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T. Yergaliev

University of Hohenheim, Stuttgart, Germany
E-mail: timyerg@gmail.com

Molybdenum and plant resistance to viral infection

Abstract. *Molybdenum takes an active part in several physiological processes necessary for the growth and development of plants and other domains of life. Molybdenum participates in numerous biochemical reactions and lack of this metal may affect the total amount of proteins in plants. More than fifty Mo-containing enzymes are currently known, although most of them were found in bacteria. Plants contain Mo-containing enzymes such as nitrate reductase, sulfite oxidase, aldehyde oxidase, xanthine dehydrogenase, and mitochondrial amidoxime reductase. Tungsten is another heavy metal, which due to highly similar physico-chemical properties with Molybdenum may be incorporated instead of the latest as enzyme cofactor, leading to its inactivation. In this article, preliminary results from a pilot experiment are shown, demonstrating the effect of Molybdenum and Tungsten treatment on Nicotiana benthamiana plants infected with Tomato Bushy Stunt Virus, which refers to viruses parasitizing economically important crops. This virus infects more than 100 species of monocotyledonous and dicotyledonous plants from more than 20 different families. Infection of plants with a viral infection occurs through mechanical damage to the root system; virions in this case can be transmitted through soil or water. It was found that Molybdenum treatment may lead to mitigation of otherwise fatal for the host viral infection.*

Key words: *Molybdenum, TBSV, plant, virus, virus resistance, infection.*

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Introduction

Molybdenum (Mo) is an essential trace mineral for animals, plants and most microorganisms [1]. The importance of molybdenum for living organisms was discovered in 1939 using tomato plants grown in a specific nutrient solution [2]. Later, molybdenum was discovered as a pterin, a cofactor, in the active center of more than fifty enzymes involved in the main redox reactions of nitrogen and sulfur, phytohormone biosynthesis and detoxification of xenobiotics. This catalytic activity converts molybdenum into an essential trace element for almost all living things [3].

Molybdenum belongs to the rare elements of group VI of the Periodic table of elements. In nature, molybdenum occurs mainly in the form of an MoO_4^{2-} anion. In addition, in soils, the molybdate anion is also the only form of molybdenum available to plants, fungi and bacteria.

As noted above, molybdenum belongs to the group of trace elements, that is, the organism needs it only in the smallest quantities. However, exceeding a certain amount can lead to toxicity of molybdenum. About 100 molybdenum compounds are listed in the US Toxic Substances Control Act [4].

Molybdenum takes an active part in several physiological processes necessary for the growth and development of plants. Lack of molybdenum reduces the total amount of proteins in plants. Molybdenum is located in the active center of aldehyde oxidase and plays an important role in plant development and adaptation to environmental stresses [5]. In plant cells, the average concentration of molybdenum is 0.2 mg/kg^{-1} dry weight and depends on the presence of molybdate in soils [6]. Molybdenum is a part of metal-containing enzymes (molybdoenzymes), which perform an important function both in the metabolism of each organism and in the cycles of carbon, nitrogen and sulfur [7]. More than fifty Mo-containing enzymes are currently known. Most of them were found in bacteria, while only seven have been identified in eukaryotes [8].

Plants contain Mo-containing enzymes such as nitrate reductase, sulfite oxidase, aldehyde oxidase, xanthine dehydrogenase, and mitochondrial amidoxime reductase [9]. In addition to pterin, there is another type of Mo-containing cofactor, which is found only in bacterial nitrogenase, forming the so-called iron-molybdenum cofactor [10].

Tungsten (W) is another heavy metal that belongs to the VI Group of the Periodic table of elements. Due to the highly similar physico-chemical properties of W and Mo, some enzymes, which use the latest as a cofactor, may incorporate W instead, leading to enzyme inactivation [11].

