*Л.Н. Гумилев атындағы Еуразия ұлттық университетінің ХАБАРШЫСЫ.*
*BULLETIN of L.N. Gumilyov Eurasian National University.*
*ВЕСТНИК Евразийского национального университета имени Л.Н. Гумилева.*
*ISSN: 2616-7034. eISSN: 2663-130X*

IRSTI 34.31.35, 34.25.01
Research article

https://doi.org/10.32523/2616-7034-2025-150-1-117-133

# Role of anthocyanins in plant resistance to virus

**M.K. Beisekova[1], A Samat[1], A.B. Kurmanbayeva[1], A.Zh. Bekturova[1], N.N. Iksat[1], S.B. Zhangazin[1], Zh.K. Masalimov*[1]**

[1]*L.N. Gumilyov Eurasian National University, Astana, Kazakhstan*

**Corresponding author: masalimov@gmail.com*

**Abstract.** Viral infections pose a serious threat to crop production in Kazakhstan and worldwide, negatively affecting the growth, development, and productivity of agricultural crops. Under conditions of multiple stresses, such as drought, extreme temperatures, soil salinity, and pathogen damage, viruses aggravate physiological changes in plants, disrupting their metabolic pathways and reducing resistance to adverse factors. Particular attention is paid to the effect of viral infections on the biosynthesis of anthocyanins, important compounds involved in plant defense mechanisms. In this work, it was shown that infection with tomato bushy stunt virus (TBSV) caused more damage to the middle leaves of the model plant compared to other leaves, and the upper leaves stopped developing. In addition, necrosis was observed in the middle leaves, which led to further programmed cell destruction (PCD). Moreover, infection with the TBSV virus led to a significant increase in hydrogen peroxide levels and accumulation of anthocyanins in *Nicotiana benthamiana* plants. These changes indicate a disturbance in the redox balance and activation of defense reactions in response to viral infection. The findings highlight the importance of studying the interaction of viruses with plants to develop strategies to improve crop resistance to viral infections and other stress factors.

**Keywords:** *N. benthamiana,* virus, TBSV, biotic stress, ROS, $H_2O_2$, anthocyanin

**Received: 11.03.2025. Accepted: 28.03.2025. Available online: 04.04.2025**

## Introduction

Viral infections pose a significant threat to agricultural production and can cause significant damage to global food security [1]. The development of viral infections in economically important crops over several generations leads not only to yield losses caused directly by the viruses themselves but also to degradation or degeneration of varieties, thereby increasing the harmful effects. In general, this phenomenon can lead to yellowing of leaves, changes in their shape and height of stems, and a decrease in vegetative mass and quality of harvest [2]. One of the deadly affecting causes is viral invasion of plant tissue, which leads to necrosis and programmed cell death (PCD). Plants experience a variety of metabolic and physiological changes during stress, including changes in shoot/root biomass, reduced photosynthesis and nutrient intake, and suppression of flowering and seed development, all of which contribute to decreased growth and productivity [3].

*Tomato bushy stunt virus* (TBSV) is a member of the Tombusviridae family and possesses a single-stranded positive RNA genome of about 4800 nucleotides encased by 180 capsid protein subunits [4]. TBSV is composed of 30 nm-diameter spherical particles with a positive-sense single-stranded RNA genome of about 4.8 nt that encodes five main open reading frames (ORFs). ORF1 and ORF2 are necessary for viral replication. ORF3 encodes the coat protein, whereas ORF4 encodes the viral movement protein, which is required for cell-to-cell mobility and symptom detection on particular host plants. ORF5 products play a role in inducing necrotic signs as well as the virus's long-distance transmission, depending on the host [5].

The virus has five unique open reading frames: p33 and p92 for the replicase, which are translated from genomic RNA; P41, the capsid protein, is translated from subgenomic RNA 1, while P22 and P19 are translated from subgenomic RNA 2. P19 is the key determinant of the virus's pathogenicity, and it suppresses RNA interference-based defensive systems [6,7].

This virus spreads naturally through contaminated seeds and propagative material, as well as through manual use of infected cutting equipment [8]. TBSV has a limited host range, affecting just a few dicotyledonous species from several families. It also affects numerous vegetable crops [9].

TBSV causes stunting, bushy growth, distortion, and necrosis in tomatoes, eggplants, and peppers. Fruits of infected plants develop necrosis and chlorotic blotching, causing significant economic damage such as production loss and decrease in the quality of commercial solanaceous crops farmed in greenhouses and fields [5].

Therefore, there is an urgent need to study in detail the defense mechanisms used by plants against viral infections. Reactive oxygen species (ROS) play a crucial role in responding to viral infections by integrating various signalling networks and activating plant defense mechanisms [10].

Reactive oxygen species (ROS) are obligatory products of metabolism in living organisms and have several types of these molecules, including the superoxide radical ($O_2$•–), hydrogen peroxide ($H_2O_2$), hydroxyl radical (•OH), singlet oxygen ($^1O_2$), peroxy radical (ROO•), and alkoxyl radicals (RO•) [11–15]. ROS production happens in plant tissue as a consequence of cell wall peroxidases, amine oxidases, NADPH oxidases, oxalate oxidases, lipoxygenases, and quinone reductase [16,17]. ROS accumulated in plant tissue under stress conditions, such as high temperature, salinity, low temperature, and biotic stresses such as virus invasion, bacterial infection, and other agents [18,19]. Excessive ROS accumulation negatively affected plant tissue

and led to significant damage, which was described as oxidative damage [19]. Nucleic acid degradation, carbohydrate, protein, and lipid peroxidation that lead to PCD.

ROS accumulation serves as a signalling molecule to activate the defense system of plants, such as non-enzymatic (tocopherol, proline) and enzymatic (ascorbate peroxidase, catalase, superoxide dismutase, peroxidase, GSH) ROS scavenging molecules [20–22]. Furthermore, reactive oxygen species damaged the photosynthetic apparatus of plants by reducing photosynthesis efficiency, stomatal conductance, and chlorophyll content [23].

Moreover, during the virus infection, Sugarcane mosaic virus (SCMV) in transgenic sugarcane was detected at a low level of ROS concentration; meanwhile, another hybrid of sugarcane showed a higher level of ROS and MDA, which are evidence of disruption of cell ultrastructure [18]. Between virus infection and ROS level, there are strong bounds, and in tobacco infected, it has determined the uricase activity, which is mainly affected by hydrogen peroxide production [24–27]. Also, cowpea cultivars treated by the ringspot virus showed higher accumulation of superoxide and superoxide dismutase activity, which serves as a converter of superoxide to hydrogen peroxide [24]. Additionally, higher levels of ROS and lipid peroxidation and protein oxidation leading to Sharka symptoms were a consequence of long-term infection of the *Plum pox virus* in *Prunus* species [25].

