Казахстана; провести сравнительную оценку урожайности кормовых культур; определить наиболее перспективные для возделывания в степной зоне Северного Казахстана кормовые культуры. Научная значимость работы заключается в получении новых данных о сравнительной урожайности различных кормовых культур в условиях степной зоны Северного Казахстана. Вклад работы: полученные данные о сравнительной урожайности различных кормовых культур в условиях степной зоны Северного Казахстана позволят повысить эффективность сельскохозяйственного производства. Практическая значимость и ценность работы заключается в разработке рекомендаций по выбору наиболее перспективных для возделывания в степной зоне Северного Казахстана кормовых культур, а также для внедрения адаптивных технологий в сельскохозяйственное производство. В работе использованы следующие методы исследования: методика опытного дела Б.А. Доспехова, метод учета растительности Д. Брауна, методика определения влажности почвы Н.М. Бакаева; лабораторные анализы; математическая обработка урожайных данных. По результатам исследований установлено, что сорго-суданковый гибрид сформировал наибольшую урожайность: 209,1 ц/га зеленой массы и 32,6 ц/га сухого вещества. Кукуруза и сорго-суданковый гибрид показали сбалансированность кормами по содержанию сырого протеина и сырой клетчатки. Пайза и африканское просо имеют меньшую, но достатточно высокую урожайность, чем кукуруза и сорго-суданковый гибрид.

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THE ALARMING PREVALENCE OF RHIZOMANIA DISEASE CAUSED BY BEET NECROTIC YELLOW VEIN VIRUS IN KAZAKHSTAN'S SUGAR BEET CULTIVATION

ANNOTATION

Rhizomania is a devastating disease affecting sugar beet crops, characterized by root swelling, necrosis and yellowing of the veins. The main agent causing rhizomania in sugar beet is the Beet Necrotic Yellow Vein Virus (BNYVV), which is transmitted by the single-celled parasitic organism Polymyxa betae, belonging to the order Plasmodiophorales. The disease exhibits increased virulence under conditions of high soil temperature and moisture, while relatively dry soil mitigates its effects. The destructive effect of rhizomania is manifested in a significant decrease in the weight of roots: infected roots weigh 10-15 times less than healthy ones. In addition, in roots infected with rhizomania, the sugar content and sugar yield are reduced, which worsens the technological quality of the raw material. To prevent the spread of the disease, it is necessary to observe strict quarantine measures when importing, exporting, transporting and storing planting material containing soil. In addition, to combat the disease it is necessary to introduce rhizomania-resistant sugar beet hybrids. Thus, understanding the interactions between viral strains and host resistance mechanisms is critical to develop effective strategies to control and maintain sugar beet yields in the face of this formidable disease.

In this study, we confirmed the presence of beet necrotic yellow vein virus (BNYVV) in beet plants (Viorica and Ardan varieties) collected from fields in the Zhetysu region. Zhetysu district is recognized as a major center for growing sugar beets in Kazakhstan. The virus was detected using a commercial real-time PCR kit (Letgen), which identified pathotypes A, B, and P. Only pathotype A was confirmed to be present.

Key words: *BNYYV, sugar beet, quarantine object, yield, qPCR*

Introduction. Rhizomania is the most devastating disease affecting sugar beet. It is characterized by a reduction in sugar content in the roots of susceptible varieties by up to 20%. However, according to reports from sugar companies, yield losses during sugar purification can reach 50 to 70% [1]. The virus can spread through the movement of contaminated soil by agricultural machinery from one field to another, or through the importation of infected seeds. The disease was first reported in the 1950s in Italy [2]. Rhizomania manifests with the following symptoms: leaf chlorosis, characterized by yellowing of veins and interveinal spaces, leaf deformation, including curling, stunted plant growth leading to a reduction in overall size, and proliferation of lateral roots, characterized by the formation of numerous small lateral roots [3].

