

**Dielectric Properties, Free Radicals, Amino Acids,
and Some Elements in Maize Tissues**

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Abstract. Data on (1) permittivity and dielectric losses, (2) free radical concentrations, and (3) potassium, sodium, magnesium, calcium, nitrogen, and amino acid contents in tissues of nodal roots, internodes, and leaves of maize plant are presented in the paper. The participation of these factors in the transport phenomenon and the degree of their cooperation in the transport process are discussed.

Studies of fundamental electric properties of plant tissues, of the status or of changes in the contents of free radicals, amino acids, and macroelements in plant parts certainly can contribute to a better understanding of the transport phenomenon in plant tissues. The aim of this paper is to characterize and assess dielectric properties of maize root, internode, and leaf tissues from the viewpoint of the transport phenomenon and to estimate differences in the concentration of free radicals, amino acids, and some macroelements.

MATERIAL AND METHODS

A 92-day-old maize plant (hybrid TA-68/48-S) which grew in the field served as experimental material in this study.

The concentration of free radicals was measured by means of EPR spectroscopy in samples prepared from nodal roots, internodes, and leaves. Plant parts were fixed in fresh state with liquid nitrogen and then lyophilized. Dried tissues were homogenized and pressed into cylinders with a diameter of 5 mm and a height of 7 to

5 mm. Measurements were carried out on an EPR spectrometer Varian E-4, with g of 2.00₅. The spectra obtained were evaluated by means of known methods of EPR spectroscopy (ALGER 1968, MICHALOV and PLÁČEK 1976).

Because samples maintain their original properties during the determination of the content of non-coupled electrons, they also were used for the study of dielectric properties. For this purpose, they were pressed to discs with a diameter of 25 mm using a pressure of 250 MPa which then served for the determination of permittivity and of the loss factor using a Q-meter Tesla BM 449G.

Amino acid content in dry matter was determined following sample hydrolysis in 6 M HCl in fused glass tubes at 105 °C for 20 h. The hydrolyzate obtained was evaporated, and amino acids in the remainder were determined using an AAA

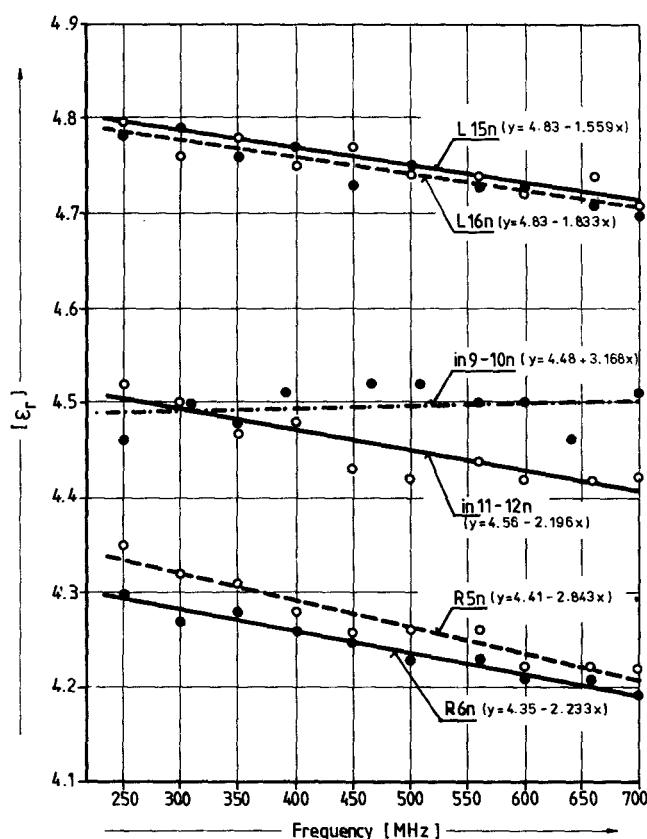


Fig. 1. Frequency dependence of relative permittivity ϵ_r of the biomass of nodal roots (the fifth and the sixth node), internodes (9-10n and 11-12n), and of leaves (the 15th and the 16th node). Symbols: L15n - the leaf on node 15; L16n - the leaf on node 16; in 9-10n - the internode between nodes 9 and 10; in 11-12n - the internode between node 11 and 12; R5n - roots formed on the fifth node; R6n - roots on the sixth node. Analytical description of linear regression of frequency relationships $y = a + bx$ is presented in parentheses which follow the symbols.

