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RELATIONSHIP BETWEEN RNA/DNA RATIO, GROWTH RATE AND ACCUMULATION OF SELENIUM IN THE CELLS OF WHEAT LEAVES UNDER THE INFLUENCE OF MINERALS ANALCIME AND TREPEL

O. I. MARTYNENKO[1], T. K. KYRYLENKO[1], N. V. ZAIMENKO[2], M. M. ANTONYUK[3], A. V. STEPANYUGIN[1], D. P. PLODNIK[4], D. M. HOVORUN[1,4]

[1]Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv;
[2]M. M. Gryshko National Botanical Gardens, National Academy of Sciences of Ukraine, Kyiv;
[3]National University of Food Technonogies, Kyiv, Ukraine;
[4]Institute of High Technologies, Taras Shevchenko National University of Kyiv, Ukraine;
e-mail: Martynenko.Olena@gmail.com

We studied specific effects of different doses of natural minerals – analcime (An) and trepel (Tr) – on the growth rate, selenium (Se) content and functional activity of the genome of wheat leaves measured by the RNA/DNA ratio. Our results show that under the influence of An and Tr, especially at low doses (25 mg/100 g sand), there is a significant increase in the content of Se, increased growth rate of leaves of wheat seedlings and decreased RNA/DNA ratio. We have found significant correlations between studied parameters. Our findings suggest that the RNA/DNA ratio can be used as a convenient, reliable indicator of the biological activity of minerals An and Tr, and for quantitative express-estimation of their impact on plant organisms.

K e y w o r d s: RNA/DNA ratio, minerals analcime and trepel, selenium, wheat.

Studying mechanisms of plant responses to the effects of abiotic factors has become of particular importance given the need to achieve many theoretical and application-oriented objectives of modern phytobiology, including among others the development of new highly effective technologies for controlled growing of plants using natural minerals. Among natural composites that have started to be used in plant growing, special attention should be paid to zeolites, in particular analcime (An) and trepel (Tr) [1, 2]. These minerals are among the most promising because they are ecologically safe natural sources of mineral nutrition for plants and, above all things, they are accessible forms of silicon (Si) [3, 4]. It is known that Si is necessary for many physiological and biochemistry processes in the plant organism, for increasing its adaptive resistance to stress-factors, etc. [5, 6]. According to existing research findings [1, 7], more than 30 different biogenic elements have been detected in An, and around 40 essential micro- and macroelements have been found in Tr. Taking into account complicated nature of silicon-containing silica rocks, the impact of each of them on the plant requires complex investigation. The lack of knowledge regarding biological effects of these natural minerals limits their broad use.

Finding quick, reliable and easily available methods of quantitative evaluation of plant response to exogenous effects has become an important task. It is known that the response of a plant organism to the effects of exogenous factors can be characterized by a number of quantitative indicators including functional state of genome and growth rate as a generally accepted genetically determined indicator of a general response to exogenous effects.

To date, there is no comprehensive information regarding plant genome response, in particular that of wheat, to the effects of natural minerals. Specificities of functional activity of the genome as a whole highly-organized structure were studied on various plants species using DNA-RNA hybridization and microarray technologies [8, 9]. The possibility of using RNA-DNA ratio for this purpose, which we experimentally established [10, 11], might significantly facilitate the determining of index of activity of the genome of plants grown under different conditions.

As reported in [12], Tr is able to decrease penetration of radionuclides in plants, in particular, ^{137}Cs 3-5 times. However, the effects of An and Tr on accumulation of micro- and macroelements in plant tissues, in particular accumulation of selenium (Se)

as an important element in plants' metabolic processes, has not been completely studied. Nowadays adequate provision of a plant organism with Se is considered to be one of the most important criteria of plant quality and utility for animals and people [13].

Cereals belong to the plants with low ability to accumulate Se, in particular optimal content of this microelement in wheat is usually within the range of 0.8-20 μg/g of dry mass [13]. It is known that Se plays a substantial role in regulating water balance and antioxidant status in cells. Selenium also increases adaptation potential and stress-resistance of higher plants and is considered one of the growth-regulating microelements [13, 14]. Therefore, it is important to gain information about the effects of natural minerals on Se accumulation in plants.