Although numerous researches demonstrated an important role of molybdenum in plants, still only a few of them are focused on the role of Mo in plants' resistance to viral infections. In this article, preliminary results from a pilot experiment are shown, demonstrating the effect of Mo and W treatment on Nicotiana benthamiana plants, infected with Tomato Bushy Stunt Virus (TBSV). TBSV is a member of the Tombusviridae family, genus Tombusvirus, group IV. Refers to viruses parasitizing economically important crops. This virus infects more than 100 species of monocotyledonous and dicotyledonous plants from more than 20 different families [12]. Infection of plants with a viral infection occurs through mechanical damage to the root system; virions, in this case, can be transmitted through soil or water.

Materials and methods

Plant material. *N. benthamiana* plants were grown in the growth room in conditions of long-day photoperiod (16-h light/8-h dark) and 75-80% relative humidity. Temperatures fluctuated from 20 to 27 C and the average temperature during the day was 25 C and 22 C at night. For lighting of growth room lamps with 2700 K and 6400 K spectrum were used.

Treatments. As treatments, 100 and 500 μM Na2MoO4·2H2O and Na2WO4·2H2O solutions, as well as their mixture, were used.

Plant inoculation. For inoculation in vitro generated transcripts of full length TBSV cDNAs were used [13]. For this, plasmids containing the inserts were linearized at the 30-end of the viral cDNA sequence by restriction of the Sma1 enzyme digest. Transcripts were synthesized using T7 RNA polymerase, and these transcripts were used for inoculation of plants as previously described [14]. Control plants were mock-inoculated by using a phosphate buffer without viral RNA. Healthy and infected plants were grown separately in the same conditions.

TBSV detection. The leaves of *N. benthamiana* were analyzed for the presence of TBSV virions. Plant tissues were homogenized in TRIS/EDTA (TE) buffer in ratio 1/2 (sample/buffer) on ice, then centrifuged at 10.000 rpm for 20 min. After 15 μl of each sample was mixed with a 6X Loading buffer. Separation of macromolecules was performed in 1% agarose gel with ethidium bromide for 45 min with Tris/Borate/EDTA (1×TBE) buffer. UV light used to detect viral particles in agarose gel. Then capillary transfer was performed onto the nitrocellulose membrane with TBSV virus-specific polyclonal antibodies.

Results and discussion

To investigate, if Mo and W application may affect the natural counterplay between plants and plant viruses, one small pilot experiment was implemented in two biological repeats. In this experiment, 14 one-month-old *N. benthamiana* plants were selected for each repeat. Among selected plants, 7 were infected with TBSV transcripts. Then, all plants were divided in pairs, one healthy and one infected in each. One pair was left untreated as a control, and to the rest the following treatments were applied: Mo 100 and 500 μM, W 100 and 500 μM, and a mixture of Mo and W 100 and 500 μM solutions. Each treated plant was daily poured with 25 ml of corresponding solution.

Usually, TBSV infection on *N. benthamiana* plants results in the appearance of first morphological signs of infection on 3 day past infection (dpi), and leads to almost complete collapse of the plant by 7 dpi. On Figure 1, photos of plants that were taken at 9 dpi are depicted.