339-Microtechna Analyzer. The contents of macroelements, that is of potassium, sodium, calcium, and magnesium, were determined in dry matter which was subjected to additional desiccation at 105 °C. Exactly weighed samples of homogenized dry matter from different plant parts were digested in nitric and perchloric acid and the material obtained was appropriately diluted for the measurement in a SP 90A Unicam Spectrometer.

RESULTS

Dielectric Properties

The relationships presented in Fig. 1 characterize the dependence of permittivity on frequency and clearly distinguish root tissues from internode and leaf tissues according to their natural location. The highest values of relative permittivity were recorded in leaf tissues and the lowest in root tissues. The results obtained also show that neighbouring leaves or roots differ very little from each other in their relative permittivity.

The situation is more interesting in the case of internodes: The internode which was closer to the base of the plant showed higher relative permittivity values which increased with increasing frequency. By contrast, in the case of an internode located at a higher level, relative permittivity values were lower and decreased with increasing frequency similarly as in cases of leaves and roots.

The values of dielectric losses (Fig. 2) decreased with increasing frequency, with the exception of tissues of the 11–12 n internode, in which the highest dielectric losses were recorded, whereas the lowest losses were recorded in tissues of the 9–10 n internode. Roots showed the lowest values of relative permittivity, leaves the highest values. In contrast to this, dielectric losses were higher in roots than in leaves in which only low dielectric losses were recorded.

Free Radicals

The concentrations of free radicals are illustrated in Fig. 3 (right upper part). The highest concentration of free radicals in the plant parts studied was recorded in roots of the fifth node. It decreased towards the internode. It again increased in leaves and also in internodes which apparently also take part in photosynthesis (MICHALOV 1985).

Macroelements

Potassium, sodium, calcium, and magnesium contents in nodal roots of the fifth and sixth node, in the internodes 9–10 and 11–12, and in the leaves belonging to nodes 15 and 16 are presented in the left part of Fig. 3. This figure shows that

potassium content exceeded the contents of the other elements in all the tissues examined, especially in roots and internodes and to a lesser extent in leaves.

Sodium content was very low in these plant parts. The highest sodium content values were recorded in root tissues and the lowest in leaf tissues. The highest calcium content was found in the roots of the fifth node, and the lowest content in internode tissues. Leaf calcium content was considerable. A similar situation was recorded in the case of magnesium, the content of which in leaves, however, was lower by approximately one half than calcium content.

Nitrogen

The highest nitrogen content was recorded in leaf tissues, the lowest in the internode 11–12 n. The internode 9–10 n could not be analyzed, because no material was left following macroelement content determination.