The increasing amount of $H_2O_2$ triggers the production of anthocyanin under various stresses. Anthocyanins are antioxidant compounds that help to mitigate oxidative stress by neutralizing reactive oxygen species (ROS) like $H_2O_2$. Besides, $H_2O_2$ might activate transcription factors controlling the expression of genes responsible for anthocyanin biosynthesis, including MYB, bHLH, and WD40 transcription factors; therefore, activation of these factors is significant for anthocyanin pathways [26]. Also, $H_2O_2$ interacts with plant hormones, including ABA, JA, and ethylene, which are known modulators of anthocyanin accumulation [27]. Many of these hormones also are induced by stress, again leading to coordinately responsive outcomes in which anthocyanin synthesis is one of the consequences.

To address this issue, plants have developed a variety of ROS scavenging mechanisms, including the induction of anthocyanin molecules, that provide protective benefits under stress [28]. One approach for conferring stress resistance on plants is the accumulation of anthocyanins. Anthocyanins are water-soluble flavonoid chemicals that give flowers, fruits, and vegetables characteristic red, purple, orange, blue, and brown colors [29]. Under stress, anthocyanin molecules accumulate in many plant tissues, providing considerable antioxidant activity and inducing plant morphophysiological and metabolic responses [30]. Pigmented leaves and crops aid in stress tolerance by increasing ROS scavenging [31]. Furthermore, different levels and concentrations of anthocyanins contribute to plant biodiversity and adaptation, with pigmented plants containing higher anthocyanin levels being more tolerant to various stress conditions and beneficial for human nutrition [32].

For example, significant anthocyanin accumulation was observed under salt stress in wheat genotypes of different colors [29]. In another case, grapevine cells under phosphate deficiency showed anthocyanin accumulation responsible for stress tolerance [33]. Similar observations of anthocyanin biosynthesis gene induction under stress conditions have been reported in various crops, conferring stress tolerance [34]. Given their antioxidant properties, anthocyanins

protect plants from adverse environmental conditions by scavenging stress-induced ROS and are considered a promising approach to enhancing plant stress tolerance [35]. However, the role of anthocyanins in the stress pathogen response remains underexplored.

This study aims to investigate the impact of viral infections on anthocyanin accumulation in plants. Understanding how viruses interact with the metabolic pathways responsible for anthocyanin synthesis can provide valuable insights into plant defense mechanisms and aid in the development of more resilient crop varieties.

## Materials and research methods

*Grows of N. benthamiana*

The *Nicotiana benthamiana* plants used in this study were cultivated in enriched soil (Volshebnaya Gryadka, produced in Russia) within a controlled growth room under artificial lighting conditions. To simulate optimal growth and development conditions, a long-day photoperiod was established using alternately installed lamps with spectra of 2700 K and 6400 K, providing 16 hours of light (day) and 8 hours of darkness (night). Seeds were placed in soil pre-moistened with distilled water, and seedlings were transferred into new individual pots 10-14 days following germination, as they grew. To avoid contamination, the planting pots were sterilized with disinfectant treatments. The growth room had a relative humidity of 75-80% and an air temperature of 23-27 ºC. Watering was conducted three times a week with a consistent amount of distilled water at the same time each day.

*Preparation of material for inoculation*

Plasmids containing TBSV cDNAs were linearized with SmaI type II restriction endonuclease from ThermoFisher Scientific according to the manufacturer's instructions. The restriction products were then purified by phenol-chloroform extraction with subsequent ethanol precipitation. The linearized plasmids served as a template for the synthesis of TBSV viral RNA transcripts. The reaction was carried out using a set of ribonucleotide mixture, reaction buffer, and T7 polymerase from ThermoFisher Scientific. The in vitro transcription products were separated and visualized in 1% agarose gel.

*Horizontal agarose gel electrophoresis*

Separated in 1% agarose gel with ethidium bromide for 40 minutes with buffer. An agarose gel was prepared using a ratio of 500 mg agarose to 50 ml of buffer containing TRIS, boric acid, and EDTA (TBE). Viral particles were detected on agarose gels using UV light. The presence of DNA in the gel was detected under ultraviolet light using a Vilber Lormat gel documentation system (France).

*Inoculation with viral material*

Inoculation was carried out by the rub-inoculation method. The transcripts obtained in vitro were mixed with phosphate buffer and carborandrum. For inoculation, 2-3 leaves from the middle tier were selected. Mechanical damage was caused by light movements, through which

matrix RNA penetrates into plant tissues and cells. N. benthamiana plants aged 30-35 days were inoculated with in vitro synthesized TBSV transcripts.

*Immunodetection of viral proteins*

To determine the presence of viral proteins, hydroxyapatite chromatography fractions from healthy and virus-infected plants were separated on 15% polyacrylamide gels and transferred to nitrocellulose membrane (Osmonics, Westborough, MA). Transfer efficiency was tested by incubating the membrane in Ponceau S solution (Sigma, St. Louis, MO). Membranes were incubated with anti-capsid protein antibody solution (1:5000) for 2 h. After washing three times, secondary antibodies conjugated to alkaline phosphatase 1:3000 were added. NBT-BCIP solution was used to visualize the formed immune complexes [36].

*Detection of $H_2O_2$ in N. benthamiana*

The upper non-inoculated leaves were examined to identify ROS components, such as H2O2, which are known for their detrimental effects on plant growth and development. For H2O2 detection, samples were extracted in 50 mM phosphate buffer (pH 7.5) at a ratio of 1:8 (w/v) and centrifuged twice at 10,000 rpm for 10 minutes. The reaction mixture for detecting H2O2 consisted of 0.85 mM 4-aminoantipyrine, 3.4 mM 3,5-dichloro-2-hydroxybenzene sulfonate, and 4.5 U/ml HRP in 2 ml of 50 mM phosphate buffer (pH 7.5) as previously described by Yesbergenova et al. [37]. Absorbance was measured after 5 minutes at 515 nm using a Spectrophotometer.

*Quantitative determination of anthocyanin content*

The upper, uninoculated leaves were sampled. Samples were homogenized with an Extraction buffer containing 45% methanol and 5% acetic acid in a ratio of 1:5. Then they were centrifuged twice at 4°C, 10,000 rpm for 10 minutes each time, and then placed on a special plate. Measured as described by Nakata and Ohme-Takagi [38]. Absorbance was measured at 530 and 637 nm in a microplate spectrophotometer, “Multiskan SkyHigh” (Thermo Fisher Scientific, USA).

