### EMIL BROOK, MARY BAKHANASHVILI

Faculty of Life Sciences, Bar-Ilan University Ramat-Gan 52900, Israel

# COLLECTION AND LEVEL OF HOMEOSTATIC IONS IN PLANT TISSUES DEPENDING ON THEIR AGES

Key words: homeostasis, ion activity, atomic-absorption spectroscopy, ion-selective ionometry

#### Abstract

Ionic composition and its peculiarities in the homeostasis of maize stem growth are considered at different stages of ontogenesis. The content of mineral elements and the activity of their ions are studied in tissues of different stem segments varying in their growth rate. Observations show that the stem growth function and the activity of ions are closely related. The period of peak growth function, especially in the first stage of ontogenesis, is characterized by an increase of ionic activity in tissues of a growing stem. The acropetal and basipetal distribution gradients of elements and the activity of their ions are determined for different organs of maize. The ionic state and the state of water in cells of maize stem tissues are found to be dependent on the functional load. Guidelines for further studies of ionic homeostasis are drawn up to elucidate the mechanism behind the formation of rapid response systems in plants.

## Introduction

To explain the mechanisms of plant growth, contemporary physical and chemical biology resorts to molecular genetics and biochemistry of macromolecules. Analyzing the results of studies, we can infer that changes in the level and rate of biosynthesis of proteins and their respective RNAs may be triggered by certain earlier events, such as changes in the parameters of ionic homeostasis in cells [2, 5, 7, 10, 12]. Cellular ionic homeostasis governs the activities and concentrations of organic and inorganic ions and water content [9]. Organic ions  $(SO_4^{2-}, NO_{3\bar{1}}, H^+, OH\bar{1}, PO_4^{2-}, etc.)$  and polyamines can appear and disappear in metabolic processes, whereas inorganic ions  $(K^+, Na^+, CaI^+, MgI^+, etc.)$  can only take part in the transport or change their activity. Ionic activity means the effective concentration of ions-a in solution, which is usually less than the analytical concentration and equal to  $a = c \cdot f$ , where c is the concentration and f is the coefficient of activity, which depends on the ionic strength of the solution and approaches 1 in extremely diluted solutions.

The ionic activity of organic elements is usually taken into account when ions of these elements go from their bound state in organic or organomineral compounds to their free active state as ions of cytosol or xylem juice.

© EMIL BROOK, MARY BAKHANASHVILI, 2006

Ionic activity is a factor vital for many important physiological functions. Changes in its level have far-reaching consequences in the functioning of an organism as a whole. The basic mineral elements participate in the synthesis of organic compounds in plant cells. On the one hand, they are the chemical elements that make up these organic compounds. On the other hand, they act as allosteric effectors and nonspecific agents, thus shaping the physical and chemical properties of ferments and their conformation without necessarily being their constituents.

However, for a long time, the ideas about the impact of mineral elements on the colloid-chemical state and organization of protoplasts were not covered by the traditional approach in plant physiology. Even though there is certain success in the studies of ionic flows, ionic gradients, and ionic pumps, which play a leading role in energy and information exchange within the cell, not much is known about the basic parameters of ionic homeostasis and its peculiarities in plants [8, 14]. This is especially true for the ionic activity and ionic state (free or bound) of various elements and the possibility of their transformation during the processes of growth and development.

Reviewing the recent studies of ionic homeostasis in animal cells [5, 6] and plant cells, we can clearly discern two main lines of research in this field [4, 11, 13]. The first estimates the role of parameters of ionic composition in the adaptive changes of membrane properties, the formation of action potential, changes of ionic equilibrium, osmoregulation, oxidation and reduction conditions, etc. The second line studies the relationship between the dynamic properties of ionic homeostasis and the system of replication of informational macromolecules.

To understand how the parameters of ionic homeostasis affect the growth process, it is necessary to carefully and comprehensively investigate the acropetal and basipetal distribution gradients of the total content of basic macroelements and the activity of their ions in different organs and tissues of plants, depending on the age of tissues and the functional loads during the process of growth and development. The copious present-day literature on plant physiology still lacks reliable *in vitro* and *in vivo* data on the total content of different elements and the activity of their ions in plant tissues.