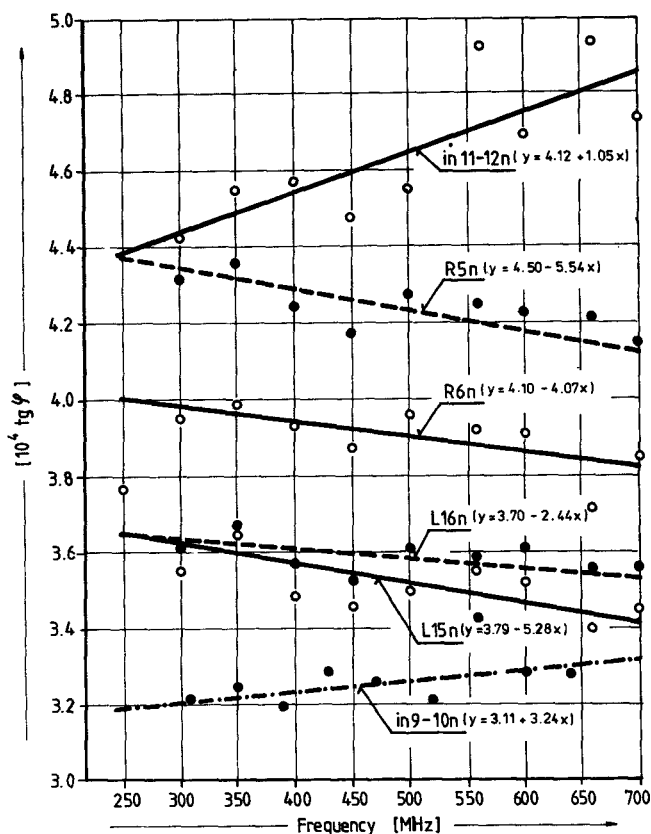


Fig. 2. Frequency dependence of dielectric losses tag ϕ of the biomass of nodal roots (the 5th and the 6th node), internodes (9–10n and 10–11n) and leaves (the 15th and the 16th node). For symbols see Fig. 1.

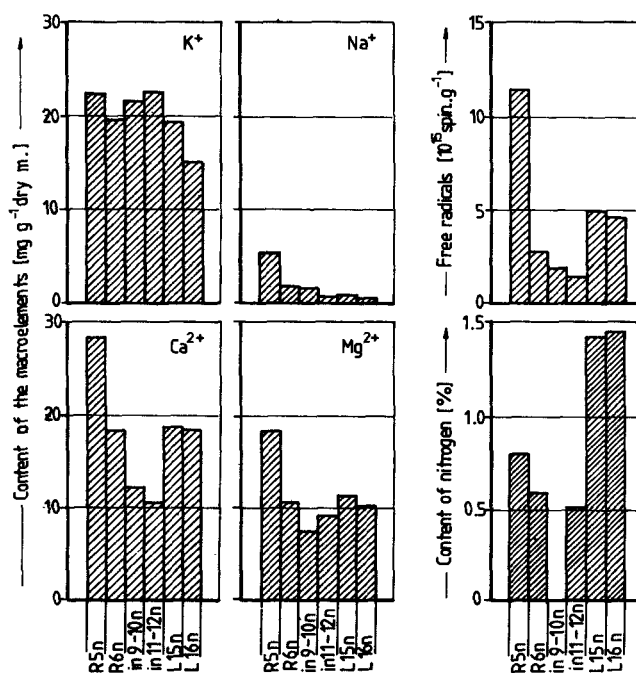


Fig. 3. The content of macroelements (K^+ , Na^+ , Ca^{2+} , Mg^{2+}) in tissues of nodal roots (the 5th and the 6th node), internodes (9–10n and 11–12n), and leaves (the 15th and the 16th node) in the left part of the figure. The symbols representing the ions are presented in the right upper part of each section of the left part of the figure. Free radical contents in the above mentioned tissues are illustrated in the right part of the figure. Nitrogen content in the tissues under investigation is illustrated in the lower section of the right part of the figure.

Amino Acids

In total 15 amino acids were determined in tissue hydrolyzates. Their contents are illustrated in Fig. 4 for five plant parts. The highest amino acid contents were recorded in leaf tissues. Amino acid content in root tissues was lower by one half and the lowest amino acid contents were found in internode tissues. Glutamic acid, aspartic acid, alanine, glycine, serine, valine, threonine, proline, phenylalanine, and isoleucine were the most abundant amino acids. Histidine, lysine, and tyrosine contents were lower. Methionine content was rather low, and the other amino acids were not detectable.