*Statistical analysis*

Each treatment option was analyzed in triplicate samples from each plant. Statistical analysis was performed using the StatPlus Professional 5.8.4.3 2018 version for Windows software package (AnalystSoft Inc., www.analystsoft.com/ru/), Student's t-test. Values were expressed as mean ±SE. P values below 0.05 were considered statistically significant. If the null hypothesis is true, the group's assessment of dyspepsia with alcohol changes should be close to the assessment of maternal dyspepsia. It should not be similar.

## Results

One-month-old *N. benthamiana* plants with initially similar morphological traits, such as height, leaf plate development, and total vegetative mass, were selected for inoculation. The plants were inoculated by treating the leaves of the middle tier with an inoculation mixture. *N. benthamiana* plants were infected in vitro with wild-type transcripts of TBSV.

Seven days after inoculation with *Tomato Bushy Stunt Virus* (TBSV), the infected plants developed characteristic symptoms indicating systemic spread of the virus and accumulation of viral proteins (Figure 1). Local chlorotic and necrotic spots and, in some cases, necrotic rings were observed on the inoculated leaves during the first 2–5 days. Some plants showed cell death at the inoculation site, which may indicate a hypersensitive response. Systemic symptoms of infection appeared on the 7th day after inoculation. Most plants showed pronounced chlorosis and mosaic coloration of the leaves, accompanied by their deformation (curling, size reduction, wrinkling). Inhibition of apical growth, shortening of internodes, and development of dwarfism were also recorded. In some cases, tissue necrosis was observed along the main veins of the leaves. The observed symptoms indicate active accumulation of viral proteins and successful systemic infection, which confirms the ability of TBSV to spread rapidly in the host plant.

**Figure 1.** Morphological signs of infection development in *N. benthamiana* plants

To confirm the presence of viral particles in N. benthamiana plants infected with wild-type TBSV, agarose gel electrophoresis and immunoblotting analyses were performed. Samples extracted from inoculated and healthy plants were subjected to 1% agarose gel electrophoresis followed by ethidium bromide staining. Visualization under UV light revealed a clear band corresponding to TBSV virions in samples obtained from infected leaves (Figure 2). To confirm the specificity of the detected particles, capillary transfer onto a nitrocellulose membrane was performed, followed by immunostaining with polyclonal antibodies specific for TBSV virion. The proposed methodological approach demonstrates high efficiency for rapid and reliable detection of TBSV virions in inoculated plants. This method can be particularly useful in cases where visual morphological signs of viral infection are weak or absent, making it a valuable tool for diagnosing viral infections in plants.

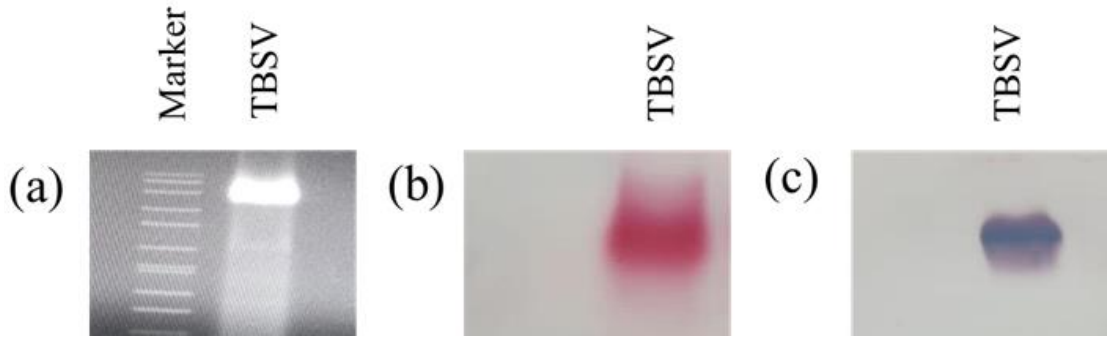
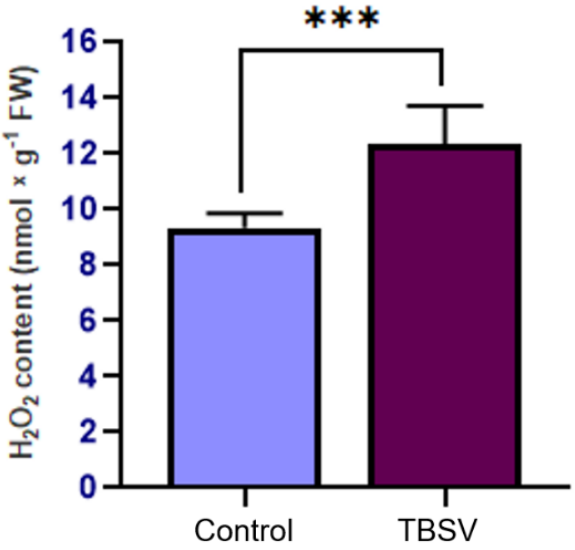



**Figure 2.** Detection of virion particles in *N. benthamiana* infected with wild-type TBSV. **(a)** Ethidium bromide staining of agarose gel after electrophoresis; **(b)** Ponceau S staining of nitrocellulose membrane; **(c)** Western blot analysis for detection of TBSV virions.

Reactive oxygen species (ROS) play a key role in plant signaling networks, participating in the regulation of many biological processes, including defense responses to pathogens. One of the most important representatives of ROS is hydrogen peroxide ($H_2O_2$), which, when accumulated in cells, can cause oxidative stress that affects the physiological state of the plant. In this study, we examined the effect of TBSV infection on the level of $H_2O_2$ accumulation in N. benthamiana plants.

To assess changes in ROS levels, experiments were conducted to quantify $H_2O_2$ in the tissues of infected and healthy plants. The results showed that TBSV infection leads to a significant increase in $H_2O_2$ concentration compared to control (healthy) plants (Figure 3).



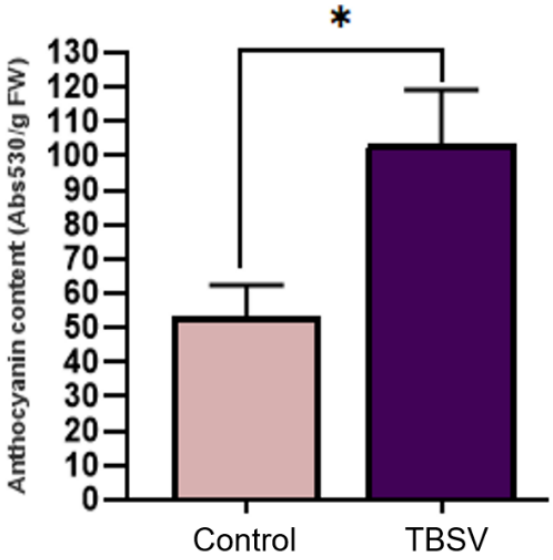

*Note:* Asterisks in the graph "***" indicate a very significant (P < 0.01); "ns" – an insignificant (P > 0.05) difference in the presented data. Statistical analysis (Student's t-test) was performed using GraphPad Prism software (v.8.01). Data are presented in relative units.