The etiological agent causing rhizomania is a phytopathogenic virus identified by Tamada and Baba in 1973 and named Beet necrotic yellow vein virus (BNYVV) [4, 5]. This is a positive-sense RNA virus transmitted by the obligate root-infecting plasmodiophorid endoparasite Polymyxa betae Keskin [6]. Currently, the virus is listed in Annex III as a quarantine pest of protected zones (PZ) under Commission Regulation (EU) 2019/2072 [2].

There are three main subgroups of the virus: A, B, and P types (7). Type A is present in most European countries, Iran, North America, China, and Japan, while Type B is often found in France and Germany, and occasionally in Sweden, China, and Japan [8, 9, 10]. BNYVV isolates designated as P-type isolates have been identified in the Pithiviers area in France [11] and in Kazakhstan [10] and are the rarest type.

The share of domestically produced sugar from sugar beet in Kazakhstan is 7%. Over the past 20 years, there has been a sharp decline in sugar beet production in the country, primarily due to the spread of Beet necrotic yellow vein virus [12]. Therefore, the identification of this virus type is a crucial step in preventing significant economic losses associated with reduced agricultural crop yields.

Materials and methods. A total of 123 sugar beet plant samples exhibiting symptoms similar to rhizomania were collected from two fields in the Zhetisu region, near the city of Taldykorgan. Of these, 60 samples were of the Viorica-KWS variety and 63 samples were of the Ardan variety.

RNA Extraction and qPCR Identification of Beet Necrotic Yellow Vein Virus. Total RNA was extracted from the sugar beet roots using the CTAB method [13, 14]. Identification of the types of Beet Necrotic Yellow Vein Virus (BNYVV) was performed using qPCR with specific primers and a FAM-labeled probe, developed by Letgen. The reaction mixture contained 10 µl of qPCR Mastermix, 2 µl of Primer-Probe Mix, and 4 µl of genomic RNA. The amplification program consisted of reverse transcription at 50°C for 10 minutes, polymerase activation at 95°C for 2 minutes, followed by 40 amplification cycles of denaturation at 95°C for 10 seconds, and annealing and extension at 60°C for 30 seconds. Fluorescence readings were taken after each elongation cycle. The analysis of the results was carried out according to the protocol provided in the kit.

Inoculation test . Preparation of inoculum: 200 mg infected tissue of sugar beet was grinded in a mortar with 1 ml potassium phosphate buffer until it becomes a homogenous mixture. The homogenate was filtered through a double layer of cheesecloth to remove debris. Carborundum powder was used as an abrasive, facilitating the entry of the virus into the leaf cells.

Preparation of Plants: three healthy sugar beet plants were selected for inoculation for every isolate. Before inoculation, the leaves were washed gently with distilled water to remove dust and allow them to air dry. The inoculum mixture (100 ul) was applied evenly to ensure good coverage onto the upper surface of the leaves. Then the leaves were gently rubbed with the swab or brush to facilitate the penetration of the virus ensuring that the carborundum powder creates small abrasions on the leaf surface without causing extensive damage. After inoculation, the plants were covered with plastic bags for 24-48 hours to maintain high humidity, which facilitates infection. Analysis of infection was performed every 3 days post inoculation by qPCR.

qPCR Identification of *Polymyxa betae*

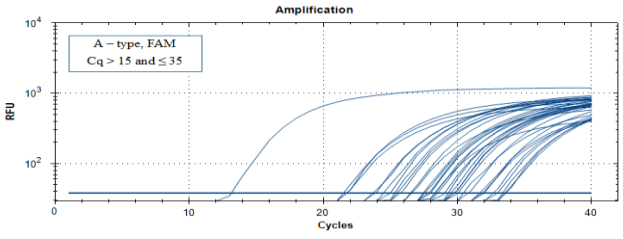
Genomic DNA from lateral roots was extracted using a plant/fungi DNA extraction kit (Norgen). The following primers and probe were used for identification of *P. betae:* forward primer: 5'-GATGGTGAACCTGCGGAAGGATCA-3'; reverse primer:5'-ACGAGGTCAACCTGCGGAAGG

ATA-3'; Probe: 5'-FAM-AGAGAGATGTGTGCGTCAGCAGGGAAG -TAMRA-3'. PCR reaction mixture was prepared in accordance with protocol supplied with Luna® Universal qPCR & RT-qPCR (NEB).