Comparison of Results from the Quantitative Viewpoint

If we start with leaf tissues, we can see that they showed the highest relative permittivity, and the highest nitrogen and amino acid contents. In contrast to this, root tissues showed the lowest relative permittivity, an amino acid content lower than

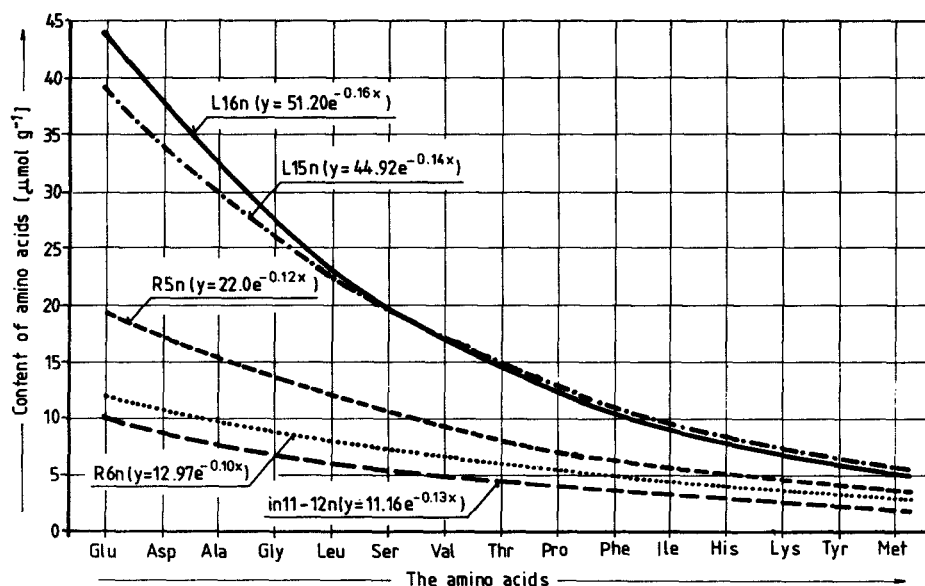


Fig. 4. Amino acid content in tissues of nodal roots (of the fifth and the sixth node), internode 11–12n, and in leaf tissues of the 15th and 16th node (for symbols see Fig. 1). Analytical expression of curves connecting peaks of different amino acids is presented in parentheses which follow the symbols.

a half, higher dielectric losses than leaf tissues and also macroelement content and free radical concentration.

The values of relative permittivity in internode tissues were approximately in the middle between relative permittivity values of leaves and roots, respectively, with a characteristic increase with increasing frequency in the 9–10 n internode and a decrease with increasing frequency in the 11–12 n internode. The difference between these two tissues manifested itself even more clearly in the case of dielectric losses: the highest losses were recorded in the 11–12 n internode and the lowest losses in 9–10 n. Dielectric losses increased in both cases with increasing frequency.

The concentration of free radicals was higher in the 9–10 n internode than in 11–12 n. Potassium and magnesium contents were higher in 11–12 n than in 9–10 n. By contrast, calcium and sodium contents were higher in the 9–10 n internode. As far as nitrogen and amino acid contents are concerned, these tissues could not be compared because of the above mentioned lack of material.

DISCUSSION

The frequency range, within which permittivity and dielectric losses were measured, belongs to the region of β -dispersion, that is to the so-called Maxwell-Wagner

dispersion which is caused by charging processes in membrane interface (PETHIG 1979, ZIMMERMANN 1982).

Maxwell-Wagner polarization plays an important part during the formation of pear-like chains, orientation and rotation of cells (ZIMMERMANN 1982).

If we compare permittivity values of our samples in the applied frequency range with permittivity values of materials which only show elastic polarization, such as for example ϵ_r (polyethylene) = 2.2–2.4; ϵ_r (polypropylene) = 2.3–2.6; ϵ_r (PTFE) = 2.0–2.2, we can see that plant tissue permittivity values are higher. We therefore assume that there are also other (relaxation) types of polarization in plant tissues. This conclusion can be supported by the fact that permittivity values decrease with increasing frequency and thus that there exist contributions to the polarization which with increasing frequency cease to follow the direction of the field and for this reason cannot contribute to permeability.