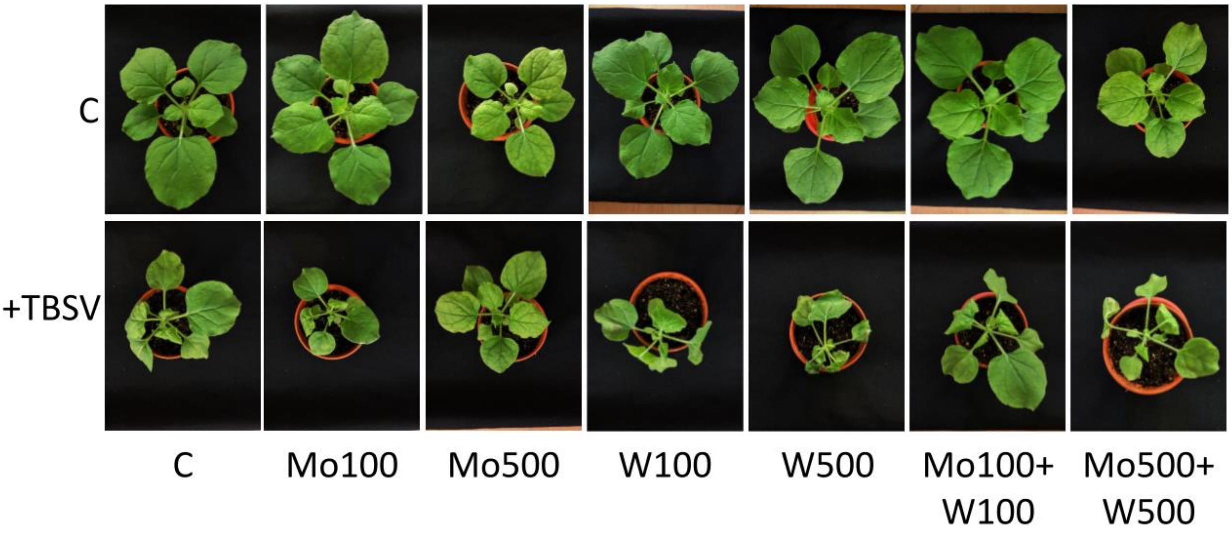

Figure 1. Photos of plants at 9 dpi. Upper and lower rows represent plants that were not infected and infected with TBSV accordingly. Treatments are indicated by columns under photos

As it shown on Figure 1, 500 μM Mo and Mo+W treatments resulted in retarded plant growth. But most interestingly, *N. benthamiana* plants, infected with TBSV and treated with 500 μM Mo, demonstrated no morphological signs of viral infection.

To test, if all inoculated with TBSV transcripts plants were successfully infected, an express method of TBSV detection in plant tissues was implemented (Figure 2) as we described earlier [15]. TBSV infection was found in all inoculated plants, although in plants, treated with 500 μM Mo the signal of virus presence in tissues was weaker by comparison with other treatments and positive control.

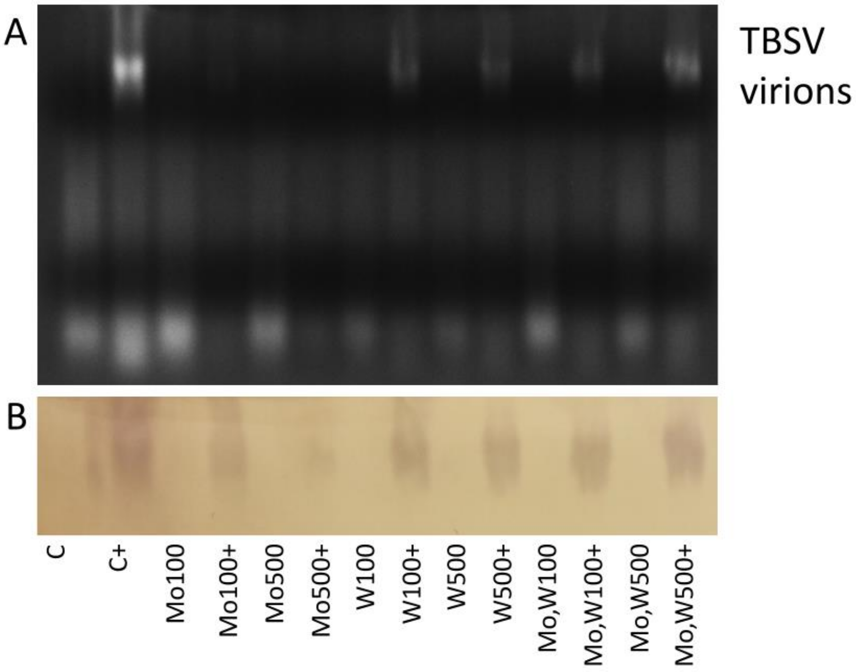

Figure 2. Express method of TBSV detection in plant tissues at 9 dpi. A) TBSV virions in agarose gel. B) Northern blot assay with TBSV antibodies for detection of TBSV virions

Over 3 weeks after inoculation, still no morphological symptoms were developed in plants infected by TBSV and treated with 500 μM Mo. Also, plants, treated with 100 and 500 μM Mo+W solutions, despite severe symptoms of infection started to recover (Figure 3). In addition, healthy plants treated with 500 Mo and 500 Mo+W solutions demonstrated some retardation in development.

