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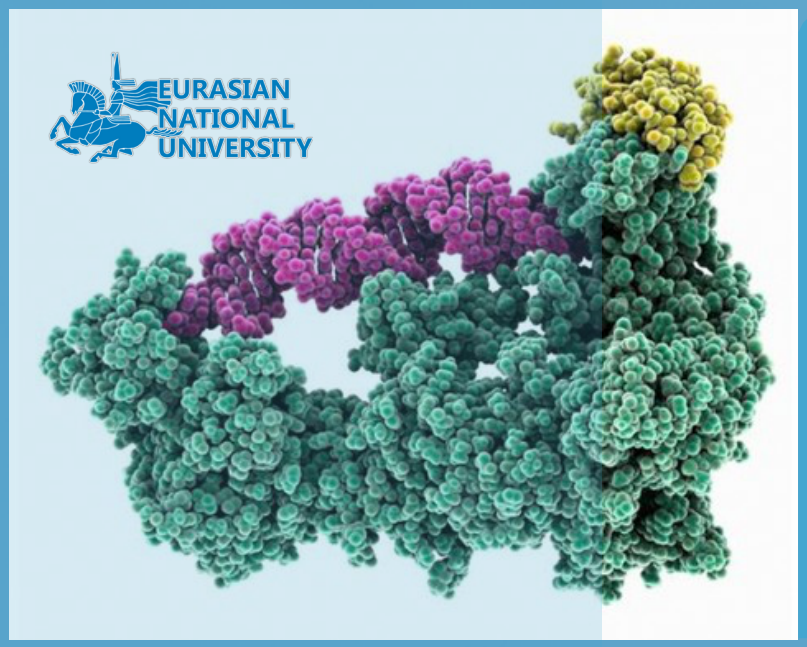
МИНИСТЕРСТВО НАУКИ И ВЫСШЕГО ОБРАЗОВАНИЯ



Л.Н. ГУМИЛЕВ АТЫНДАҒЫ
ЕУРАЗИЯ ҰЛТТЫҚ УНИВЕРСИТЕТІ

ЕВРАЗИЙСКИЙ НАЦИОНАЛЬНЫЙ
УНИВЕРСИТЕТ ИМЕНИ
Л.Н. ГУМИЛЕВА

"ОМАРОВ ОҚУЛАРЫ: XXI ҒАСЫРДЫҢ БИОЛОГИЯ ЖӘНЕ БИОТЕХНОЛОГИЯСЫ" АТТЫ ХАЛЫҚАРАЛЫҚ ҒЫЛЫМИ ФОРУМНЫҢ БАЯНДАМАЛАР ЖИНАҒЫ

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Жинақ «Омаров оқулары: XXI ғасыр биология және биотехнологиясы» атты халықаралық ғылыми форумына қатысушылардың баяндамаларымен құрастырылған. Бұл басылымда биология, биотехнология, молекулалық биология және генетиканың маңызды мәселелері қарастырылған. Жинақ ғылыми қызметкерлерге, PhD докторанттарға, магистранттарға, сәйкес мамандықтағы студенттерге арналған.

Сборник составлен по материалам, представленным участниками международного научного форума «Омаровские чтения: Биология и биотехнология XXI века». Издание освещает актуальные вопросы биологии, биотехнологии, молекулярной биологии и генетики. Сборник рассчитан на научных работников, PhD докторантов, магистрантов, студентов соответствующих специальностей.

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эффективность и стабильность этих кислот, увеличивая их практическую полезность в сельском хозяйстве. Более того, интеграция насыщенных монокарбоновых кислот с другими средствами биоконтроля или культурными практиками может обеспечить синергетический эффект и улучшить общие стратегии борьбы с заболеваниями. Кроме того, продолжающиеся исследования молекулярных механизмов, лежащих в основе противовирусной активности насыщенных монокарбоновых кислот, могут выявить новые мишени для вмешательства и привести к разработке более эффективных решений для биоконтроля.