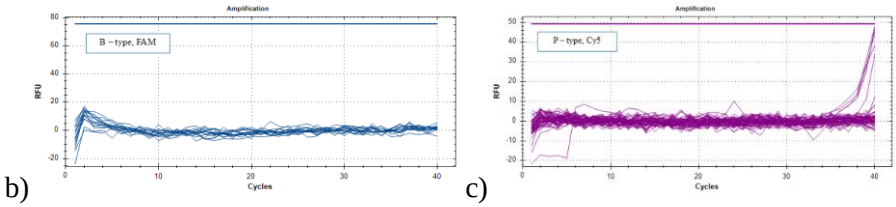
Results and discussion. BNYVV is a major concern for sugar beet producers globally due to its significant economic impact. The virus causes rhizomania, a disease that severely stunts root growth and deforms the roots, leading to substantial losses in both yield and sugar content. Early detection of BNYVV is essential to implement control measures and prevent large-scale financial losses. BNYVV is spread by the soil-borne fungus *Polymyxa betae*, and once a field is infected, the virus can remain in the soil for many years. Early detection is crucial to prevent the virus from spreading to new fields and regions. By identifying and managing infected areas promptly, the spread can be contained, protecting future crops and maintaining soil health.

The present study revealed that out of all 123 samples tested for the presence of Beet Necrotic Yellow Vein Virus type A, 106 samples were found to be infected. Among these, the infection rate for the Viorica variety was 53.7%, while for the Ardan variety it was 46.3%. The remaining 17 samples tested negative, suggesting the absence of BNYVV type A infection, figure 1.

No samples were found to be infected with BNYVV types B or P during the course of the study.



a)



b) c)

Figure 1 – м Results of qPCR Analysis

a) Results for type A virus; b) Results for type B virus; c) Results for type P virus. Ct value of less than 35 was considered positive in accordance with the test system protocol.

There are several reasons for the spread of BNYVV. One of them is that soil containing virulent spores of *Polymyxa betae* might have been introduced to other vegetable crops brought from outside, such as seed potatoes [15, 16].

Testing of lateral roots of positive samples for BNYVV revealed contamination by *Polymyxa betae*, figure 2.