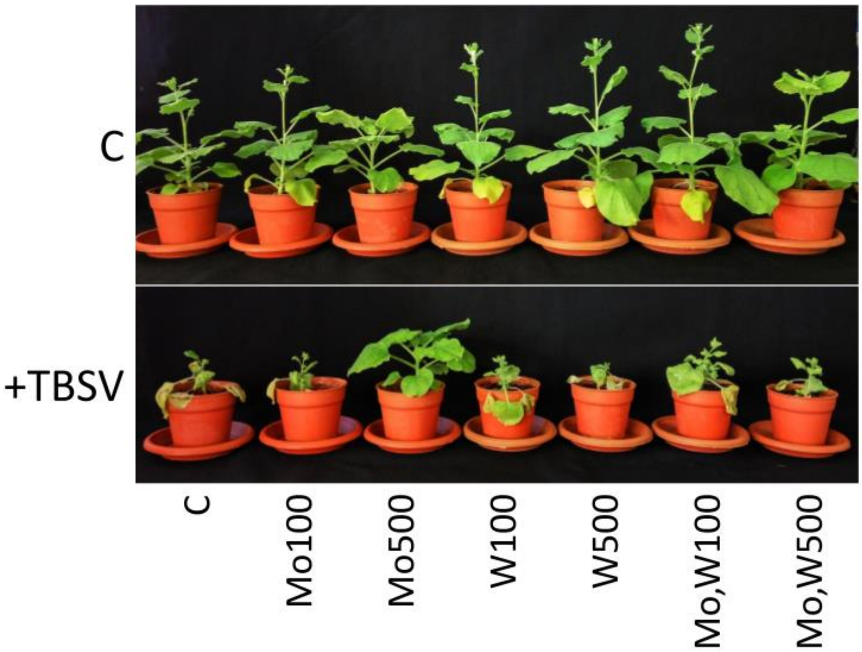

Figure 3. Photos of plants at 22 dpi

As shown on Figure 4, plant, treated with 500 µM Mo, was not affected by TBSV infection at 45 dpi (Fig. 4, B), and had no morphological differences compared to plant, treated with the same solution, but not infected with TBSV transcripts (Fig. 4, C). Both infected and non-infected 500 µM Mo treated plants flowered 3-4 days later than healthy control and healthy plants, treated with 100 µM Mo, 100 and 500 µM Mo and Mo+W solutions. Plants, treated with 100 µM Mo, and 100 and 500 µM Mo+W solutions despite severe symptoms survived TBSV infection, although demonstrated significant retardation in development. Positive control, or plant, infected with TBSV infection without any treatments, did not recover with time.

