ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XXVIII, 1 SECTIO C 1973

Instytut Biologii UMCS
Zakład Fizjologii Roślin

Adam PASZEWSKI and Tadeusz ZAWADZKI

Bioelectrical Potentials in *Lupinus angustifolius* L. Shoots *

Bioelektryczne potencjały w łodygach *Lupinus angustifolius* L.

Биоэлектрические потенциалы в стеблях *Lupinus angustifolius* L.

INTRODUCTION

The experiments carried out in our laboratory on the biopotentials of coleoptiles of *Avena sativa* L. and other higher plants (12, 13, 14, 15) have given results similar to those obtained by other workers (4, 5, 3, 6, 7, 8, 9, 10, 11, 16, 17).

The following problems have been directly related to biopotentials: the distribution of potentials in coleoptiles, the polarity of coleoptiles and the geoelectric effect.

It seems that the coleoptiles — which have been the standard material in physiological research — are not suitable as research material in electrophysiological experiments because of their weak reaction to stimuli.

In works related to action potentials in plants there is a very considerable similarity as far as the amplitude is concerned while the wide range of frequencies from 1/hour (5) to 1/min (18) and 1/sec (1) having been found.

Irregarding the time scale the shape of the curves was always similar. This shape is also very similar to the curves obtained at the stimulation of nerves, although with nerves the order of 1 to 10^3 Hz and the amplitude is of the order of 10^2 μV to 100 mV. In the present work the resting

* This work was supported by the Biochemical and Biophysical Comittee of the Polish Academy of Sciences.

and action potentials of *Lupinus angustifolius* L. have been observed. The plants were stimulated electrically by square current pulses of 9 V for 30 sec or chemically by diethyl ether.

MATERIALS AND METHODS

Plant material. The research was carried out on 16-day old plants of *Lupinus angustfiolius* L., variety Wielkopolski, crop 1970. The lupin seeds were placed in Mitscherlich vessels and incubated under stable conditions of light, temperature and humidity. Sixteen-day old plants were placed separately in test tubes with Knopp nutrient (the roots sumberged) and placed in the measuring chamber.

Apparatus. The block-diagram of the apparatus in shown in Fig. 1 A.

Electrodes. Calomel, nonpolarizing, liquid-contact electrodes were used, Fig. 1 B.

Method. The potentials differences were measured after three hours of adaptation in the measuring chamber. Three modes of measurement were performed: a) potential of plants in a normal, i. e. nonstimulated, condition, b) potentials of plants stimulated chemically, c) potentials of plants stimulated electrically. The potentials of plants in a normal condition were measured in order to obtain a base for the experiments b) and c). The chemical stimulus was given in the form of a tampon of cotton woll soaked in diethyl ether. The tampon was placed on the upper end of the shoot, about 14—16 cm above the electrode 1, Fig. 1 C. We assume that the moment when the tampon was applied was the beginning of the stimulation period. The tampon remained on the plant until action potentials ceased to be registered, i.e. after about 30—45 minutes. It was not possible to determine the end of the period of stimulation because of the rapid evaporation of ether.

Action potentials related to chemical stimulation were also measured in a modified way using the three-channel recorder. Such an arrangement allowed for the continuous measurement of potentilas (every three seconds) at three points on the shoot, Fig. 1 D. Action potentials related to electrical stimulus were measured by introducing a separate stimulating system isolated from the measuring system (Fig. 1 A and 1 E).

In each of these experimental series 15 plants were examined. Every experiment lasted 12 hours from 8th hour to 20th hour.

RESULTS AND DISCUSSION

The measurements of the potentials showed that nonstimulated plants were characterised by noticeably stable values during the 12-hour measuring period. The potential values measured were different in separate specimens and fluctuated within the limits of -80 to $+50$ mV with 2/3 of the results lying within the limits of -10 to -40 mV. One of the characteristics curves of resting potentials can be seen in Fig. 2 A. This was