Polarization is dependent on specific mass from the viewpoint of the number of polarizable particles per volume unit, which in our case was dependent on mechanical properties of the material and on the pressure applied when compacting the material. Because the same pressure was applied during the preparation of all samples, sample compressibility was the main factor which influenced the number of polarizable particles per volume unit.

Dielectric losses occur because the electric field acts on ions or permanent dipoles (macromolecules) at a point and in membrane. These forces, when acting at molecular level, result in ion movement and in partial rotation of polar molecules (ZIMMERMANN 1982).

The decreases in dielectric losses with increasing field frequency in R5 n, R6 n, L15 n, and L16 n tissues may be the result of relaxation. That means that in the frequency range examined there still exist polarization abilities of tissues which can follow to a certain extent changes in the electric field. This ability is associated with relaxation times which, as shown by MICHALOV and REMIŠ (1982), can differ according to tissues, and are closely associated with the configuration of particles in the material under investigation. This need not be valid in the case of internode tissues, where the opposite situation can be expected to exist.

It has been shown that all plant tissues contain free radicals (MICHALOV and PLÁČEK 1982, MICHALOV 1985, MICHALOV *et al.* 1987). Roots show the highest, leaves lower, and internodes the lowest free radical contents. What is of interest is the fact that this order corresponds to the contents of calcium and magnesium, which elements are known to have several important physiological functions. Calcium, among others, plays an important part in the maintenance of membrane integrity (MONK *et al.* 1989); in the root, it maintains functional integrity of root cell membranes, especially of the plasmalemma, which is a so-called site of ion absorption processes (LAÜCHLI and EPSTEIN 1970), and acts as a permeable and selective factor (ETHERTON 1967, MENGEL and HELAL 1967). Magnesium is indispensable for respiration processes, as well as for the activity of bending cells and

is also an activator of some important enzymes which play an important part in active transport and in photosynthesis (PRICE 1970).

These examples show that the concentration of free radicals in plant tissues can either increase or decrease with the intensity of oxidation processes and with enzymatic activity (KOZLOV 1968) which are closely associated with other metabolic processes and thus also with the transport phenomenon.

High potassium contents recorded in all plant parts reflect the importance of potassium ions in the plant. Potassium ion is generally considered to be the contra-ion of sodium ion in the process in which the sodium ion is actively transported out of the cell and a corresponding amount of potassium ions enters the cell. High passive diffusion potentials are the result of higher potassium concentrations inside the cells and higher sodium concentrations outside the cells (HODGKIN and HUXLEY 1952). HASKELL *et al.* (1968) showed that potassium is the sole cation which determines the diffusion potential across a permeable membrane.

HOPE (1965), KERNAN (1965), and POOLE (1966) assumed that the electrogenic pump could cause an asymmetry in ion distribution which under conditions of high variability of temperature and of internal and external concentrations of potassium ions can lead to a situation in which the membrane potential behaves as a potassium diffusion potential. This requires a prevalence of potassium ions inside plant cells and suggests a considerable involvement of potassium ions in the transport process.

The presence of sodium ions, although at lower concentrations, is of great importance, first of all from the viewpoint of osmotic balance in the cell. Second, sodium ion concentration influences in each case the membrane potential value. Third, with respect to a common structural basis of cell membranes, it can be assumed that sodium ions take part in the excitation of the plant cell membrane (MCROBBIE 1962, SPANSWICK and WILLIAMS 1964, SPANSWICK *et al.* 1967).