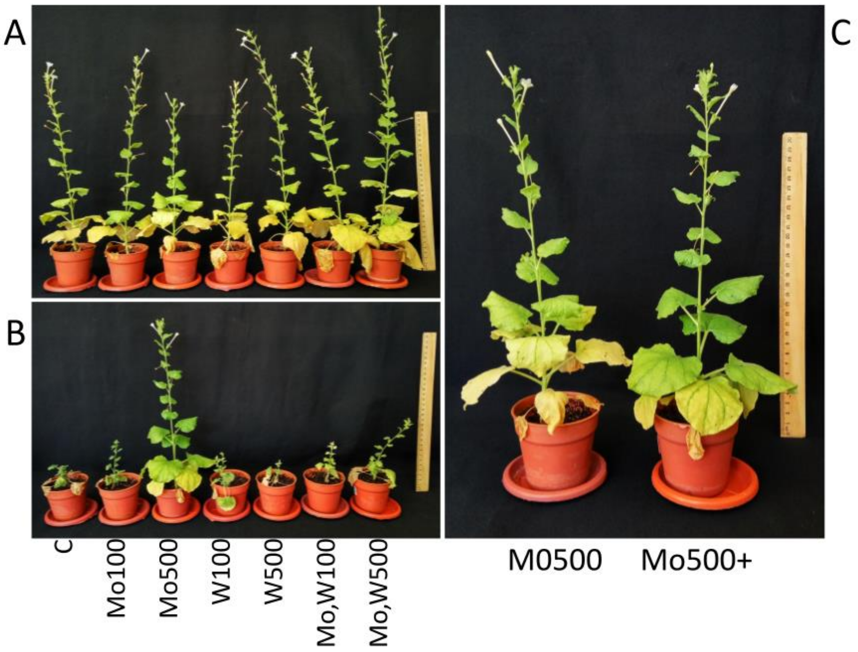

Figure 4. Photos of plants at 45 dpi. A) Healthy plants. B) Plants, inoculated with TBSV transcripts. C) Plants, treated with 500 µM Mo solution, healthy on the left side and infected with TBSV on the right

Conclusion

Molybdenum application may mitigate symptoms of otherwise fatal viral infections in plants. Additional experiments should be implemented to investigate the interactions between molybdenum applications, Mo-dependent enzymes and viral infections.

References

1. "Metals Ions in Biological System: Volume 39: Molybdenum and Tungsten: Their Roles in Biological Processes": Routledge & CRC Press [Electronic resource]. URL: https://www.routledge.com/Metals-Ions-in-Biological-System-Volume-39-Molybdenum-and-Tungsten-Their/Sigel-Sigel/p/book/9780367396299 (Accessed: May 15, 2021).

2. Arnon D.I., Stout P.R. Molybdenum as an Essential Element For Higher Plants // Plant Physiol. -1939. -Vol. 14. № 3. -P. 599-602. DOI: 10.1104/pp.14.3.599.

3. Tejada-Jiménez M., Chamizo-Ampudia A., Galván A., Fernández E., and Llamas Á. Molybdenum metabolism in plants // Metallomics. -2013. -Vol. 5. № 9. -P. 1191-1203. DOI: 10.1039/C3MT00078H.

4. Jonmaire P. Molybdenum in Industrial Toxicology. John Wiley & Sons Inc., 2015. 1340 p. DOI: 10.1002/9781118834015.ch24.

5. Naqib S.A., Jahan M.S. The Function of Molybdenum and Boron on the Plants // Plants. J Agri Res. -2017. -Vol. 2. -№ 3. -P. 1-8. DOI: 10.23880/OAJAR-16000136.

6. Gupta U. C. Boron, molybdenum and selenium status in different plant parts in forage legumes and vegetable crops // J. Plant Nutr. -1991. -Vol. 14. -№ 6. -P. 613-621. DOI: 10.1080/01904169109364228.

7. Stiefel E.I. The biogeochemistry of molybdenum and tungsten // Met. Ions Biol. Syst. -2002. -Vol. 39. -P. 1-29.

8. Hille R., Nishino T., Bittner F. Molybdenum enzymes in higher organisms // Coord. Chem. Rev. -2011. -Vol. 255. -№ 9. -P. 1179-1205. DOI: 10.1016/j.ccr.2010.11.034.

9. Omarov R.T.,Sagi M., Lips S.H. Regulation of aldehyde oxidase and nitrate reductase in roots of barley (*Hordeum vulgare L.*) by nitrogen source and salinity // J. Exp. Bot. -1998. -Vol. 49. -№ 322. -P. 897-902. DOI: 10.1093/jxb/49.322.897.

10. Mendel R.R. Cell biology of molybdenum in plants // Plant Cell Rep. -2011. -Vol. 30. -№ 10. -P. 1787-1797. DOI: 10.1007/s00299-011-1100-4.

11. Mendel R.R., Kruse T. Cell biology of molybdenum in plants and humans // Biochim. Biophys. Acta. -2012. -Vol. 1823. -№ 9. -P. 1568-1579 DOI: 10.1016/j.bbamcr.2012.02.007.

12. Martelli G. P., Gallitelli D., and Russo M., “Tombusviruses,” in The Plant Viruses: Polyhedral Virions with Monopartite RNA Genomes, R. Koenig, Ed. Boston, MA: Springer US, 1988. -P. 13-72. DOI: 10.1007/978-1-4613-0921-5_2.