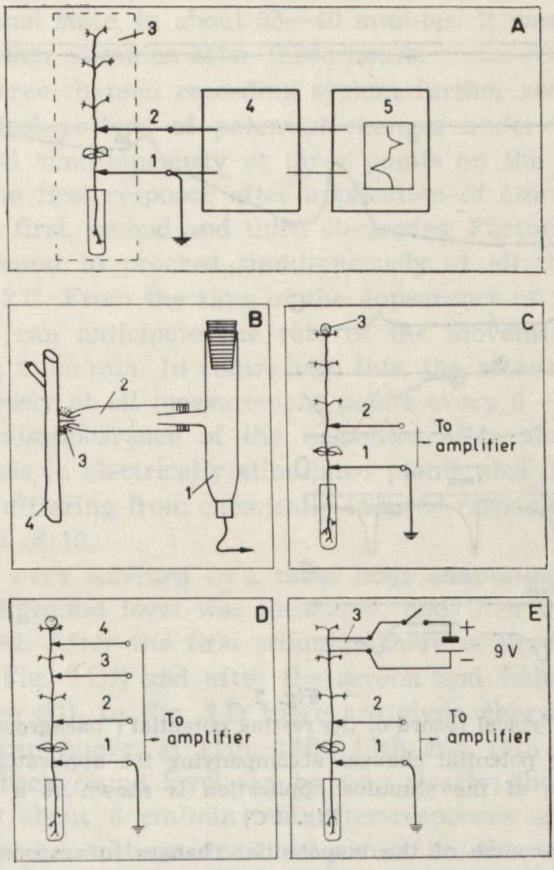

Fig. 1

A — Block-diagram of the apparatus; 1 and 2 — calomel electrodes, 3 — chamber, 4 — amplifier ($R_{in}=10^{14}\Omega$), 5 recorder

B — Diagram showing the contact of the electrode with plant; 1 — calomel electrode, 2 — glass tube filled up with 0.1% KCl, 3 — the camel wool pinsel wet with 0.1% KCl insuring liquid contact with plant, 4 — the plant

C — The way of the application of a chemical stimulus. 1 and 2 — calomel electrodes distant 6 cm, 3 — cotton wool tampon impregnated with ether

D — The way of the application of a chemical stimulus using the 3-channel recording apparatus. 1, 2, 3 and 4 — calomel electrodes

E — The way of the application of electrical stimulus. 1 and 2 — calomel electrodes, 3 — stimulating device consisting of two steel inserted electrodes and a battery

considered a sufficient background for the examination of action potentials.

The action potentials of plants stimulated with ether were charasteristic, and occurred in 13 of the 15 experiments performed. The charac-

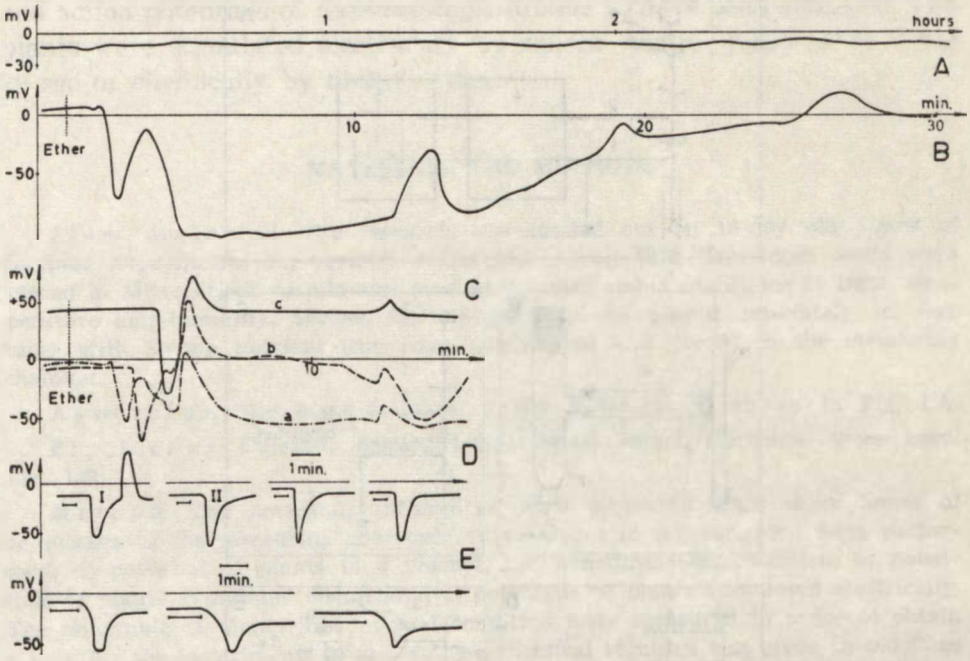

Fig. 2

A — Typical record of the resting potential ("background")

