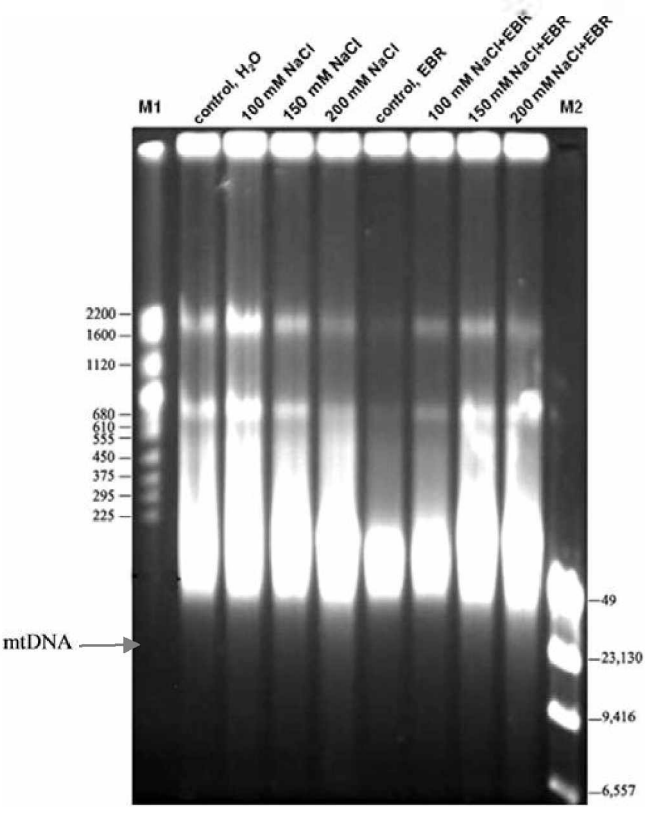
УДК 234.57

EFFECT OF 24-EPIBRASSINOLIDE ON SALINITY INDUCED CHANGES IN THE DNA INTEGRITY OF ROOT TIP CELLS OF BARLEY SEEDLINGS

A. Shkrabkou, postgraduate student
A. Gorbach, 5 course
Supervisor – ***V. Egorova***, PhD in Biology Sciences
Belarusian State Pedagogical University named after M. Tank

Salt stress is one of the most serious problems in agriculture in arid and semi-arid areas. A high concentration of NaCl greatly reduces growth of both the shoot and the root [1]. It is well known that abiotic factors can activate the programmed cell death (PCD).This process could help plants survive during adverse stresses by means of eliminating cells, tissues or organs that render a plant more vulnerable to its environment [4].



Analysis of high-molecular weight DNA by PFGE.
Barley seedling were grown for 2 days in the stress-free nutrient medium and transferred into nutrient medium with salt stress for 5 days (100, 150, 200 mM NaCl). High molecular weight DNA was prepared and analysed by PFGE (Stained with ethidium bromide, pulse period = 60 sec., U = 175 V., t°C = 10-12°C, continuance – 12 h). M1 – marker Pulse marker 225-2200 kb (Sigma); M2 – marker λ/λHind III.

PCD is characterized by cell shrinkage, membrane blebbing, re-organization of the cell nucleus and chromatin fragmentation. At the DNA level, fragmentation occurs in two consecutive steps. The early stage of the apoptotic process is characterized by fragmentation of the DNA into 50–200, 300-500, 800 and 1200 kb fragments resulting from cleavage of the matrix-attached domains [5]. In most cases, this 'domain' fragmentation is followed by cleavage of DNA into nucleosomal linker regions yielding a typical DNA ladder on conventional agarose gels [5].

Brassinosteroids (BRs) are the plant hormones with pleiotropic effects, which influence different physiological processes such as growth, seed germination, rhizogenesis, senescence and leaf abscission [3]. A remarkable feature of BRs is their potential to increase resistance of plants to a wide spectrum of abiotic stresses, such as: low and high temperatures, drought, high salinity [2].

The aim of the work was investigation the nuclear DNA integrity in root tip cells of barley under the physiological salt stress conditions by Pulsed Field Gel Electrophoresis (PFGE). Also it was analyzed the influence of 24-Epibrossinolid (EBR) which possess adaptive properties on this process. The research objects were root tips of spring barley seedlings in salt stress.

The stress induction in barley seedlings was caused by their cultivation in the controlled conditions with the influence of so-

dium chloride in concentration 100, 150 and 200 mM for 5 days (seven-day seedlings). During the increasing of salt solution concentration from 100 mM and above the inhibition of seedlings growth processes were initiated. It was evinced in the gradual descent of length and weight of root and seedlings.

Effective action of EBR, which was introduced into the seeds by their solution treatment with concentration 10^{-9} M during 18 h, was evinced at 150 mM salt stress. The DNA integrity was analyzed by PFGE. The untreated barley seedlings exposed to saline stress were characterized by specific endonucleolitic cleavage of the genome into 300-500, 800 and 1200 kb fragments, which may arise from the release of chromatin loops from the nuclear scaffold. At treatment seeds of barley with 10^{-9} M EBR, abatement of these fragments formation intensity is observed. The observed specific chromatin fragmentation suggests that early stage of PCD might be activated in root tip cells of barley in response to salt stress. These fragments were detected even in case of 50 mM of the salt stress. In the same time there were no any visible signs of seedling growth processes inhibition. However, it should be taken in consideration that barley seedlings are characterized by the maintenance of the full seedling viability despite extensive fragmentation of DNA into pieces of chromatin loop size. Analysis of high-molecular-weight DNA by pulsed-field-gel electrophoresis showed that the majority of DNA strand breaks were repaired by 24-epibrassinolid application.

These findings also evidence the efficient PFGE method use for the initial stages of the plants PCD identification in the salt stress.

References

1. Katsuhara M., Kawasaki T. *Salt Stress Induced Nuclear and DNA Degradation in Meristematik Cells of Barley Roots.* Plant Ceel Physiology. 37(2): 169 – 173 (1996).
2. Bajguz A., Hayat Sh., *Effects of brassinosteroids on the plant responses to environmental stresses.* Plant Physiology and Biochemistry. 47: 1 – 8 (2009).
3. Khripach V., Zhabinskii V., A. De Groot. *Twenty Years of Brassinosteroids: Steroidal Plant Hormones Warrant Better Crops fo XXI Century.* Annals of Botany. 86: 441 – 447 (2000).
4. Huh G. H., Damsz B., Matsumoto T. K., Reddy M. P., Rus A. M, Ibeas J., Narasimhan M. L., Bressan R. A., Hasegawa P. M. *Salt causes ion disequilibrium-induced programmed cell death in yeast and plants.* Plant Journal. 29: 649 – 659 (2002).
5. Fojtova M., Kovarik A. *Genotoxic effect of cadmium is associated with apoptotic changes in tobacco cells.* Plant, Cell and Environment 23: 531 – 537 (2000).