13. Knorr D.A., Mullin R.H., Hearne P.Q., Morris T.J. De novo generation of defective interfering RNAs of Tomato bushy stunt virus by high multiplicity passage // Virology. -1991. -Vol. 181. -№ 1. -P. 193-202. DOI: 10.1016/0042-6822(91)90484-S.

14. Hearne P.Q., Knorr D.A., Hillman B.I., Morris T.J. The complete genome structure and synthesis of infectious RNA from clones of Tomato bushy stunt virus // Virology. -1990. -Vol. 177. -№ 1. -P. 141-151. DOI: 10.1016/0042-6822(90)90468-7.

15. Suleimenova Zh. Z., Kutzhanova A., Yergaliyev T.M., Batyrshina Zh.S., Omarov R.T. The development of express method for detection viral diseases in plants // Bull. LN Gumilyov Eurasian Natl. Univ. Biosci. Ser. -2018. -Vol. 124. -P 65-69. DOI: 10.32523/2616-7034-2018-124-3-65-69.

Т. Ергалиев

Гогенгейм университеті, Штутгарт, Германия

Молибден және өсімдіктердің вирустық инфекцияға төзімділігі

Аңдатпа. Молибден өсімдіктердің және басқа салаларының өсуі мен дамуына қажетті бірнеше физиологиялық процестерге белсенді қатысады. Молибден көптеген биохимиялық реакцияларға қатысады және бұл металдың жетіспеуі өсімдіктердегі ақуыздардың жалпы санына әсер етуі мүмкін. Қазіргі уақытта құрамында Мо бар елуден астам ферменттер белгілі, дегенмен олардың көпшілігі бактериялардан табылған. Өсімдіктерде нитратредуктаза, сульфитоксидаза, альдегид оксидаза, ксантин дегидрогеназа және митохондриялық амидоксимредуктаза сияқты Мо бар ферменттер бар. Вольфрам – да ауыр металл, ол молибденмен өзінің физикалық-химиялық қасиеттеріне байланысты өте ұқсас келеді. Мұндағы ферменттің жанама факторы ретінде соңғысының орнына қосылуы мүмкін, бұл оның инактивациясына әкеледі. Бұл мақалада пилоттық эксперименттің алдын-ала нәтижелері келтірілген.

Түйін сөздер: молибден, TBSV, өсімдік, вирус, вирусқа төзімділік, жұқпалылық.

Т. Ергалиев
Гогенгеймский университет, Штутгарт, Германия

Молибден и устойчивость растений к вирусной инфекции

Аннотация. Молибден принимает активное участие в нескольких физиологических процессах, необходимых для роста и развития растений и в других сферах жизни. Молибден участвует во многих биохимических реакциях, и недостаток этого металла может повлиять на общее количество белков в растениях. В настоящее время известно более пятидесяти ферментов, содержащих Мо, хотя большинство из них было обнаружено в бактериях. Растения содержат Мо-содержащие ферменты, такие как нитратредуктаза, сульфитоксидаза, альдегидоксидаза, ксантиндегидрогеназа и митохондриальная амидоксимредуктаза. Вольфрам - еще один тяжелый металл, который из-за очень схожих физико-химических свойств с молибденом может быть включен вместо последнего в качестве кофактора фермента, что приводит к его инактивации. В этой статье показаны предварительные результаты пилотного эксперимента, демонстрирующего влияние обработки молибденом и вольфрамом на растения Nicotiana benthamiana, зараженные вирусом томатного кустарника, который относится к вирусам, паразитирующим на экономически важных культурах. Этот вирус поражает более 100 видов однодольных и двудольных растений из более чем 20 различных семейств. Заражение растений вирусной инфекцией происходит через механическое повреждение корневой системы; вирионы в этом случае могут передаваться через почву или воду. Было установлено, что лечение молибденом может привести к смягчению последствий, в противном случае смертельных для хозяина вирусной инфекции.

Ключевые слова: молибден, TBSV, растение, вирус, вирусоустойчивость, инфекция.