The aim of this work is to investigate interdependencies between changes of functional state of the genome of wheat seedlings using RNA/DNA ratio, growth rate and Se accumulation under different doses of An and Tr.

Materials and Methods

The effects of different doses of An and Tr were studied on the model of leaves of wheat seedlings (*Triticum vulgare* (Will)). Plants grown in laboratory conditions on a solid substrate (sand) at the temperature of 20-23 °C, relative air humidity of 70–85% and with natural light. We used calibrated and previously moistened wheat seeds to obtain control samples of plant material. Such seeds for experimental purposes were powdered (P) before planting or planted into sand with natural minerals An and Tr at concentrations of 25, 50, 100, 150 i 200 mg per 100 g of sand.

We used 8 days-old seedlings, which on the 8th day exhibited the increase in the indicators of functional activity of the genome and growth rate of leaves according to the schedule of time distribution of functional activity of genomic DNA in the process of wheat growth [10]. The above findings supported the appropriateness of using these plants in experiments to detect changes in the indicators and Se content in wheat seedlings at different doses of An and Tr.

The growth rate of wheat leaves was determined according to [15] based on the data on the daily growth rate of the aboveground part of 8 days-old seedlings. Thirty wheat plants (7 and 8 days-old) of control and experiment samples were collected and photographed, and the length and growth rate of their leaves were measured using NIH Image 1.59 Software.

The content of Se in the wheat leaves, treated by mixture of concentrated HNO_3 and $HClO_4$ acids, was measured following fluorometric method using 2.3-diaminonaphtaline [16]. The measurement of Se content was performed on the fluorescence spectrophotometer Turner (USA) with 365 nm wave length of stimulating light and 525 nm wave length of radiation. Concentration of this microelement was measured using calibrating graph with $R^2 = 0.98$. To plot a calibrated graph, a standard sample of selenium with concentration of 0.1 mg Se/cm^3 was used. To detect the accuracy and error of selenium measurement we used standard "Ishtar-selenium" sample with concentration of 40 μg /ml. Computer processing of data was performed with the use of software Scion Image.

Spectrophotometric evaluation of quantitative content of nucleic acids (NA) directly in leaves of wheat seedlings was performed according to the procedure [17] with some modifications of the conditions of consecutive hydrolysis of the same plant sample by different chemical factors (0.5 M KON at 67 °C, 1 hour, and then 0.5 M $HClO_4$ at 90 °C, 20 min) [18] with further spectrophotometric analysis of alkaline (RNA) and acid (DNA) fractions in a standard way. The obtained results allowed estimating the RNA/DNA ratio.

For the control and experimental samples of seedlings we conducted three rounds of experiments for each of the three independent experiments.

Statistical analysis of the data was performed using STATA 11.0 software.

Results and Discussion

We report the results of the study of natural minerals' effects on wheat leave growth rate and Se content in these tissues in Fig. 1. It appears that the values of the mentioned parameters in experimental plants significantly exceed the control ones. Seedlings, whose seeds were powdered by An and Tr before planting, have shown an increase in growth rate at the level 25% (An) and 37% (Tr), and Se accumulation at the level 3.2% (An) and 9.7% (Tr). After adding a certain amount of these natural minerals to the substrate (in particular, a dose of 25 mg per 100 g of sand), we observed a significant increase in growth rate and Se content in comparison with control plants. Particularly, the growth rate in experimental plants exceeded growth rate in the control

plants 2.9 times when An was added and 3.5 times when Tr was used, Se content increased 2.5 times and 3.7 times when An and Tr were used, respectively. However, when the doses of An and Tr increased within the range of 50-200 mg per 100 g of substrate we observed a decrease in values of the parameters in experimental plants, although their values were still higher than in the control ones: the indicator of growth rate was 3.6-2.2 cm (An) and 3.0-1.8 cm (Tr); the indicator of Se content, μg/g dry mass changed from 7 to 4.2 under the effect of An and from 9.2 to 1.5 under the effect of Tr. Within the doses range of 25-100 mg minerals per 100 g of substrate and compared to An, Tr has appeared to be the most effective stimulator of growth processes and Se accumulation in wheat seedlings leaves. The observed similarity of dynamics of Se accumulation and growth rate under the influence of two minerals supports existing findings regarding the role of selenium in plant growth processes [14, 19]. It should be noted that compared to An, an increase in doses of Tr led to a more prominent decrease of growth rate and Se content in wheat leaves. The established change in these parameters is among the first described effects of An and Tr mineral components on the plant organism.