The purpose of this study is to determine the parameters of ionic homeostasis in maize and their peculiarities in tissues with various functions, such as division, elongation, and differentiation in the process of growth. The acropetal and basipetal distribution gradients of nitrogen, phosphorus, potassium, calcium, and magnesium mineral in plant tissues and organs were determined and compared with the gradients of total content of these elements and their ionic activity. In new tissues of maize, we saw high ionic activity of  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $K^+$  in the division phase. These tissues were characterized by changing the phase of water.

The main objective of the present study was to confirm the already measured homeostatic total content and ionic activity of different elements in certain plant organs and tissues and carry out mathematical analysis of data as described by Afifi and Eizen [1].

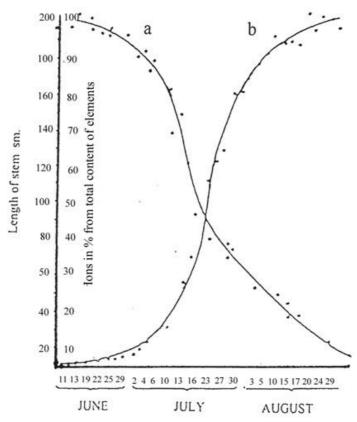


Fig. 1. Correlation between ion activity (a) and growth (b) in tissues of maize stem at different stages of ontogenesis

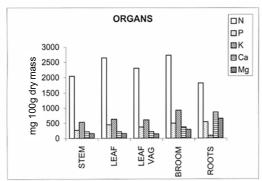
# **Materials and Methods**

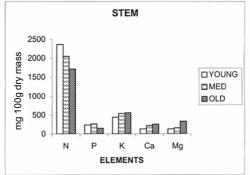
In our field, vegetative, and laboratory experiments, we studied the «Dnepropetrovksaya 247» variety of maize. The samples of maize were selected in the period when stem internodes appear during intercalary growth.

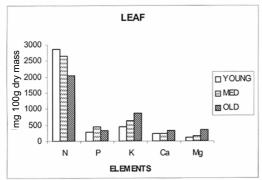
The modern methods of atomic-absorption spectroscopy (AAS) and ion-selective ionometry were used for analyzing the general content and ion activity of mineral nutrition elements in plant samples. We used magnetic resonance imaging (MRI) to study the phase of water in tissues of the maize as described by Buzukashvili and Gordetsky [3].

# **Results**

For many years, we studied the correlation between the growth function of maize stem and the total content of different elements and the activity of their ions. The studies show that despite the high heterogeneity of the total content of elements in stem tissues, stem growth function is closely related with ionic activity. Indeed, the curve of ionic activity in the ontogenesis of maize stem (Fig. 1, *a*) and the curve of growth (Fig. 1, *b*) have the same *S*-like shape but are of opposite signs.







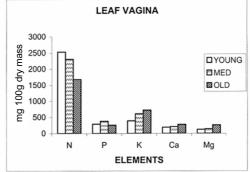


Fig. 2. Acropetal and basipetal gradients of distribution of basic macroelements in organs of maize plants dependent on age

The peak growth period is characterized by the increasing activity of ions in tissues of the growing stem, especially in the first stage of ontogenesis.

We measured the acropetal and basipetal gradients of distribution of basic mineral elements (Fig. 2) and the activity of their ions (Table) in organs of different ages and in tissues of the growth zones of maize stem internodes with different functional states of cells (such as division, elongation, and differentiation). The data presented in Fig. 2 show that, unlike the basipetal gradients of nitrogen and phosphorus in maize organs, the acropetal gradients of the total content distribution of calcium, magnesium, and potassium are not always the same within tissues of the same age. Moreover, the widely accepted concept that calcium and magnesium are elements with acropetal distribution gradients, was not confirmed in the analysis of the ionic activity of these elements.

We can see significant changes in the total content and the ionic activity of calcium and magnesium in internode cells undergoing division, elongation, and differentiation at all ages and in all tiers of the stem. These changes suggest that calcium and magnesium should be considered with more discretion as poorly reutilized elements.