**Figure 3.** Determination of hydrogen peroxide accumulation in the leaves of *N. benthamiana*

The obtained data allow us to conclude that the increase in the accumulation of hydrogen peroxide ($H_2O_2$) in response to a viral attack may be one of the key components of the defense response of *N. benthamiana* plants. Hydrogen peroxide, as one of the most stable reactive oxygen species (ROS), plays an important role in the formation of an oxidative burst, which in turn contributes to the activation of additional plant defense mechanisms.

The interaction of the virus with the host plant is a complex process involving many physiological mechanisms, including the regulation of ROS levels, the functioning of the antioxidant system, and the accumulation of stress markers such as malondialdehyde (MDA), proline, and anthocyanins. The accumulation of ROS in response to a viral infection activates signaling cascades, which leads to the initiation of defense reactions. Anthocyanins probably act as ROS scavengers, protecting cells from oxidative damage and increasing plant resistance to pathogens. Therefore, the determination of the level of anthocyanin accumulation under the influence of TBSV was investigated.

As a result of the experiments, it was found that in *N. benthamiana* plants, in response to viral infection with wild-type TBSV, there is a significant accumulation of anthocyanin in the leaves compared to extracts obtained from control, uninfected plants (Figure 4). These results suggest that anthocyanins may play an important role in plant defense systems by being activated in response to virus infection and participating in the neutralization of oxidative stress. Thus, the accumulation of $H_2O_2$ and anthocyanins in response to virus infection is an important element of the defense response of *N. benthamiana* plants, highlighting their role in enhancing resistance to pathogens.




*Note:* Asterisks in the graph "***" indicate a very significant (P < 0.01); "ns" – an insignificant (P > 0.05) difference in the presented data. Statistical analysis (Student's t-test) was performed using GraphPad Prism software (v.8.01). Data are presented in relative units.

**Figure 4.** Determination of anthocyanin accumulation in the leaves of *N. Benthamiana*

## Discussion

As a result of successful inoculation, plants exhibited TBSV-specific disease symptoms at 7 days post-inoculation, indicating systemic spread of the virus and accumulation of viral proteins (Figure 1). The middle leaves of model plant more damaged in comparison with other leaves, and the tops of the leaves stopped development. Additionally, in middle leaves has observed necrosis which leading to further PCD of plants.

In the majority of cases observed, the interaction between viruses and cultivated crop plants has a detrimental impact on host morphology and physiology, leading to diseases [39]. Similarly, in plants including *Lycopersicon esculentum L*. and *C. annuum*, infected by TYLCV observed the internode reduction, curling and dwarfing of leaves [40].

Plants have evolved complex signaling and defensive mechanisms to cope with stressful environments. One of the first plant reactions to pathogen invasion is a considerable increase in reactive oxygen species (ROS) levels [41]. TBSV infected plants showed the higher accumulation of hydrogen peroxide level in leaves in comparison with not infected plants. (Figure 2). Similar results where hydrogen peroxide over produced during infection of plum pox virus (PPV) in *Prunus armeniaca L.* and *Prunus persica L.* [42]. Furthermore, the hydrogen peroxide level strongly correlated with lipid peroxidation, electrolyte leakage and protein oxidation [43,44]. Excessive amount of hydrogen peroxide influenced to the activation of ROS scavenging enzymes including SOD. Interestingly, apoplastic SOD participated to generation of secondary cell wall [45]. Simultaneously, the as an ROS scavenging enzymes, the level of phenolic compounds including anthocyanin, increased response to oxidative damage and it pointing out the critical role of anthocyanin as antioxidative function [46].

As demonstrated our results, anthocyanin content increased during the infection with TBSV, whereas in control plants remains, whereas in control plants remains unchanged. Similarly, trends were observed in Grape leaves *(Vitis vinifera L.)* infected by *Grapevine leafroll-associated virus 3 (GLRaV-3).* It suggests that anthocyanin were sensitive to stress conditions than other phenolic compounds. Additionally, anthocyanin is one of the key players of the oxidative defense system in vivo [47]. Moreover, hydrogen peroxide accumulated in different parts of plants, whereas anthocyanin accumulated only in vacuoles, and it was determined in *Malus domestica Borkh*. In vitro ROS scavenging function of anthocyanin was affected actively than in vivo [47]. Also, during the stress condition, anthocyanin content accumulated in adaxial and abaxial cells, which are located near to meshophilic cells [48]. Grape leaves *(Vitis vinifera L.)* infected with grapevine leafroll disease (GLD) exhibited downward curling of leaf margins and increased anthocyanin biosynthesis, causing a reddish-purple coloration [49].

In recent years, there has been an increasing interest in researching the role of anthocyanins in plants, particularly in terms of their response to biotic stressors such as virus infections [46, 48]. Previous research has demonstrated that virus infection can boost anthocyanin levels in a range of plants [48]. These findings indicate that anthocyanins may play a significant role in plant defense systems by activating in response to viral infection.

Previously, Tsuyoshi Inukai investigated the effect of anthocyanins in *Brassicaceae* species infected with turnip mosaic virus [50]. It has previously been discovered that turnip mosaic

virus significantly reduces anthocyanin production in Brassica rapa leaves, and that such leaves become infected while anthocyanin-rich leaves on the same plants are rarely affected. The authors indicate that anthocyanin accumulation dramatically inhibited turnip mosaic virus infection, implying that it acts as a chemical barrier against the virus, demonstrating the protective role of anthocyanins [50].

Another study that demonstrates the link between viral infections and anthocyanin accumulation is that of Xiang-Ru Chen, who evaluated the effect of the *Brassica yellows* virus movement protein on anthocyanin concentrations in plants [51]. Chen demonstrated that the viral movement protein increases anthocyanin accumulation, resulting in the development of purple leaf symptoms in Arabidopsis thaliana. This study sheds light on how viral proteins influence plant metabolic pathways, particularly those involved in anthocyanin production, and how this links to the obvious signs of viral infection [51].