B — Curve of the potential changes accompanying the application of a chemical stimulus. The time of the stimulus application is shown as a dotted line (see Fig. 1 C)

C — Typical time-course of the biopotential changes in response to a chemical stimulus (see Fig. 1 D); curve a — changes recorded with electrode 4, curve b — with electrode 3, curve c — with electrode 2

D — Typical time-course of the biopotential changes in response to the electrical stimulation (9 V for 30 sec). The time of the application of the stimulus is shown as a short line under each curve

E — Typical time-course of the biopotential changes appearing after electrical stimulation in different conditions (for explanation see text)

teristic curve is shown in Fig. 2 B. The shape of the curve (amplitude, frequency and type of changes, as well as the amount and temporal arrangement of peaks) was repeated in almost all measurements. The existence of a latency period of a few minutes and the regular repetition of after-responses with diminishing amplitude is also interesting. Ether is less definite as a stimulus than electricity, which may have led to the irregularity in responses. The return of the potentials to the background level should be noticed. An entirely normal potential value is reached after a gradual diminution in amplitude, suggesting that the plant re-

turns to a normal state, in about 35—40 minutes. It was possible to apply the next ether stimulus after three hours.

With the three-channel recording system further results were obtained. The typical pattern of potential changes under the stimulus of ether, measured simultaneously at three points on the shoot, is shown in Fig. 2 C. The first response after application of ether appeared succesively at the first, second and third electrodes. Further responses, however, were found to proceed simultaneously at all the electrodes as shown in Fig. 2 C. From the time of the appearance of responses at the electrodes one can anticipate the rate of the movement of excitation which is about 2 cm/min. In contrast to this, the after-responses occurred simultaneously at all measurement points every 6—7 minutes, until the complete disappearance of the responses after about 40 minutes. Action potentials in electrically stimulated plants also showed characteristic changes, differing from chemically induced responses, and observed in 12 cases out of 15.

The plants were subdued to a three hour adaptation period, during which the background level was measured, and then the electrical stimulus was used. After the first stimulus there occurred the first type of the curve (Fig. 2 DI) and after the second and following stimuli — the second type (II). In Fig. 2 D there are given characteristic sections from the 12 hour curve, at 11th, 12th, 13th and 14th hour. The rapid return to the background level can be seen clearly; the impulse conduction speed is about 5 cm/min and after-responses are not observed. The experimental conditions described above, i.e. the value and time of application of the stimulus (whether chemical or electrical), the application method seem to be the optimum ones. Application of the stimuli every ten minutes, for example, gave no response. Application of an electrical stimulus every thirty minutes gave responses as shown in Fig. 2 E. The response is weaker and more deformed. In these conditions the stimulus must be longer for a response to be obtained. This suggests that refraction time in plants may be determined. The same is probably true for the other parameters. The determination of their threshold and maximal or optimal values could supply material for an attempt for a theoretical explanation of the conduction of stimuli in plants.

On comparing of the results obtained by other workers with ours some correlations were revealed (Fig. 3). C l a r k (5) etherizing coleoptiles of *Avena* noted changes in potentials of the order of 1/hour — Fig. 3 A. The experiment described here has given similar results except that the frequencies measured were of the order of 1/min — Fig. 3 B. B u r e s et al. (2) described the results of the electrical stimulation of the sartorius muscle of *Rana esculenta* — Fig. 3 D. For comparison, in

Fig 3 C are shown the results obtained from the electrical stimulation of the lupin shoot. There is an obviously similar form and amplitude with a difference in the frequency.