Along with determining the growth rate of wheat leaves and their Se content, we studied sensitivity of plants to the change of doses of An and Tr at the level of their genome response. RNA/DNA ratio was used to evaluate general functional activity of

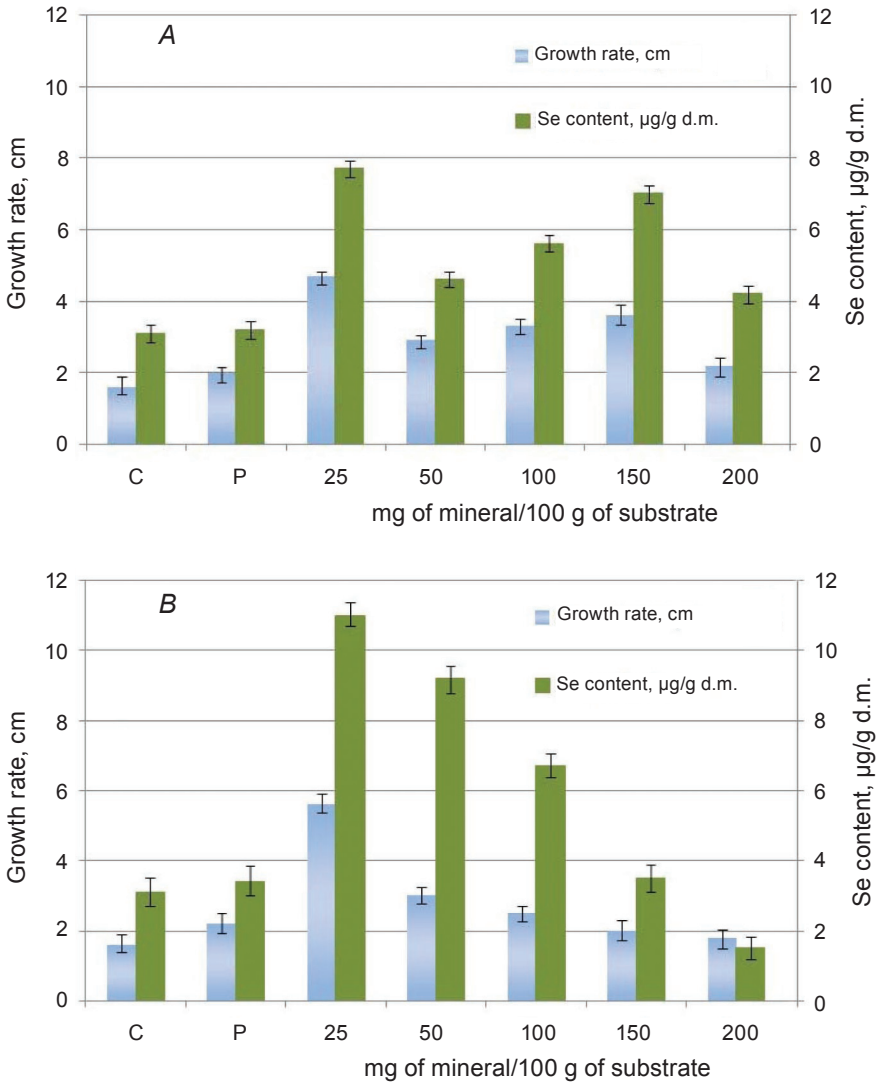

Fig. 1. The impact of analcime (A) and trepel (B) on the growth rate of wheat seedling leaves on the 8th day and their Se accumulation. C – control, P – powdering