According to Linga R. Gutha, the symptoms of grapevine leafroll disease in red-fruited wine grape *(Vitis vinifera L.)* cultivars are green veins and crimson to reddish-purple discoloration in the interveinal portions of the leaves [49]. He proposed that the reddish-purple hue observed in symptomatic leaves was due to anthocyanin accumulation, which might be caused by up-regulation of genes involved in their biosynthesis. This concept highlights the importance of anthocyanins as a potential indicator of grapevine leafroll disease symptoms and proposes a genetic response to viral infection [49].

## Conclusion

In this work, it was found that TBSV viral infection leads to an increase in the level of hydrogen peroxide accumulation and also contributes to the accumulation of anthocyanins in TBSV-infected N. benthamiana plants. As is known, hydrogen peroxide and anthocyanin play an indispensable role in the implementation of various mechanisms of plant resistance to pathogens and other adverse environmental factors. The results presented in this work allow us to better understand the action of plant defense mechanisms and can be used to develop new methods for increasing the resistance of crops to viral infections.

## Author Contributions

**M.B., A.S.,** and **A.K.** – conceptualization; **M.B.** and **A.S.** – data curation; **M.B., A.S.,** and **A.K. –** formal analysis; **M.K., A.B., N.I.,** and **S.Zh.** – investigation; **M.K., A.K., A.S.,** and **Zh.M.** – methodology; **M.B.** – visualization; **M.B., A.S.,** and **A.K.** - writing – original draft; **M.B., A.S.**, **A.K., S.Zh., A.B., N.I.,** and **Zh.M. –** writing – review & editing; **S.Zh., A.B., N.I.**, and **Zh.M**. – project administration; **M.B., A.K.,** and **Zh.M.** – supervision**; Zh.M** - funding acquisition and resources. All authors have read and agreed to the published version of the manuscript.

## Funding

This work was supported by the BR21882269 “Use of genome editing technology to increase productivity economically important cultivated plants” task 3 “Development of a CRISPR/Cas13 gene editing system to ensure antiviral resistance of plants” of the Committee on Science of the Republic of Kazakhstan for 2023-2025.

**Acknowledgments**

We thank the laboratory of the plant biotechnology named professor R.T Omarov for providing research support and our warmed thanks to our students Satu Albina and Kumargazy Karina for their research assistance.

**Conflicts of Interest**

The authors declare no conflicts of interest.

**Compliance with ethical standards**

This article does not contain a description of studies performed by the authors involving people or using animals as objects.

**References**

1. Yang X, Li Y, Wang A. Research Advances in Potyviruses: From the Laboratory Bench to the Field. Annual Review of Phytopathology. 2021;59, 1–29. https://doi.org/10.1146/annurev-phyto-020620-114550

2. Atkinson NJ. Urwin, PE. The Interaction of Plant Biotic and Abiotic Stresses: From Genes to the Field. Journal of experimental botany. 2012; 63, 3523–3543. dhttps://doi.org/10.1093/jxb/ers100

3. Iksat N, Masalimov Z, Omarov R. Plant Virus Resistance Biotechnological Approaches : From Genes to the CRISPR/Cas Gene Editing System. Journal of Water and Land Development. 2023; 57. https://doi.org/10.24425/jwld.2023.145345

4. Manabayeva SA, Shamekova M, Park et al. Differential Requirements for Tombusvirus Coat Protein and P19 in Plants Following Leaf versus Root Inoculation. Virology 2013; 439, 89–96. https://doi.org/10.1016/j.virol.2013.01.011

5. Hafez ESE., Saber GA, Fattouh FA. Tomato Bushy Stunt Virus (TBSV) Infecting Lycopersicon Esculentum. Zeitschrift für Naturforschung C. 2010; 65, 619–626. https://doi.org/10.1515/znc-2010-9-1015

6. Scholthof HB. The Tombusvirus-Encoded P19: From Irrelevance to Elegance. Nat Rev Microbiol. 2006; 4, 405–411. https://doi.org/10.1038/nrmicro1395

7. Omarov R, Sparks K, Smith L, et al. Biological Relevance of a Stable Biochemical Interaction between the Tombusvirus-Encoded P19 and Short Interfering RNAs. Journal of Virology. 2006; 80, 3000–3008. https://doi.org/10.1128/jvi.80.6.3000-3008.2006

8. Nawaz HH, et al. A research review on Tomato bushy stunt virus disease complex // J. Nat. Sci. Res. 2014;4:18–23.

9. Ali AS, Fattouh FA, Fathy RM. Physiological responses to infection by Tomato bushy stunt virus in different host plants // International Journal of Agriculture and Crop Sciences. SABI Publications. 2015;8(3):438–448. https://www.researchgate.net/publication/349588801

10. Mittler R., Zandalinas S.I., Fichman et al. Reactive Oxygen Species Signalling in Plant Stress Responses. Nat Rev Mol Cell Biol. 2022;23:663–679. https://doi.org/10.1038/s41580-022-00499-2

11. Foyer C.H., Noctor G. Redox Signaling in Plants. Antioxidants & Redox Signaling. 2013;8:2087–2090. https://doi.org/10.1089/ars.2013.5278

12. Vaahtera L, Brosche M, Wrzaczek M, Kangasjärvi J. Specificity in ROS Signaling and Transcript Signatures. Antioxidants & redox signaling. 2014; 21:1422–1441. https://doi.org/10.1089/ars.2013.5662

13. Considine MJ, María Sandalio L, Helen Foyer C. Unravelling How Plants Benefit from ROS and NO Reactions, While Resisting Oxidative Stress. Annals of Botany. 2015;116:469–473. https://doi.org/10.1093/aob/mcv153

14. Dietz K-J. Efficient High Light Acclimation Involves Rapid Processes at Multiple Mechanistic Levels. Journal of experimental botany. 2015;66:2401–2414. https://doi.org/10.1093/jxb/eru505

15. Mignolet-Spruyt L, Xu E, Idänheimo N, et al. Spreading the News: Subcellular and Organellar Reactive Oxygen Species Production and Signalling. Journal of experimental botany. 2016;67:3831–3844. https://doi.org/10.1093/jxb/erw080

16. Survila M, Davidsson PR, Pennanen, et al. Peroxidase-Generated Apoplastic ROS Impair Cuticle Integrity and Contribute to DAMP-Elicited Defenses. Frontiers in Plant Science. 2016;7:1945. https://doi.org/10.3389/fpls.2016.01945

17. Kärkönen A, Kuchitsu K. Reactive Oxygen Species in Cell Wall Metabolism and Development in Plants. Phytochemistry. 2015;112:22–32. https://doi.org/10.1016/j.phytochem.2014.09.016