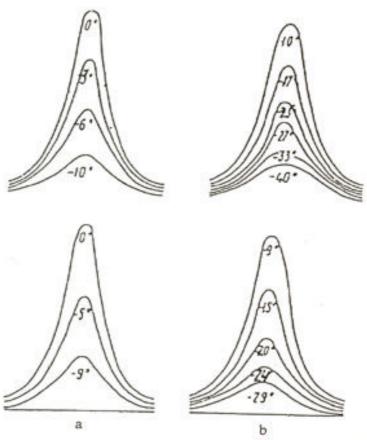


Fig. 3. MRI spectrum of water tissue cells of  $5^{\rm th}$  (above) and  $7^{\rm th}$  (below) maize internodes with different functional loads: a- cells of differentiation zone; b- cells of division zone (above) and elongation zone (below)

### **Discussion**

The results of our experiments show the lack of correlation between the total content of elements and the ionic activity in tissue cells independent of age or functional state. Elevated activity of  $Ca^{2+}$ ,  $Mg^{2+}$ , and  $K^+$  ions was detected in tissues those cells which were predominantly in the phase of division. These tissues were also characterized by changing the phase of water (Fig. 3). In actively dividing cells (the meristem), we found water that did not freeze at temperatures below  $-40\,^{\circ}C$ . In cells of the differentiation and elongation zones, water freezes at  $-9\,^{\circ}C$  and  $-29\,^{\circ}C$ , respectively. Phase transitions of water and changes in the mobility of water molecules can be attributed to changes in ionic activity in actively dividing cells, especially the activity of ions with a high affinity to hydration. Cells of the elongation zone were also characterized by sharply increasing activity of  $Ca^{2+}$  ions, although the total content of this element in this zone was quite low. This may be associated with the participation of calcium ions in the exchange of hydrogen ions during the elongation of cell shells.

The results show that for calcium, the main part of which is in cells and especially in their shells and cytoplasm membranes, which is in the bound state, very sharp changes into the ionic form dependent on growth activity, was observed. This ability of elements to change from the bound state to the active form enables their participation in the formation of a regulatory rapid response system in cells during cell interactions through abrupt changes of pH, rH, and membrane potential, which determine the permeability of membranes and the activity of ferments. Changes in the ionic activity may underlie the formation of action potentials and cause the rapid response and coordination of physiological functions of a whole organism in quickly changing or extreme conditions.

The facts of significant violations in the behavior of parameters of ionic homeostasis, such as the increased activity of univalent and bivalent ions in actively dividing cells, deserve special attention and further investigation.

Stresses and violations of the vital functions of organisms are accompanied by abrupt changes in the parameters of ionic homeostasis, which lead to the reduplication of macromolecules and division of cells. All these aspects of ionic homeostasis in plants are not studied enough and call for further investigation.