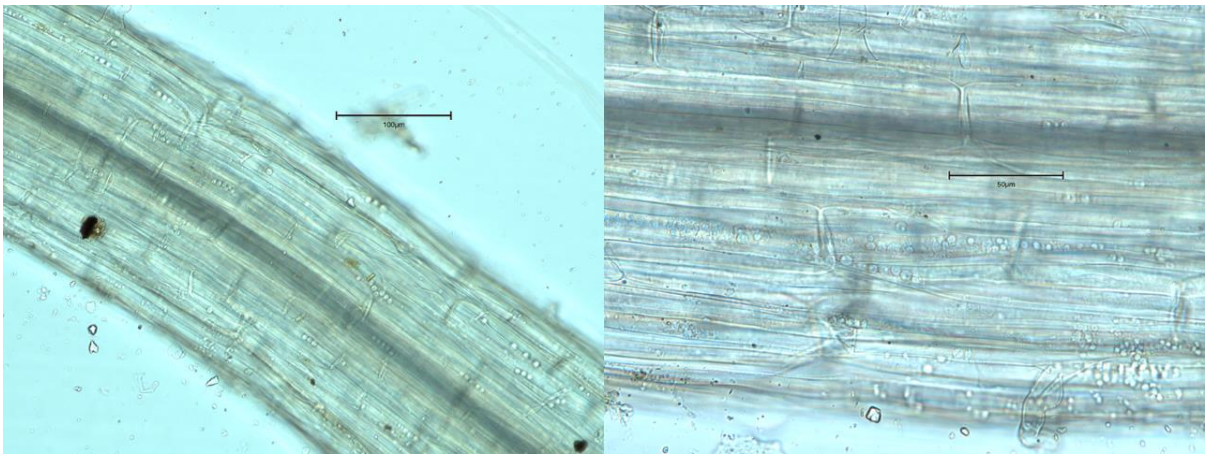


Figure 2 – Microscopic examination of lateral roots of sugar beet plants in researched field. Zoospores are presented at 40X magnifications, the samples were stained with methylene blue.

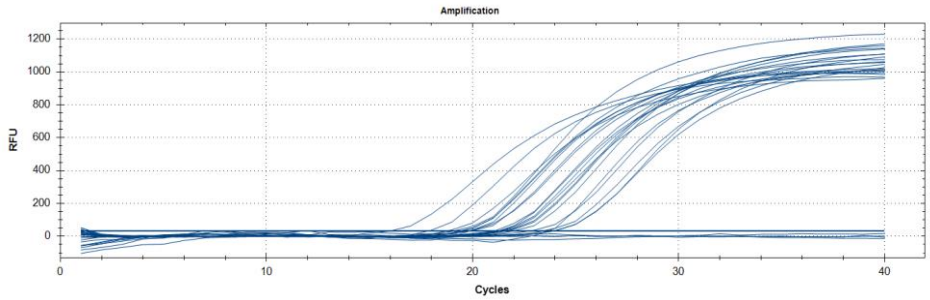


Figure 3 – Results of qPCR detection of *P. betae* in 18 samples of sugar beet lateral roots.

Polymyxa betae acts as the vector for BNYVV. The virus is not capable of infecting plants directly but relies on *P. betae* to gain entry into the plant's root cells. Zoospores encyst on the root hairs of sugar beets and penetrate the root cells, introducing BNYVV into the plant during this process. Infected roots exhibit necrosis and reduced functionality, which significantly impacts water and nutrient uptake, leading to overall poor plant health and reduced yield. The lifecycle of *P. betae* includes the formation of resting spores called cystosori, which can harbor the virus for over a decade. These spores remain dormant in the soil or dried plant roots and are activated under favorable

conditions, such as high soil moisture, which often results from excessive rainfall or irrigation. *P. betae* in tested roots was also confirmed by qPCR, figure 3.

After entering the root cells by *P. betae*, BNYVV begins to replicate and spread from cell to cell through plasmodesmata. The virus's movement proteins, such as the P42 protein, are crucial in facilitating this cell-to-cell movement by modifying plasmodesmata to allow the passage of viral particles. BNYVV's cysteine-rich suppressor proteins inhibit the plant's gene silencing pathways, allowing the virus to replicate and spread without being targeted by the host's antiviral responses.

The A strain of virus was used for inoculation test of sugar beet plants to confirm biological activity of viral isolates and identification of precise strain presence, figure 4. Testing different hybrids, including Tisserin, MN 7001, and the hybrid Aksu, revealed mild symptoms of infection, such as chlorosis and yellowing of leaves.

Figure 4 – Inoculation test of sugar beet plants by strain A of Beet necrotic yellow vein virus. MN 7007, Aksu, and Tisserin were analyzed for the symptoms of infection 7 days post inoculation.

Investigating plant viruses through inoculation tests is a key method in plant virology. These tests help researchers understand various aspects of plant-virus interactions, including virus identification, host range, disease symptoms, and the mechanisms of virus transmission.