Заключение

Насыщенные монокарбоновые кислоты представляют собой многообещающий класс средств биоконтроля для борьбы с вирусными заболеваниями растений в сельском хозяйстве. Их антимикробные свойства широкого спектра действия, низкое воздействие на окружающую среду и потенциал для интеграции в стратегии устойчивого управления болезнями делают их привлекательной альтернативой химическим пестицидам. Однако необходимы дальнейшие исследования для оптимизации их эффективности, методов применения и экологической совместимости. С продолжающимися достижениями в области биотехнологии и агрономии насыщенные монокарбоновые кислоты открывают большие перспективы для внесения вклада в развитие более устойчивых сельскохозяйственных систем.

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Impact of combined abiotic and biotic stresses on ROS accumulation in *Nicotiana* benthamiana

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Abstract

The effect of biotic and abiotic stress reduces and limits crop yields. In this study, molybdenum created an abiotic stress condition, while biotic stress was generated by inoculation of *Tomato Bushy Stress Virus* (TBSV) in *Nicotiana benthamiana*. TBSV is a model system in virological studies of the activity of plant viruses, especially in most cases the model host plant is *Nicotiana benthamiana*. Under various stress conditions, reactive oxygen species (ROS) components, especially hydrogen peroxide (H2O2), are accumulated, damaging cellular components (nucleic acids, lipids and proteins). In response to stress, plants have developed various mechanisms to cope with these threats. The purpose of this study is to demonstrate the effect of the combined effect of molybdenum and TBSV on N. benthamiana and its ROS accumulation.

Keywords: TBSV, N.benthamiana, ROS, Molybdenum, H2O2, antioxidants

Introduction

Typically, viruses are virulent and could cause diseases in crops and plants growing in both open spaces or greenhouses for food production. One of the most studied ones is the *Tomato bushy virus* (TBSV) which belongs to the *Tombusviridae* family of spherical RNA viruses and was first isolated in 1935 from tomatoes in Ireland [1]. The length of TBSV is about 4800nt and about 30nm in diameter [2], which is wrapped by 180 identical subunits of 41kDa capsid protein by containing 5 different open reading frames such as p33, p92, p41, p22 and p19 [3]. TBSV causes a huge impact on plant physiology such as necrosis, brushy growth, leaf deformation, stunting, withering [3] and other mozaic symptoms that significantly reduce crop yields. For that reason, crop and plant protection from viruses is essential for the development of agriculture. Some trace elements such as copper, zinc etc. are found to have antiviral properties [4]. Another trace element is molybdenum. It has the key role in the synthesis of molybdoenzymes such as aldehyde oxidase (AO), xanthine dehydrogenase (XDH), and nitrate reductase (NR) and as sulfite oxidase (SOX) by forming molybdenum cofactor (Moco) [5]. Both Mo toxicity and efficiency are harmful to plant growth and development and have clear symptoms [6]. Plants and crops with Mo deficiency usually develop an altered phenotype, it includes lesions and some morphological differences in leaves [7]. On the other hand main symptom of Mo toxicity is yellow chlorosis, with additional brownish tints starting in the youngest leaves.

Significantly raised ROS production, called oxidative (respiratory) burst is one of the main plant responses to the virus invasion [8]. It includes the production of reactive oxygen species such as hydrogen peroxide (H2O2), singlet oxygen (1O2), the hydroperoxyl radical (HO2·), the superoxide anion O2, the hydroxyl radical (OH-) and others. The production of ROS acts as a part of the defence system, more specifically they act as antimicrobial agents and have a key role in signal transduction and gene expression during defence mechanisms however, the highly accumulated ROS is toxic to all organisms [9]. After significant accumulation of ROS, different fat-soluble and non-soluble antioxidants take part in plants' defence against oxidative stress or highly accumulated ROS. To minimize oxidative damage caused by excessive levels of ROS, plants have a complex antioxidant system which contains non-enzymatic antioxidants and various antioxidant enzymes [3]. These antioxidant enzymes have a key role in the scavenging accumulation of ROS generations under stress conditions in plants. The enzymatic defence system contains antioxidants such as catalase (CAT), sodium dismutase (SOD), glutathione reductase (GR) and others [10]. The initial line of defence system against oxidative damage is formed by SOD, it catalyzes the dismutating of superoxide radicals (O•−2) turning it into O2 and H2O2. Whereas, catalase catalyses a reaction of dismutation of hydrogen peroxide to water and oxygen [11] and GR is used in catalyzing the reduction of glutathione disulfide (GSSG) to glutathione (GSH) [12].