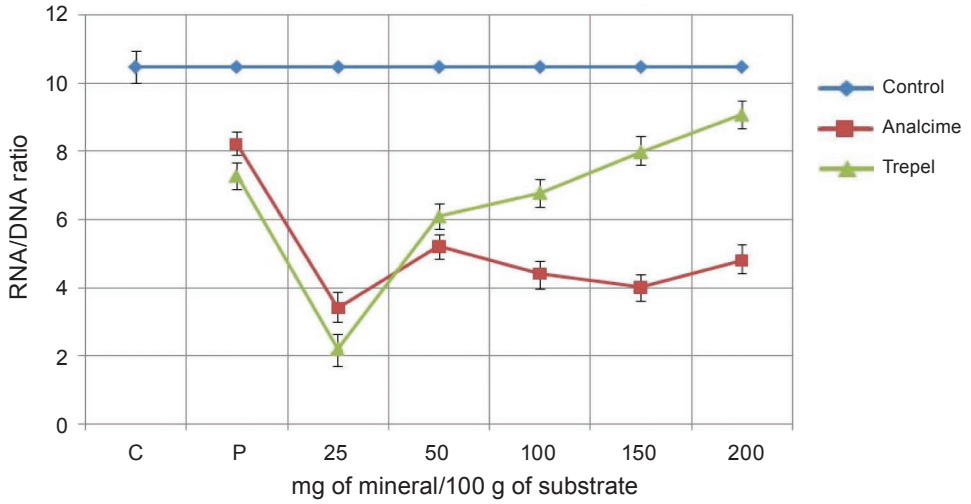

Fig. 2. The impact of different doses of analcime (An) and trepel (Tr) on the RNA/DNA ratio in wheat seedling leaves

genomic DNA [10, 11, 20]. The results of monitoring the dynamics of RNA/DNA ratio in leaves of seedling grown under different content of An and Tr in sand are presented in Fig. 2. It is important to note that general functional state of genome of leaves in control seedlings and in the seedlings grown from the seeds powdered by the minerals was not significantly different. But the level of genome functioning decreased under influence of both minerals used in low doses (25 mg per 100 g of substrate) and gradually increased, but not exceeded control values, in the range of doses of 50-200 mg per 100 g of sand. Within this range we also observed differences between An and Tr effects on the rate of increase of genome activity characterized by the RNA/DNA ratio. Thus, the obtained results prove that the effect of different doses of natural minerals causes significant changes, which are detected at the level of genome functioning and which may be estimated by the RNA/DNA ratio.

Comparing the indicators of genome activity (RNA/DNA ratio) with relevant indicators of Se content and growth rate has showed the existence of opposite direction of changes in the values of these parameters in all studied wheat plants. This result is confirmed by the data of the correlation analysis (Table). It shows that parameters "growth rate" – "RNA/DNA ratio" and "Se content" – "RNA/DNA ratio" are related and characterized by a significant negative coefficient of linear correlation. However, high positive correlation was observed between values of "growth rate" and "Se content". Given significant correlation between these parameters found in our study, the change in one parameter allows us to predict the nature of change in others.

The obtained results prove that wheat is sensitive to the effects of natural minerals An and Tr and is fully suitable plant system for studying biological effects of chemical agents, which may occur at different levels of plant organism organization.

Coefficients of linear correlation (r) between indicators of growth rate, Se content and RNA/DNA ratio in the leaves of control and experimental wheat seedlings under the effect of An and Tr

Indicators	Growth rate	Se content	RNA/ DNA
	Control		
Growth rate	1.0		
Se content	0.965**	1.0	
RNA/DNA ratio	-0.990**	-0.960**	1.0
	Analcime		
Growth rate	1.0		
Se content	0.959**	1.0	
RNA/DNA ratio	-0.880*	-0.944*	1.0
	Trepel		
Growth rate	1.0		
Se content	0.860*	1.0	
RNA/DNA ratio	-0.986**	-0.918*	1.0

*$P < 0.05$; ** $P < 0.01$

This direction of research has a practical implication for establishment of optimal doses of minerals, which provides a possibility to improve ways to regulate growth processes and, as a consequence, to improve productivity and quality of agricultural plants. Using the RNA/DNA ratio has allowed us in a relatively short period of time to determine differences in the character of genome functional activity under influence of different doses of An and Tr, which opens possibilities to study relevant changes at its structural level.