- 1. Afifi A., Eizen S. Systematic Analysis with the Employment of Computer. M.: Nauka, 1992.
- 2. *Buzukashvili E.* Gradients of localization and activity of ions, main macroelements in the organs and tissue of maize depending of their ages and functional role // Report Academy of Science of Ukraine. 1986. **B**, N 3. P. 51—54.
- 3. *Buzukashvili E., Gordetsky A.* Methodical aspects of researches on the peculiarities of ionic homeostasis and conditions of water and plant tissues // Ukr. Botan. Journ. 1987. 44, N 4. P. 25—29.
- 4. *Clemens S.* Molecular mechanisms of plant metal tolerance and homeostasis // Planta. 2001. 212. P. 475—486
- 5. *Kafiani K., Malenkov A.* The role of the ion homeostasis of cell in phenomenon of growth and development // Successes of Modern Biology. Moscow, 1976. **81**. P. 445—463.
- 6. *Lane M., Gardner DK*. Regulation of ionic homeostasis by mammalian embryos // Semin Reprod Med. 2000. **18**. P. 195—204.
- 7. Lott JNA., Greenwood JS., Batten GD. Mechanisms and regulation of mineral nutrient storage during seed development // Kigel J., Galili G. (eds.). Seed development and Ggermination. New York: Marcel Dekker, 1995. P. 215—235.
- 8. *Maeshima M*. Tonoplast transporters: Organization and function // Annu Rev. Plant. Physiol. Plant. Mol. Biol. 2000. **152**. P. 469—497.
- 9. *Malenkov A*. Problems and perspectives of the biophysics // Priroda. 1981. 7. P. 73—84.
- 10. Marschner H. Mineral nutrition of higher plants. -London; San Diego: Acad. Press, 1995.
- 11. *Niu X., Bressan R.A., Hasegawa P.M.*, Pardo JM. Ion homeostasis in NaCl stress environments // Plant Physiol. 1995. **109**. P. 735—742.
- 12. *Shaul O.* Magnesium transport and function in plants: the tip of the iceberg // Bio Metals. 2002. **15.** P. 309—322.
- 13. Shaul O., Hilgemann D.W., de-Almeida-Engler J., Van Montagu M., Inze D., Galili G. Cloning and characterization of a novel Mg<sup>2+</sup>/H<sup>+</sup> exchanger // EMBO J. 1999. 18. P. 3973—3980.
- 14. Vahmistrov D. Ion regime of plants: problems of evolution // The new directions in plant physiology. —M.: Nauka, 1985.

Recommended for publication by L.I. Musatenko

Submitted 29.08.2005

Е. Брук, М. Баханашвілі

Бар-Іланський університет, Рамат-Ган, Ізраїль

# НАБІР ТА РІВЕНЬ ГОМЕОСТАТИЧНИХ ІОНІВ У РОСЛИННИХ ТКАНИНАХ ЗАЛЕЖНО ВІД ВІКУ

Застосовуючи методи іоноселективних електродів у поєднанні з методом атомно-адсорбційної спектроскопії встановлено градієнти розподілу основних мінеральних елементів та активність їх іонів у рослинах кукурудзи, які відрізняються за станом тканин. Методом ядерно-магнітного резонансу також встановлена активність води у різноякісних тканинах. Показано, що періодам з найактивнішою ростовою функцією відповідає підвищена активність органогенних фонів у тканинах. Встановлено набір та рівень деяких гомеостатичних іонів, а також підвищена активність води у молодих тканинах.

Обговорюється здатність елементів переходити від зв'язаного стану в активну іонну форму, що забезпечує можливість їх участі у формуванні регулярних систем швидкого реагування у клітинах та в разі міжклітинних взаємодій шляхом миттєвих змін рH, гH, мембранного потенціалу, які визначають проникність мембран та активність ферментів.

Kл ю ч о в і с л о в а: гомеостаз, активність іонів, атомно-абсорбційна спектроскопія, іоноселективна іонометрія

Э. Брук, М. Баханашвили

Бар-Иланский университет, Рамат-Ган, Израиль

### НАБОР И УРОВЕНЬ ГОМЕОСТАТИЧЕСКИХ ИОНОВ В РАСТИТЕЛЬНЫХ ТКАНЯХ В ЗАВИСИМОСТИ ОТ ВОЗРАСТА

Применяя методы ионоселективных электродов в сочетании с методом атомно-адсорбционной спектроскопии, установлены градиенты распределения основных минеральных элементов и активности их ионов в растениях кукурузы, различающихся функциональным состоянием тканей. Методом ядерно-магнитного резонанса определена также активность воды в разнокачественных тканях. Показано, что периодам с наиболее активной ростовой функцией соответствует повышенная активность органогенных ионов в тканях. Установлены набор и уровень некоторых гомеостатических ионов, а также повышенная активность воды в молодых новообразующихся тканях. Обсуждается способность элементов переходить из связанного состояния в активную ионную форму, что обеспечивает возможность их участия в формировании регуляторных систем быстрого реагирования в клетках и при межклеточных взаимодействиях за счет мгновенных изменений рН, гН, мембранного потенциала, определяющих проницаемость мембран и активность ферментов.

Ключевые слова: гомеостаз, активность ионов, атомно-абсорционная спектроскопия, ион-селективная ионометрия