It is well-known that ROS significantly accumulated in plants under stress [13], it was studied in various model plants such as Arabidopsis thaliana [14] and *Nicotiana benthamiana*. In

this study, we are going to identify the combined effect of Mo and viral particles (TBSV) on *Nicotiana benthamiana* for the first time.

Materials and methods

2.1. Plant material

The *N. benthamiana* plant leaves utilized in this study were cultivated in a greenhouse under a long-day photoperiod consisting of 16 hours of light and 8 hours of darkness. Daily checks were conducted to monitor humidity and temperature, which remained consistent, with temperatures ranging between 25°C during the day and 22°C at night. The cultivation of *N. benthamiana* benefited from the favourable light intensity provided by spectrum lamps, approximately 2700K.

2.2. Treatment of *N.benthamiana* with molybdenum

To treat with Mo, a solution concentration of 2.5 mM was applied, and watered for 5 days after 30 days of planting, with regular checks on greenhouse conditions. The special solution, comprising Mo and DDW per litre, was prepared. Following treatment, physiological parameters were documented.

2.3. Plant inoculation by TBSV

To inoculate with TBSV, additional plants were cultivated for 30 days before being inoculated. A mechanical method involving fingertip rubbing was employed for effective inoculation. After 37 days, the plant leaves were homogenized in TE buffer and centrifuged at 4°C, 10,000 rpm for 30 minutes. The resulting samples were collected and filtered through colony chromatography. Subsequently, these samples were used to infect the target plant (*N. benthamiana*) for 7 days, with physiological parameters recorded. Healthy and infected plants were then grown separately under identical conditions.

2.4. Detection of H2O2 in plant samples

The upper non-inoculated leaves were examined to identify ROS components, such as H2O2, known for its detrimental effects on plant growth and development. Samples underwent homogenization with PB buffer and were centrifuged twice at 4°C, 10,000 rpm for 10 minutes each time and then subjected to a special plate containing a mixture of AAP (4-Aminoantipyrine), BHS and HRP (Horseradish peroxidase) reagents. Following the protocol, the samples were mixed with a specific buffer and compared with standards. Detection was performed using a spectrophotometer, with periodic checks on the results. The varying sample amounts were determined based on the intensity of light relative to wavelength.

Results

3.1. Detection of viral infection of inoculated plants

N. benthamiana plants were treated with 2.5 mM molybdenum solution for 7 days. The effect of the treatment is shown in Figure 1: no dramatic changes were observed between the control and treated plants. The length of the molybdenum plant was slightly shorter than the healthy one, indicating the effect of molybdenum on plant growth. The leaves of the plants were large and bright green, which resulted in good photosynthesis.

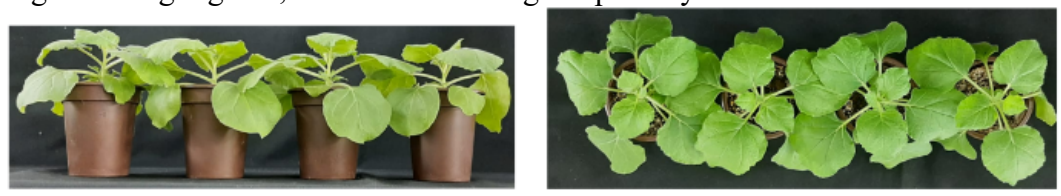

Figure 1. The effect of 2.5mM Mo treatment in *N.benthamiana* development.