Changes in the protein lining of pores, caused by a change in passive permeability of cells for the ions under investigation, might result in changes in genetic composition of the organism. Because the process of these changes is based on passive permeability of cells, JENNINGS (1967) considered passive permeability of cells to be a genetic control which might be one of the possible roles of proteins in the transport process. The carrier function represents another function according to SUTCLIFFE (1962), DENNIS and MIERNYK (1982), and KYLE and ARNTZEN (1983). SUTCLIFFE (1962) suggested that amino acids could function as salt carriers in the transport process. It is generally known that asparagine and glutamine and in legumes also arginine are the main transport forms of nitrogen in plants (IVANKO and INGVERSEN 1971, PATE 1971).

As we have obtained an amino acid content pattern in plant tissues similar to that known from animal physiology (LEE and VIDAVER 1981), it seems to be appropriate to ask if there also exists in plant tissues an amino acid transport system involved, as we have seen, in the transport of salts or of nitrogen?

To conclude, we can state that the transport phenomenon in an intact plant is

determined by several forces of different origin and different magnitude. According to the functional specialization of each plant tissue, either forces of electric origin or those of chemical origin can predominate. The action of forces of these two groups is difficult to differentiate.

REFERENCES

- ALGER, R. S.: Electron Paramagnetic Resonance. Techniques and Applications. – Interscience Publ., New York–London–Sydney 1968.
- DENNIS, D. T., MIERNYK, J. A.: Compartmentation on nonphotosynthetic carbohydrate metabolism. – *Annu. Rev. Plant Physiol.* **33** : 27–50, 1982.
- ETHERTON, B.: Steady state sodium and rubidium effluxes in *Pisum sativum* roots. – *Plant Physiol.* **42** : 685–690, 1967.
- HASKELL, J. A., HARVEY, W. R., CLARK, R. M.: Active transport by the *Cecropia* midgut. V. Loss of potassium transport during larval-pupal transformation. – *J. exp. Bot.* **48** : 25–37, 1968.
- HODGKIN, A. L., HUXLEY, A. F.: A quantitative description of membrane current and its application to conduction and excitation in nerve. – *J. Physiol.* **117** : 500–544, 1952.
- HOPE, A. B.: Ionic relations of cells of *Chara australis*. X. Effects of bicarbonate ions on electrical properties. – *Aust. J. biol. Sci.* **18** : 789–801, 1965.
- IVANKO, Š., INGVERSEN, J.: Investigation on the assimilation of nitrogen by maize roots and the transport of some major nitrogen compounds by xylem sap. I. Nitrate and ammonia and assimilation in the nitrogen fractions of nitrogen starved maize roots. – *Physiol. Plant.* **24** : 359–365, 1971.
- JENNINGS, D. H.: Electrical potential measurements, ion pumps and root exudation – comment and model explaining cation selectivity by the roots. – *New Phytol.* **66** : 357–369, 1967.
- KERNAN, R. P.: *Cell*. – Butterworths, London 1965.
- KOZLOV, Y. P.: Svobodnoradikalnye Protssessy v Biologicheskikh Sistemakh. [Free Radical Processes in Biological Systems.] In Russ. – In: *Biofizika*. Pp. 103–159. Izd. Vysshaya Shkola, Moskva 1968.
- KYLE, D. J., ARNTZEN, C. J.: Thylakoid membrane protein phosphorylation selectivity alters the local membrane surface charge near the primary acceptor of photosystem II. – *Photobiochem. Photobiophys.* **5** : 11–25, 1983.
- LÄUCHLI, A., EPSTEIN, E.: Transport of potassium and rubidium in plant roots. The significance of calcium. – *Plant Physiol.* **45** : 639–641, 1970.
- LEE, J. W., VIDAVER, G. A.: Active Ca^{2+} transport by membrane vesicles from pigeon erythrocytes. Simulation by amino acids, ATP, GTP, P and some other cell constituents. – *Biochim. biophys. Acta* **643** : 421–434, 1981.
- MURPHY, E. A. C.: Ionic relations of *Nitella translucens*. – *J. gen. Physiol.* **45** : 861–878, 1962.
- MENGEL, K., HELAL, M.: Der Einfluss der austauschbaren Ca^{2+} junger Gerstenwurzeln auf den Fluss von K^+ und Phosphat – eine Interpretation des Viets-Effektes. – *Z. Pflanzenphysiol.* **57** : 223–234, 1967.
- MICHALOV, J.: The influence of *Ustilago maydis* on free radicals concentration in *Zea mays* tissues. – *Phytopathol. Z.* **112** : 355–358, 1985.
- MICHALOV, J., ERDELSKÁ, O., PECLAR, C.: Changes in concentrations of free radicals and amino acids in developing maize inflorescences and fruits. – *Biol. Plant.* **29** : 279–282, 1987.
- MICHALOV, J., PLAČEK, J.: Concentration of free radicals in the root system of *Zea mays*. – *Z. Pflanzenphysiol.* **78** : 293–306, 1976.
- MICHALOV, J., PLAČEK, J.: Free radicals in the root system and stem of *Zea mays* – *J. exp. Bot.* **33** : 511–514, 1982.
- MICHALOV, J., REMÍŠ, D.: Dielectric characteristic of young maize plant biomass. – *Bioelectrochem. Bioenerg.* **9** : 15–21, 1982.