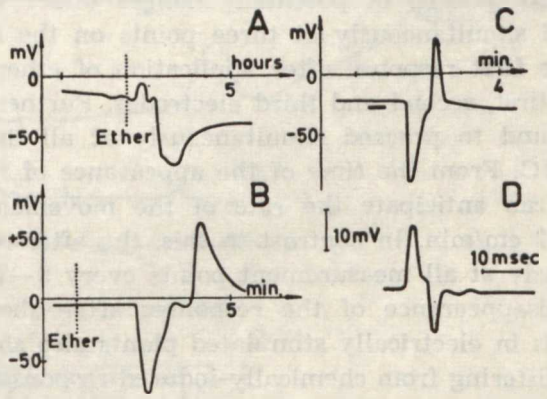

Fig. 3. Comparison of the results obtained in the present study with the data of other authors (see text)

Considering the small number of works discussing the action potentials in higher plants, this comparison is based exclusively on analogies. Nevertheless, the problem may be put in the from of a hypothesis, that the mechanism of conduction is similar in plants and nerves.

CONCLUSIONS

1. Characteristic changes in action potentials occur in young lupin shoots stimulated chemically and electrically.

2. The biopotential changes caused by a chemical stimulus are different from the changes caused by an electrical stimulus. (see Fig. 2 B and D).

3. The characteristics of both changes reveal a far-going analogy with changes observed in other plant species and in animal muscles and nerves stimulated in the same manner.

4. It is possible to measure the characteristic parameters of the stimulating proceses in plants, such as: latency time, relaxation time, refraction time, the threshold value of the stimulus etc., which would allow for a quantitative description of the phenomenon, and would give a basis for a theoretical explanation of the conducting mechanism.

REFERENCES

1. Bulychev A., A., Andrianov V. K., Kurella G. A., Litvin F. F.: Transmembrane Potential of Higher Plant's Cells and Chloroplasts. Fiziol. rast. **18**, 248—256 (1971).
2. Bures J., Petran M., Zachar J.: Electrophysiological Methods in Biological Research. Publishing House of the Czechoslovak Academy of Sciences. Prague 1960, 252—255.
3. Cholodny N. G., Sankewitsch E. Ch.: Influence of Weak Electric Currents upon the Growth of the Coleoptile. Plant Physiol. **12**, 385—408 (1937).
4. Clark W. G.: Electrical Poarity and Auxin Transport. Plant Physiol. **12**, 409—440 (1937).
5. Clark W. G.: Electrical Polarity and Auxin Transport. Plant Physiol. **13**, 529—552 (1938).
6. Grahm L., Hertz C. H.: Measurements of the Geoelectric Effect in Coleoptiles by a New Technique. Physiol. Plant. **15**, 96—114 (1962).
7. Grahm L., Hertz C. H.: Measurements of the Geoelectric Effect in Coleoptiles. Physiol. Plant. **17**, 186—201 (1964).
8. Lund E. J.: Bioelectric Field and Growth. Unic. of Texas Press, Austin 1947.
9. Parkinson K. J.: Bioelectric Potentials of Intact Green Plants. II. Analysis of the Changes in the Measured Bioelectric Potentials of *Avena sativa* L. Coleoptiles Brought about by the Application of the Measuring Contacts. J. exp. Bot. **17**, 309—319 (1966).
10. Parkinson K. J.: Bioelectric Potentials of Intact Green Plants. III. Effect of Jacketing a Region of the Plant Tissue between the Measuring Contacts with Mineral Salt Solutions. J. exp. Bot. **19**, 658—666 (1968).
11. Parkinson K. J., Banbury G. H.: Bioelectric Potentials of Intact Green Plants. I. Measurement of the Bioelectric Potentials of Coleoptiles of *Avena sativa* L., var. „Barnwell". J. exp. Bot. **17**, 297—308 (1966).
12. Paszewski A.: The Application of the Electroencephalograph for the Measurements of Changes of Electric Potential Differences in Plants. Acta Soc. Bot. Pol. **28**, 373—378 (1959).
13. Paszewski A.: Elektrischer Widerstand der Haferkoleoptile. Studia Biophysica. **8**, 227—234 (1968).
14. Paszewski A., Królikowska Z.: Investigation of Electric Potentials in Plants. Ann. Univ. Mariae Curie-Skłodowska sectio C **16**, 141—154 (1961).
15. Paszewski A., Zawadzki T.: The Effect of Physiological Drought on Bioelectrical Potentias in *Avena sativa* L. Coleoptiles. Studia Biophysica **1**, 103—105 (1966).
16. Schrank A. R.: Changes in Electrical Polarity in the *Avena* Coleoptile as an Antecedent to Hormone Action in Geotropic Response. Plant Physiol. **20**, 133—136 (1945).
17. Schrank A. R.: Effect of Mechanical Stimulation on the Electrical and Curvature Responses in the *Avena* Coleoptile. Plant Physiol. **20**, 344—358 (1945).
18. Синюхин А. М., Столярек Я.: Изменения ритмических колебаний биопотенциалов в онтогенезе колеоптиля кукурузы. Доклады Акад. Наук СССР **137**, 725—728 (1961).