N. benthamiana plants were then mechanically inoculated with TBSV at 7 dpi (days post-inoculation). They were provided with a water supply every other day, 30 ml of tap water. Control plants were treated with the same amount of DDW. Visual observation of inoculated plants had its own peculiarities. The leaves of the infected plant wilted and the apical region suffered from necrosis, as shown in Figure 2. Significant retardation of plant development was also observed, indicating that TBSV had entered the plant and had a negative impact on the

whole plant's growth system. Parameters of the greenhouse room where the plants were grown: temperature 26.8C, humidity 38%.

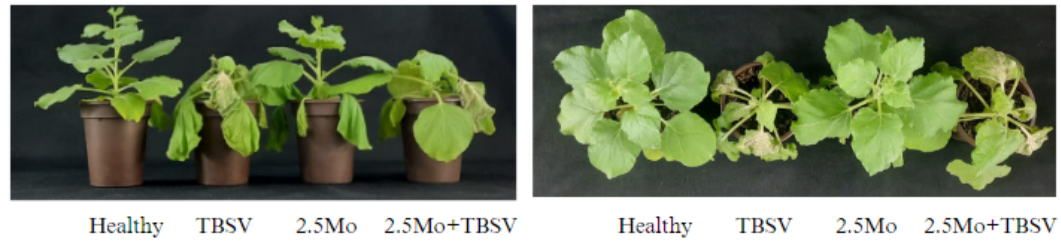

Figure 2. After *N. benthamiana* inoculation at 7 dpi.

3.2. Detection of total H2O2 in *N.benthamiana*

Hydrogen peroxide (H2O2) is a constituent of reactive oxygen species (ROS) responsible for inducing oxidative bursts under various stresses, including biotic and abiotic factors. Its detrimental impact on essential cellular components such as nucleic acids, lipids, and proteins stems from its relatively high stability and mobility within plant tissues. Spectrophotometry was employed to determine the final results. The effect of combined exposure to molybdenum metal and TVSV virus is shown in Figure 3.

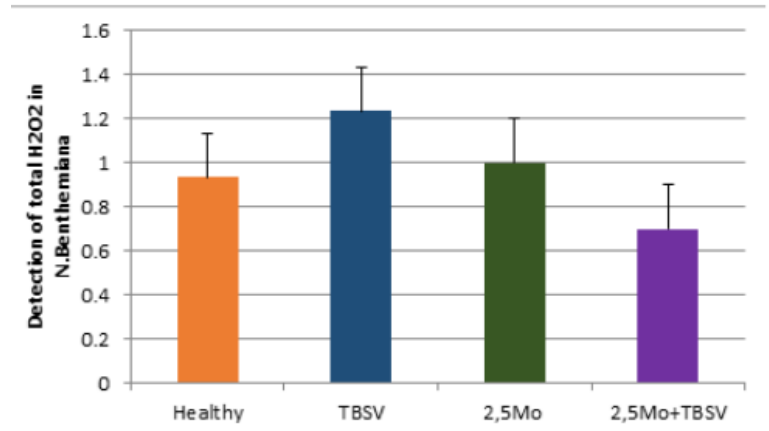

Figure 3. Detection of H2O2 levels in upper-inoculated *N.benthamiana* leaves by spectrophotometer.

As shown in Figure 3, when inoculated with the TBSV, the accumulation of hydrogen peroxide increases compared to the control. With a Molybdenum concentration of 2.5 mM, the accumulation of hydrogen peroxide increases slightly. Interestingly, the combined effect of 2.5 mM molybdenum with the virus decreases with the accumulation of hydrogen peroxide.

Conclusion

In conclusion, with the virus and molybdenum, the generation of hydrogen peroxide increases, while with the combination the effect of the virus plus metal decreases. A study of the combined effect of molybdenum and the TBSV showed a decrease in the level of accumulation of hydrogen peroxide generation compared to the control.

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