity to electric apoplastic resistance in parts of organs or tissues, as in parallel branches of an electrical circuit (JAFFE and NUCCITELLI 1977, OKAMOTO *et al.* 1984). E_{tr} also depends on electric polarity of single cells in tissues and organs, in which the electric cell dipol is multiplied by the cell number in file (JAFFE and NUCCITELLI 1977, RAVEN 1979). As shown in Table 3, E_{tr} also depends markedly on ion concentration in the place of salt bridge contact. Therefore it is necessary to ensure contact renewal or to prevent its drying during E_{tr} measurement. In aboveground organs, a very considerable component of E_{tr} is formed by the drop in potential on their surface – perhaps on the cuticle. It is stabilized after several hours of solution contact. This drop in potential should influence both E_m and E_{tr} to the same extent in intact plants.

The opposite polarity of the E_m and E_{tr} changes and their similar pattern may serve as a clue to explaining the nature of E_{tr}. The low absolute value of E_{tr} apparently indicates that the transmembrane potential differences are not included directly in the E_{tr} value. Thus, E_{tr} predominantly represents the potential difference in apoplast between the places of salt bridge contacts (OKAMOTO *et al.* 1984). The opposite polarity of changes in E_m and E_{tr} may be explained by a complementarity of symplast and apoplast ion fluxes causing electrophysiological changes. For example, a slight net efflux of cations from protoplasts to apoplast through the plasmalemma will cause a protoplast hyperpolarization and apoplast depolarization simultaneously. The algebraic sum of both changes represents the resulting E_m hyperpolarization whereas the apoplast depolarization (against the roots) represents the change in E_{tr}. It is therefore rather surprising that this principle is valid in both aerial and aquatic media (*cf.* ZAWADSKI and TREBACZ 1985, POLEVOÏ 1986).

The measurement of E_{tr} in intact *C. rubrum* plants includes the contribution of roots, stem and leaf. Only the leaf is responsible for E_{tr} changes comparable with those of E_m with an opposite polarity. The contribution of hypocotyl together with roots was much lower than that of leaf after changing light and darkness (ADAMEC and KREKULE 1989b). It might explain the rather small differences found between E_m and E_{tr} changes in intact plants.

The present results justify the use of E_{tr} measurements in place of E_m in studying electrophysiological processes. The advantages are a low electrical plant resistance (*ca.* 2 MΩ in *C. rubrum*), high E_{tr} stability, and the possibility of making long-term

E_{tr} measurements in intact plants or excised leaves and changing the flowing solution. Further, there is no need of any micromanipulator and microelectrodes. The simultaneous E_m and E_{tr} measurement in the same plant represents only a small modification of a common E_m measurement (Fig. 1). This method could be used to advantage for studying the irritability and interorgan communications in higher plants (see MONTAVON 1984, ZAWADSKI and TREBACZ 1985). We used it for studying the photoperiodic flower induction in *C. rubrum* (ADAMEC and KREKULE 1989a, b).

REFERENCES

ADAMEC, L., KREKULE, J.: Changes in membrane potential in *Chenopodium rubrum* during the course of photoperiodic flower induction. – Biol. Plant. **31** : 336–343, 1989a.

ADAMEC, L., KREKULE, J.: Changes in transorgan electric potential in *Chenopodium rubrum* during the course of photoperiodic flower induction. – Biol. Plant. **31** : 344–353, 1989b.

DUNLOP, J., BOWLING, D. J. F.: The movement of ions to the xylem exudate of maize roots. III. The location of the electrical and electrochemical potential differences between the exudate and the medium. – J. exp. Bot. **22** : 453–464, 1971.

JAFFE, L. F., NUCCITELLI, R.: Electrical controls of development. – Annu. Rev. Biophys. Bioeng. **6** : 445–476, 1977.

MONTAVON, M.: Lumière et biopotentiels chez l'épinard (*Spinacia oleracea* L. cv. Nobel): phytochrome, communication interorganique et floraison. – PhD. Thesis, No. 2099. Univ. Genève 1984.

MONTAVON, M., HORWITZ, B. A., GREPPIN, H.: Far-red light-induced changes in intracellular potentials of spinach mesophyll cells. Interaction with red light. – Plant Physiol. **73** : 671–676, 1983.

NEWMAN, I. A., BRIGGS, W. R.: Phytochrome-mediated electric potential changes in oat seedlings. – Plant Physiol. **50** : 687–693, 1972.

NOVAK, B., BENTRUP, F. W.: An electrophysiological study of regeneration in *Acetabularia mediterranea*. – Planta **108** : 227–244, 1972.

NOVAK, B., GREPPIN, H.: High-frequency oscillations and circadian rhythm of the membrane potential in spinach leaves. – Planta **144** : 235–240, 1979.

OKAMOTO, H., ICHINO, K., KATOU, K.: Radial electrogenic activity in the stem of *Vigna sesquipedalis*: involvement of spatially separate pumps. – Plant Cell Environ. **1** : 279–284, 1978.

OKAMOTO, H., KATOU, K., MIZUNO, A., KOJIMA, H., MATSAMURA, Y., ONO, Y.: Osmotic and hormonal control of electrogenic ion pumps and growth in pea hypocotyl segments by pressurized intra-organ perfusion. – In: CRAM, W. J., JANÁČEK, K., RYBOVÁ, R., SIGLER, K. (ed.): Membrane Transport in Plants. Pp. 447–452. Academia, Praha 1984.

POLEVOĬ, V. V.: Rol Auksina v Sistemakh Regulyatsii u Rasteniĭ. [The Role of Auxin in Regulatory Systems in Plants.] – Nauka, Leningrad 1986.

RAVEN, J. A.: The possible role of membrane electrophoresis in the polar transport of IAA and other solutes in plant tissues. – New Phytol. **78** : 285–291, 1979.

STARRACH, N., FREUDLING, C., MAYER, W. E., GRADMANN, D.: Aged preparations are electrophysiologically more representative of intact tissue than are freshly excised preparations. – Planta **160** : 88–90, 1984.

ULLMANN, J., SEIDLOVÁ, F., KREKULE, J., PAVLOVÁ, L.: *Chenopodium rubrum* as a model plant for testing the flowering effects of PGRs. – Biol. Plant. **27** : 367–372, 1985.

ZAWADSKI, T., TREBACZ, K.: Extra- and intracellular measurements of action potentials in the liverwort *Conocephalum conicum*. – Physiol. Plant. **64** : 477–481, 1985.