Significant correlation links established among studied parameters provide ground to consider the RNA/DNA ratio as an indicator of biological activity of exogenous factors, in particular, of natural minerals An and Tr, and quantitative evaluation of their impact on plants.

ВЗАЄМОЗВ'ЯЗОК МІЖ СПІВВІДНОШЕННЯМ РНК/ ДНК, ШВИДКІСТЮ РОСТУ ТА АКУМУЛЯЦІЄЮ СЕЛЕНУ В КЛІТИНАХ ЛИСТКІВ ПШЕНИЦІ ЗА ДІЇ МІНЕРАЛІВ АНАЛЬЦИМУ І ТРЕПЕЛУ

О. І. Мартиненко[1], Т. К. Кириленко[1], Н. В. Заіменко[2], М. М. Антонюк[3], А. В. Степанюгін[1], Д. П. Плодник[4], Д. М. Говорун[1,4]

[1]Інститут молекулярної біології і генетики НАН України, Київ;
[2]Національний ботанічний сад ім. М. М. Гришка НАН України, Київ;
[3]Національний університет харчових технологій, Київ, Україна;
[4]Інститут високих технологій Київського національного університету імені Тараса Шевченка, Україна;
e-mail: Martynenko.Olena@gmail.com

Досліджено особливості впливу різних доз природних мінералів анальциму (Ан) і трепелу (Тр) на швидкість росту, вміст селену (Se) та активність геному листків пшениці, охарактеризовану співвідношенням РНК/ДНК. Показано, що за дії Ан і Тр, особливо в низьких дозах (25 мг/100 г субстрату (пісок)), спостерігається істотне зростання вмісту Se, підвищення швидкості росту листків проростків та зниження показника РНК/ДНК. Виявлено значну кореляцію між досліджуваними параметрами. Показано, що співвідношення РНК/ДНК можна використовувати як зручний, надійний показник біологічної активності мінералів Ан і Тр та для кількісної експрес-оцінки їх впливу на рослинні організми.

Ключові слова: співвідношення РНК/ДНК, мінерали анальцим і трепел, селен, пшениця.

ВЗАИМОСВЯЗЬ МЕЖДУ СООТНОШЕНИЕМ РНК/ ДНК, СКОРОСТЬЮ РОСТА И АККУМУЛЯЦИЕЙ СЕЛЕНА В КЛЕТКАХ ЛИСТЬЕВ ПШЕНИЦЫ ПОД ВОЗДЕЙСТВИЕМ МИНЕРАЛОВ АНАЛЬЦИМА И ТРЕПЕЛА

О. И. Мартыненко[1], Т. К. Кириленко[1], Н. В. Заименко[2], М. Н. Антонюк[3], А. В. Степанюгин[1], Д. П. Плодник[4], Д. Н. Говорун[1,4]

[1]Институт молекулярной биологии и генетики НАН Украины, Киев;
[2]Национальный ботанический сад им. Н. Н. Гришко НАН Украины, Киев;
[3]Национальный университет пищевых технологий, Киев, Украина;
[4]Институт высоких технологий Киевского национального университета имени Тараса Шевченко, Украина;
e-mail: Martynenko.Olena@gmail.com

Исследованы особенности влияния различных доз природных минералов анальцима (Ан) и трепела (Тр) на скорость роста, содержание селена (Se) и активность генома листьев пшеницы, охарактеризованную соотношением РНК/ДНК. Показано, что под влиянием Ан и Тр, особенно при низких дозах (25 мг/100 г субстрата (песок)), наблюдается значительное увеличение содержания Se, повышение скорости роста листьев проростков и снижение соотношения РНК/ДНК. Установлена значительная корреляция между исследуемыми параметрами. Показано, что соотношение РНК/ДНК можно использовать как удобный, надежный показатель биологической активности минералов Ан и Тр, а также для количественной экспресс-оценки их влияния на растительные организмы.

Ключевые слова: соотношение РНК/ДНК, минералы анальцим и трепел, селен, пшеница.

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