18. Akbar S, Wei Y, Yuan Y, et al. Gene Expression Profiling of Reactive Oxygen Species (ROS) and Antioxidant Defense System Following Sugarcane Mosaic Virus (SCMV) Infection. BMC Plant Biology. 2020;20:1–12. https://doi.org/10.1186/s12870-020-02737-1

19. Maurya AK. Oxidative Stress in Crop Plants. Agronomic crops: volume 3: stress responses and tolerance. 2020:349–380. https://doi.org/10.1007/978-981-15-0025-1-18

20. Kumar V, Khare T, Sharma M, et al. ROS-Induced Signaling and Gene Expression in Crops under Salinity Stress. Reactive oxygen species and antioxidant systems in plants: role and regulation under abiotic stress. 2017:159–184. https://doi.org/10.1007/978-981-10-5254-5-7

21. Hasanuzzaman M, Bhuyan MB, Zulfiqar F, et al. Reactive Oxygen Species and Antioxidant Defense in Plants under Abiotic Stress: Revisiting the Crucial Role of a Universal Defense Regulator. Antioxidants. 2020;9:681. https://doi.org/10.3390/antiox9080681

22. Wongshaya P, Chayjarung P, Tothong C, et al. Effect of Light and Mechanical Stress in Combination with Chemical Elicitors on the Production of Stilbene Compounds and Defensive Responses in Peanut Hairy Root Culture. Plant Physiology and Biochemistry. 2020;157:93–104. https://doi.org/10.1016/j.plaphy.2020.10.015

23. Shakirova F, Allagulova CR, Maslennikova D, et al. Salicylic Acid-Induced Protection against Cadmium Toxicity in Wheat Plants. Environmental and experimental botany. 2016;122:19–28. https://doi.org/10.1016/j.envexpbot.2015.08.002

24. Montalbini P. Enhanced Uricase Activity in Tobacco Mosaic Virus Infected Tobacco Leaves. Plant Science. 1991;74:261–265. https://doi.org/10.1016/0168-9452(91)90055-D

25. Doke N, Ohashi Y. Involvement of an O2− Generating System in the Induction of Necrotic Lesions on Tobacco Leaves Infected with Tobacco Mosaic Virus. Physiological and Molecular plant pathology 1988;32:163–175. https://doi.org/10.1016/S0885-5765(88)80013-4

26. Király L, Hafez Y, Fodor J, et al. Suppression of Tobacco Mosaic Virus-Induced Hypersensitive-Type Necrotization in Tobacco at High Temperature Is Associated with Downregulation of NADPH Oxidase and Superoxide and Stimulation of Dehydroascorbate Reductase. Journal of General Virology. 2008;89:799–808. https://doi.org/10.1099/vir.0.83328-0

27. Moeder W, Yoshioka K, Klessig DF. Involvement of the Small GTPase Rac in the Defense Responses of Tobacco to Pathogens. Molecular Plant-Microbe Interactions. 2005;18:116–124. https://doi.org/10.1094/MPMI-18-0116

28. Zhu L, Liao Y, Zhang T, et al. Reactive Oxygen Species Act as the Key Signaling Molecules Mediating Light-Induced Anthocyanin Biosynthesis in Eucalyptus. Plant Physiology and Biochemistry. 2024;212:108715, https://doi.org/10.1016/j.plaphy.2024.108715.

29. Gu K-D., Wang C-K., Hu D-G. et al. How Do Anthocyanins Paint Our Horticultural Products? Scientia Horticulturae. 2019;249:257–262. https://doi.org/10.1016/j.scienta.2019.01.034

30. Cohen SP, Leach JE. Abiotic and Biotic Stresses Induce a Core Transcriptome Response in Rice. Sci Rep. 2019;9:6273. https://doi.org/10.1038/s41598-019-42731-8

31. Cavaiuolo M, Cocetta G, Ferrante A. The Antioxidants Changes in Ornamental Flowers during Development and Senescence. Antioxidants. 2013; 2:132–155. https://doi.org/10.3390/antiox2030132

32. Anum H, Li K, Tabusam J, et al. Regulation of Anthocyanin Synthesis in Red Lettuce in Plant Factory Conditions: A Review. Food Chemistry. 2024;458:140111.https://doi.org/10.1016/j.foodchem.2024.140111

33. Mbarki S, Sytar O, Zivcak M, et al. Anthocyanins of Coloured Wheat Genotypes in Specific Response to SalStress. Molecules. 2018;23:1518. https://doi.org/10.3390/molecules23071518

34. Yin Y, Borges G, Sakuta M, et al. Effect of Phosphate Deficiency on the Content and Biosynthesis of Anthocyanins and the Expression of Related Genes in Suspension-Cultured Grape (Vitis Sp.) Cells. Plant Physiology and Biochemistry 2012;55:77–84. https://doi.org/10.1016/j.plaphy.2012.03.009

35. Zheng X-T, Yu Z-C, Tang J-W, et.al. The Major Photoprotective Role of Anthocyanins in Leaves of Arabidopsis Thaliana under Long-Term High Light Treatment: Antioxidant or Light Attenuator? Photosynth Res. 2021;149:25–40. https://doi.org/10.1007/s11120-020-00761-8

36. Yergaliyev TM, Nurbekova Z, Mukiyanova G, et.al. The Involvement of ROS Producing Aldehyde Oxidase in Plant Response to Tombusvirus Infection. Plant Physiology and Biochemistry. 2016;109:36–44. https://doi.org/10.1016/j.plaphy.2016.09.001

37. Yesbergenova Z, Yang G, Oron E, et.al. The Plant Mo-Hydroxylases Aldehyde Oxidase and Xanthine Dehydrogenase Have Distinct Reactive Oxygen Species Signatures and Are Induced by Drought and Abscisic Acid. The Plant Journal. 2005; 42:862–876. https://doi.org/10.1111/j.1365-313X.2005.02422.x

38. Nakata M, Ohme-Takagi M. Quantification of Anthocyanin Content. Bio-protocol 2014;4:e1098–e1098.

39. Hafez ESE, Saber GA, Fattouh FA. Tomato Bushy Stunt Virus (TBSV) Infecting Lycopersicon Esculentum. Zeitschrift für Naturforschung C. 2010;65:619–626. https://doi.org/10.1515/znc-2010-9-1015

40. Morilla G, Janssen D, García-Andrés S, et.al. Pepper (Capsicum Annuum) Is a Dead-End Host for Tomato Yellow Leaf Curl Virus. Phytopathology®. 2005;95:1089–1097. https://doi.org/10.1094/PHYTO-95-1089