References

1. “Metals Ions in Biological System: Volume 39: Molybdenum and Tungsten: Their Roles in Biological Processes”: Routledge & CRC Press. [Electronic resource]. Available at: https://www.routledge.com/Metals-Ions-in-Biological-System-Volume-39-Molybdenum-and-Tungsten-Their/Sigel Sigel/p/book/9780367396299 (Accessed: 15.05.2021).

2. Arnon D.I., Stout P.R. Molybdenum as an Essential Element For Higher Plants. Plant Physiol. 14(3), 599-602 (1939) doi: 10.1104/pp.14.3.599.

3. Tejada-Jiménez M., Chamizo-Ampudia A., Galván A., Fernández E., and Llamas Á. Molybdenum metabolism in plants. Metallomics. 5(9), 1191-1203 (2013) doi: 10.1039/C3MT00078H.

4. Jonmaire P. Molybdenum in Industrial Toxicology. John Wiley & Sons Inc. 1340 (2015) doi: 10.1002/9781118834015.ch24.

5. Naqib S.A., Jahan M.S. The Function of Molybdenum and Boron on the Plants. Plants. J Agri Res. 2(3), 1-8 (2017). doi: 10.23880/OAJAR-16000136.

6. Gupta U. C. Boron, molybdenum and selenium status in different plant parts in forage legumes and vegetable crops. J. Plant Nutr. 14(6), 613-621 (1991) doi: 10.1080/01904169109364228.

7. Stiefel E.I. The biogeochemistry of molybdenum and tungsten. Met. Ions Biol. Syst. 39, 1-29 (2002).

8. Hille R., Nishino T., Bittner F. Molybdenum enzymes in higher organisms. Coord. Chem. Rev. 255(9-10), 1179-1205 (2011) doi: 10.1016/j.ccr.2010.11.034.

9. Omarov R.T.,Sagi M., Lips S.H. Regulation of aldehyde oxidase and nitrate reductase in roots of barley (*Hordeum vulgare L.*) by nitrogen source and salinity. J. Exp. Bot. 49(322), 897-902 (1998) doi: 10.1093/jxb/49.322.897.

10. Mendel R.R. Cell biology of molybdenum in plants. Plant Cell Rep. 30(10), 1787-1797 (2011) doi: 10.1007/s00299-011-1100-4.

11. Mendel R.R., Kruse T. Cell biology of molybdenum in plants and humans. Biochim. Biophys. Acta. 1823(9), 1568-1579 (2012) doi: 10.1016/j.bbamcr.2012.02.007.

12. Martelli G. P., Gallitelli D., and Russo M., “Tombusviruses,” in The Plant Viruses: Polyhedral Virions with Monopartite RNA Genomes, R. Koenig, Ed. Boston, MA: Springer US. 1988, pp. 13-72. doi: 10.1007/978-1-4613-0921-5_2.

13. Knorr D.A., Mullin R.H., Hearne P.Q., Morris T.J. De novo generation of defective interfering RNAs of Tomato bushy stunt virus by high multiplicity passage. Virology. 181(1), 193-202 (1991) doi: 10.1016/0042-6822(91)90484-S.

14. Hearne P.Q., Knorr D.A., Hillman B.I., Morris T.J. The complete genome structure and synthesis of infectious RNA from clones of Tomato bushy stunt virus. Virology. 177(1), 141-151 (1990) doi: 10.1016/0042-6822(90)90468-7.

15. Suleimenova Zh. Z., Kutzhanova A., Yergaliyev T.M., Batyrshina Zh.S., Omarov R.T. The development of express method for detection viral diseases in plants. Bull. LN Gumilyov Eurasian Natl. Univ. Biosci. Ser. 124, 65-69 (2018) doi: 10.32523/2616-7034-2018-124-3-65-69.

Сведения об авторе:

T. Yergaliev - PhD (Biology), postdoctoral researcher at the University of Hohenheim, Emil-Wolff-Str. 6-10 70593, Stuttgart, Germany.

Т. Ергалиев - PhD (Биология), Гогенгейм университетінің докторантурадан кейінгі зерттеушісі, Эмиль-Вульф-Стр. 6-10 70593, Штутгарт, Германия.