STRESZCZENIE

Badano elektryczną aktywność (zmiany biopotencjałów) w łodygach 16-dniowych siewek *Lupinus angustifolius* L., odmiany Wielkopolski ze zbiorów 1970 r. Rośliny stymulowano chemicznie lub elektrycznie. Bodziec chemiczny (eter) lub elektryczny (prostokątne impulsy 9 V przez 30 sek.) podawano miejscowo w górne partie łodyg. Kalomelowe elektrody pomiarowe umieszczano w środkowych i dolnych częściach łodyg.

Wartości biopotencjałów występujące w łodygach roślin nie stymulowanych przyjęto jako tło do badań potencjałów czynnościowych. Charakterystyczne krzywe potencjałów czynnościowych (amplituda rzędu 50—80 mV i częstość ok. 1/min.) zarejestrowano w 80% przeprowadzonych eksperymentów. Krzywe te powtarzają się regularnie z nieznacznymi zmianami amplitudy i częstości dla poszczególnych osobników. Zaobserwowano zjawisko „zmęczenia" u roślin stymulowanych. Powtórne uzyskanie „odpowiedzi" na bodziec może nastąpić po odczekaniu ok. 1 do 3 godz. Kształt krzywych biopotencjałów czynnościowych zależy od odległości elektrod pomiarowych i od miejsca podania bodźca, zachowuje się jednak typowy dla danego bodźca charakter zmiany.

Uzyskane wyniki wykazują daleko idące analogie do wyników uzyskiwanych w podobnych warunkach na innych gatunkach roślin. Kształt tych krzywych jest bardzo zbliżony do kształtu typowych krzywych zmian potencjałów czynnościowych w nerwach lub włóknach mięśniowych. Różnica występuje tylko w szybkości zmian (ok. 100 razy wolniej u roślin).

РЕЗЮМЕ

Исследовали электрическую активность (изменения биопотенциалов) в стеблях 16-дневных сеянцев *Lupinus angustifolius* L., сорт великопольский, урожая 1970 г. Растения стимулировали химическим или электрическим способом. Химический (эфир) или электрический раздражители (прямоугольные импульсы, 9 V в течение 30 сек) действовали на верхнюю часть стеблей. Каломельные измерительные электроды были расположены в середине и внизу стеблей.

Значения биопотенциалов, выступающих в стеблях нестимулированных растений, были использованы для исследований потенциалов действия. Характерные кривые потенциалов действия (амплитуда ряда 50—80 mV, частота около 1/мин.) зарегистрированы в 80% проведенных экспериментов. Эти кривые для исследованных растений повторяются

регулярно с незначительными изменениями амплитуды и частоты. Биопотенциалы действия, связанные с химическим раздражителем, имеют совершенно другую характеристику, чем биопотенциалы, связанные с электрическим раздражителем. У стимулированных растений наблюдали явление „усталости". Вторичный „ответ" на раздражитель может наступить после 1—3 час. Форма кривых потенциалов действия зависит от расстояния между электродами и местом раздражения. Однако при этом сохраняется типичный для данного раздражителя характер изменения.

Полученные результаты обнаруживают значительное сходство с результатами, полученными на других видах растений. Форма этих кривых очень близка к форме кривых изменений потенциалов действия в нервах или мышечных волонках. Разницы выступают только в скорости изменений (у растений медленнее приблизительно в 100 раз).