- MONK, L., MCPHAIL, D. B., GOODMAN, B. A., DAVIES, H. V. : An electron spin resonance investigation of internal rust spot, a physiological disorder of the potato tuber. – *Free Rad. Res. Commun.* **5** : 345–350, 1989.
- PATE, J. S. : Movement of nitrogenous solutes in plants. – *Atomic Agency* **341** : 165–187, 1971.
- PETHIG, R. : *Dielectric and Electronic Properties of Biological Materials*. – John Wiley and Sons, Chichester – New York 1979.
- POOLE, R. J. : The influence of the intracellular potential on potassium uptake by beetroot tissue. – *J. gen. Physiol.* **49** : 551–563, 1966.
- PRICE, C. A. : *Molecular Approaches to Plant Physiology*. – McGraw-Hill Book Company, New York 1970.
- SPANSWICK, R. M., STOLAREK, J., WILLIAMS, E. J. : The membrane potential of *Nitella translucens*. – *J. exp. Bot.* **18** : 1–16, 1967.
- SPANSWICK, R. M., WILLIAMS, E. J. : Electrical potentials and Na, K and Cl concentrations in the vacuole and cytoplasm of *Nitella translucens*. – *J. exp. Bot.* **15** : 193–206, 1964.
- SUTCLIFFE, J. E. : *Mineral Salts Absorption in Plants*. – Pergamon Press, New York–Oxford–London–Paris 1962.
- ZIMMERMANN, U. : Electric field-mediated fusion and related electrical phenomena. – *Biochim. biophys. Acta* **694** : 227–277, 1982.

BOOK REVIEW

NICHIPOROVICH, A. A. (ed.) : *FOTOSINTEZ I PRODUKSIONNYI PROTSESS*. [PHOTOSYNTHESIS AND THE PRODUCTION PROCESS.] – Nauka, Moskva 1988, 278 pp. 4.20 Rbl. [In Russian]

. The book contains new results of studies and outlines the perspectives of the theory of plant photosynthetic activity as the basis of the plant production process. The main features of the theory are explained by its author A. A. Nichiporovich in the first introductory paper. The following 30 contributions written by coworkers or pupils of A. A. Nichiporovich, are a further elaboration of the production theory. They are arranged in five parts concerning the regulatory systems of primary photosynthetic processes on the chloroplast-cell level, metabolic aspects of plant photosynthetic activity, the role of light and growth in plant photosynthetic activities and productivity, systems of optimization of photosynthetic activity and productivity, and genetic aspects of photosynthetic plant activities in relation to productivity.

The theory of plant photosynthetic productivity generalizes our fundamental knowledge and it stimulates research on different levels. Unfortunately, the book was written only in Russian. It is of interest for plant physiologists, biochemists, biophysicists, agronomists and geneticists.

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