41. Mittler R, Vanderauwera S, Suzuki N, et al. ROS Signaling: The New Wave? Trends in Plant Science. 2011;16:300–309. https://doi.org/10.1016/j.tplants.2011.03.007

42. Diaz-Vivancos P, Rubio M, Mesonero V, et al. The Apoplastic Antioxidant System in Prunus: Response to Long-Term Plum Pox Virus Infection. Journal of Experimental Botany. 2006;57:3813–3824. https://doi.org/10.1093/jxb/erl138

43. Hernández JA, Rubio M, Olmos E, et.al. Oxidative Stress Induced by Long-Term Plum Pox Virus Infection in Peach (Prunus Persica). Physiologia Plantarum. 2004; 122:486–495. https://doi.org/10.1111/j.1399-3054.2004.00431.x

44. Hernández JA, Díaz-Vivancos P, Rubio M, et.al. Long-Term Plum Pox Virus Infection Produces an Oxidative Stress in a Susceptible Apricot, Prunus Armeniaca, Cultivar but Not in a Resistant Cultivar. Physiologia Plantarum. 2006;126:140–152. https://doi.org/10.1111/j.1399-3054.2005.00581.x

45. Karlsson M, Melzer M, Prokhorenko I, et.al. Hydrogen Peroxide and Expression of hipI-Superoxide Dismutase Are Associated with the Development of Secondary Cell Walls in Zinnia Elegans. Journal of Experimental Botany. 2005;56: 2085–2093. https://doi.org/10.1093/jxb/eri207

46. Zhang KM, Yu H-J, Shi K, et.al. Photoprotective Roles of Anthocyanins in Begonia Semperflorens. Plant science. 2010;179:202–208. https://doi.org/10.1016/j.plantsci.2010.05.006

47. Bi X, Zhang J, Chen C, et.al. Anthocyanin Contributes More to Hydrogen Peroxide Scavenging than Other Phenolics in Apple Peel. Food Chemistry. 2014; 152: 205–209. https://doi.org/10.1016/j.foodchem.2013.11.088

48. Merzlyak MN, Chivkunova OB, Solovchenko AE, et.al. Light Absorption by Anthocyanins in Juvenile, Stressed, and Senescing Leaves. Journal of Experimental Botany. 2008; 59:3903–3911. https://doi.org/10.1093/jxb/ern230

49. Gutha LR, Casassa LF, Harbertson JF, et.al. Modulation of Flavonoid Biosynthetic Pathway Genes and Anthocyanins Due to Virus Infection in Grapevine (Vitis Vinifera L.) Leaves. BMC Plant Biology. 2010;10:1–18. https://doi.org/10.1186/1471-2229-10-187

50. Inukai T, Kim H, Matsunaga W, et.al. Battle for Control of Anthocyanin Biosynthesis in Two Brassicaceae Species Infected with Turnip Mosaic Virus. Journal of Experimental Botany. 2023;74:1659–1674. https://doi.org/10.1093/jxb/erac502

51. Chen X-R, Wang Y, Zhao HH, et.al. Brassica Yellows Virus' Movement Protein Upregulates Anthocyanin Accumulation, Leading to the Development of Purple Leaf Symptoms on Arabidopsis Thaliana. Scientific Reports. 2018;8:16273. https://doi.org/10.1038/s41598-018-34591-5

## Өсімдіктің патогенге төзімділігіндегі антоцианиндердің рөлі

**М.К. Бейсекова[1], А. Самат[1], А.Б. Курманбаева[1], А.Ж. Бектурова[1], Н.Н. Иқсат[1], С.Б. Жангазин[1], Ж.К. Масалимов*[1]**

*[1]Л.Н. Гумилев атындағы Еуразия ұлттық университеті, Астана, Қазақстан*

**Аңдатпа.** Вирустық инфекциялар Қазақстанда және бүкіл әлемде ауыл шаруашылығы дақылдарының өсуіне, дамуына және өнімділігіне теріс әсер етіп, өсімдік шаруашылығына үлкен қауіп төндіреді. Құрғақшылық, экстремалды температура, топырақтың тұздануы және ауру қоздырғыштарының инвазиясы сияқты көптеген стресс жағдайында вирустар өсімдіктердің метаболизм жолдарын бұзу және қолайсыз факторларға төзімділігін төмендету арқылы физиологиялық өзгерістерді күшейтеді. Өсімдіктердің қорғаныс механизмдеріне қатысатын маңызды қосылыстардың антоцианиндердің биосинтезіне вирустық инфекциялардың әсеріне ерекше назар аударылады. Бұл жұмыста қызанақтың бұталы ергежейлігінің вирусын (TBSV) зақымдау үлгісі өсімдіктің ортаңғы жапырақтарын басқа жапырақтармен салыстырғанда көбірек зақымдайтыны, ал үстіңгі жапырақтардың өспейтіндігі көрсетілген. Сонымен қатар, ортаңғы жапырақтарда некроз байқалады, бұл одан әрі бағдарламаланған жасушалардың жойылуына

(ПКД) әкеледі. Сонымен қатар, TBSV инфекциясы *Nicotiana benthamiana* өсімдіктерінде сутегі асқын тотығы деңгейінің айтарлықтай жоғарылауына және антоцианиннің жиналуына әкеледі. Бұл өзгерістер тотығу-тотықсыздану тепе-теңдігінің бұзылуын және вирустық инфекцияға жауап ретінде қорғаныс реакцияларының белсендірілуін көрсетеді. Нәтижелер өсімдіктің вирустық инфекцияларға және басқа да стресс факторларына төзімділігін арттыру стратегияларын әзірлеу үшін вирус пен өсімдіктердің өзара әрекеттесуін зерттеудің маңыздылығын көрсетеді.
**Түйін сөздер:** *N. benthamiana*, вирус, TBSV, биотикалық стресс, ОБТ, $H_2O_2$, антоцианин

## Роль антоцианов в устойчивости растений к патогену

**М.К. Бейсекова[1], А. Самат[1], А.Б. Курманбаева[1], А.Ж. Бектурова[1], Н.Н. Иксат[1], С.Б. Жангазин[1], Ж.К. Масалимов*[1]**

*[1]Евразийский национальный университет им. Л.Н. Гумилева, Астана, Казахстан*

**Аннотация.** Вирусные инфекции представляют серьёзную угрозу для растениеводства в Казахстане и во всём мире, оказывая негативное влияние на рост, развитие и продуктивность сельскохозяйственных культур. В условиях множественных стрессов, таких, как засуха, экстремальные температуры, засоление почв и поражение патогенами, вирусы усугубляют физиологические изменения в растениях, нарушая их метаболические пути и снижая устойчивость к неблагоприятным факторам. Особое внимание уделяется влиянию вирусных инфекций на биосинтез антоцианов – важных соединений, участвующих в защитных механизмах растений. В данной работе показано, что при заражении вирусом кустистой карликовости томатов (TBSV) средние листья модельного растения повреждаются сильнее по сравнению с другими листьями, а верхние листья остановились в развитии. Кроме того, в средних листьях наблюдается некроз, что приводит к дальнейшему программируемому клеточному разрушению (PCD). Более того, инфекция вирусом TBSV приводит к значительному повышению уровня перекиси водорода и накоплению антоцианов в растениях *Nicotiana benthamiana.* Эти изменения свидетельствуют о нарушении окислительно-восстановительного баланса и активации защитных реакций в ответ на вирусную инфекцию. Полученные данные подчеркивают важность изучения взаимодействия вирусов с растениями для разработки стратегий повышения устойчивости сельскохозяйственных культур к вирусным инфекциям и другим стрессовым факторам.
**Ключевые слова:** *N. benthamiana,* вирус, TBSV, биотический стресс, АФК, $H_2O_2$, антоциан

**Сведения об авторах:**

***Бейсекова Молдир Кудиярбековна*** **–** магистр технических наук, постдокторант, старший преподаватель кафедры биотехнологии и микробиологии, Евразийский национальный университет им. Л.Н. Гумилева, 010000, Астана, Казахстан.

***Самат Абай*** **–** магистр естественных наук, PhD студент кафедры общей биологии и геномики, Евразийский национальный университет им. Л.Н. Гумилева, 010000, Астана, Казахстан.

***Курманбаева Асылай Бактыбаевна –*** доктор философии (PhD), ассоциированный профессор кафедры биотехнологии и микробиологии, Евразийский национальный университет им. Л.Н. Гумилева, 010000, Астана, Казахстан.

***Бектурова Асемгуль Жамбуловна –*** кандидат биологических наук, старший препадователь кафедры биотехнологии и микробиологии, Евразийский национальный университет им. Л.Н. Гумилева, 010000, Астана, Казахстан.

***Нургул Нурканаткызы Иксат –*** доктор философии (PhD), постдокторант, и.о. доцента кафедры биотехнологии и микробиологии, Евразийский национальный университет им. Л.Н. Гумилева, 010000, Астана, Казахстан.

***Жангазин Саян Берикович –*** заместитель декана по научной работе факультета естественных наук, доктор философии (PhD), ассоциированный профессор кафедры биотехнологии и микробиологии, Евразийский национальный университет им. Л.Н. Гумилева, 010000, Астана, Казахстан.

***Масалимов Жаксылык Каирбекович –*** автор-корреспондент, кандидат биологических наук, доктор философии (PhD), ассоциированный профессор, заведующий кафедрой биотехнологии и микробиологии, Евразийский национальный университет им. Л.Н. Гумилева, 010000, Астана, Казахстан.

**Авторлар туралы мәліметтер:**

***Бейсекова Молдир Кудиярбековна –*** техника ғылымдарының магистрі, постдокторант Л.Н. Гумилев атындағы Еуразия ұлттық университетінің биотехнология және микробиология кафедрасының аға оқытушысы, 010000, Астана, Қазақстан.

***Самат Абай –*** жаратылыстану ғылымдарының магистрі, Л.Н. Гумилев атындағы Еуразия ұлттық университетінің жалпы биология және геномика кафедрасының PhD докторанты, 010000, Астана, Қазақстан.

***Курманбаева Асылай Бактыбаевна –*** PhD, Л.Н. Гумилев атындағы Еуразия ұлттық университетінің биотехнология және микробиология кафедрасының қауымдастырылған профессоры, 010000, Астана, Қазақстан.

***Бектурова Асемгуль Жамбуловна –*** биология ғылымдарының кандидаты, Л.Н. Гумилев атындағы Еуразия ұлттық университетінің биотехнология және микробиология кафедрасының аға оқытушысы, 010000, Астана, Қазақстан

***Нұргүл Нұрқанатқызы Иқсат –*** PhD, постдокторант, Л.Н. Гумилев атындағы Еуразия ұлттық университетінің биотехнология және микробиология кафедрасының доценті м.а., 010000, Астана, Қазақстан.

***Жангазин Саян Берикович –*** жаратылыстану ғылымдары факультеті деканының ғылыми жұмыстар жөніндегі орынбасары, философия ғылымдарының докторы (PhD), Л.Н. Гумилев атындағы Еуразия ұлттық университетінің биотехнология және микробиология кафедрасының қауымдастырылған профессоры, 010000, Астана, Қазақстан.

***Масалимов Жаксылык Каирбекович*** – хат-хабар авторы, биология ғылымдарының кандидаты, PhD, қауымдастырылған профессоры, Л.Н. Гумилев атындағы Еуразия ұлттық университетінің биотехнология және микробиология кафедрасының меңгерушісі, 010000, Астана, Қазақстан.

**Authors' information:**

***Beisekova Moldir Kudiyarbekovna*** **–** MSc, postdoctoral student, senior lecturer, Department of Biotechnology and Microbiology, L.N. Gumilyov Eurasian National University, 010000, Astana, Kazakhstan.

***Samat Abay*** **–** MSc, PhD student, Department of General Biology and Genomics, L.N. Gumilyov Eurasian National University, 010000, Astana, Kazakhstan.

***Kurmanbaeva Asylai Baktybaevna*** **–** PhD, Associate Professor, Department of Biotechnology and Microbiology, L.N. Gumilyov Eurasian National University, 010000, Astana, Kazakhstan.

***Bekturova Asemgul Zhambulovna*** **–** candidate of biological sciences, senior lecturer of the department of biotechnology and microbiology, Eurasian National University named after L.N. Gumilyov, 010000, Astana, Kazakhstan

***Nurgul Nurkanatkyzy Iksat*** **–** PhD, postdoctoral student, acting associate Professor, Department of Biotechnology and Microbiology, L.N. Gumilyov Eurasian National University, 010000, Astana, Kazakhstan.

***Zhangazin Sayan Berikovich*** **–** Deputy Dean for Research, Faculty of Natural Sciences, Doctor of Philosophy (PhD), Associate Professor, Department of Biotechnology and Microbiology, L.N. Gumilyov Eurasian National University, 010000, Astana, Kazakhstan.

***Masalimov Zhaksylyk Kairbekovich*** **–** Corresponding author, candidate of biological sciences, PhD, Associate Professor, head of the department of biotechnology and microbiology of the Eurasian National University named after L.N. Gumilyov, 010000, Astana, Kazakhstan.