Previously performed inoculation tests by BNYVV showed different response of sugar beet genotypes to virus. The symptoms included the extensive proliferation of lateral roots, giving the root system a "bearded" appearance, the vascular tissue in the roots becomes necrotic, leading to blackening and decay of the root tissue, infected plants often have significantly smaller main roots compared to healthy plants, which can severely impact yield, yellowing along the veins of leaves, in severe cases, necrosis (death of tissue) can occur along the veins of the leaves, and the leaves may become generally chlorotic (yellowed), which indicates a disruption in normal chlorophyll production and function. The symptoms are more noticeable when the virus has systemically spread throughout the plant.

It is hypothesized that the presence of one virus type in a region partially prevents the spread of other types of BNYVV due to competition between different strains of *Polymyxa betae*. Based on this, it can be concluded that the absence of type B and P viruses in the samples studied is likely due to the presence of type A virus [17].

Sustainable agricultural practices are vital for long-term food security and environmental health. Detecting BNYVV early supports sustainable farming by allowing farmers to adopt biological control methods and reduce reliance on chemical treatments. This not only helps manage the virus but also promotes overall soil and crop health [18].

The Rz1 and Rz2 genes play a crucial role in conferring resistance against Beet necrotic yellow vein virus (BNYVV) in certain sugar beet cultivars. The Rz1 and Rz2 genes, which are derived from the wild beet species Beta maritima, confer resistance against BNYVV through different mechanisms

[19]. The Rz1 gene provides a broad-spectrum resistance against various BNYVV strains, while the Rz2 gene specifically targets certain pathotypes or strains of the virus. The Rz1 gene encodes a protein that belongs to the CC-NB-LRR (coiled-coil, nucleotide-binding, leucine-rich repeat) class of resistance proteins. These proteins are involved in recognizing specific pathogen-derived molecules, known as avirulence factors, and triggering a hypersensitive response (HR) in the plant. The HR is a localized cell death response that restricts the spread of the virus and prevents systemic infection [20].

The Rz2 gene, on the other hand, encodes a protein that is structurally distinct from the CC-NB-LRR class of resistance proteins. The exact mechanism by which Rz2 confers resistance against BNYVV is not fully understood, but it is believed to involve a different signaling pathway or a different mode of recognizing the viral components.

The interaction between the Rz1 and Rz2 genes and BNYVV is governed by the gene-for-gene model of plant-pathogen interactions. In this model, resistance is conferred when a specific resistance (R) gene in the plant recognizes a corresponding avirulence (Avr) gene or factor from the pathogen. The recognition of the Avr factor by the R gene triggers a defense response in the plant, leading to resistance against the pathogen [21].

In the case of BNYVV, the Rz1 gene is thought to recognize a specific avirulence factor encoded by the virus, while the Rz2 gene recognizes a different avirulence factor or interacts with a different viral component. When either the Rz1 or Rz2 gene is present in a sugar beet cultivar, it can recognize the corresponding BNYVV avirulence factor and activate defense responses, conferring resistance against the virus [22].

The deployment of sugar beet cultivars carrying the Rz1 and Rz2 resistance genes has been instrumental in managing BNYVV infections in sugar beet production areas. However, the emergence of new BNYVV strains or pathotypes that can overcome these resistance genes poses a continuous challenge. Ongoing research efforts are focused on identifying additional resistance genes and understanding the molecular mechanisms underlying the plant-virus interactions, with the aim of developing more durable and broad-spectrum resistance against BNYVV [23].

Detecting viruses and developing resistant sugar beet cultivars is crucial for maintaining the economic viability, food security, and environmental sustainability of sugar beet production, as well as ensuring the long-term sustainability of the crop in the face of climate change and evolving viral diseases [24].

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РЕЗЮМЕ

Ризомания — разрушительное заболевание, поражающее посевы сахарной свеклы, характеризующееся набуханием корней, некрозом и пожелтением жилок. Основным агентом

вызывающим изыманию сахарной свеклы является вирус некротического пожелтения жилок (BNYYV), переносчиком которого является одноклеточный паразитический организм Polymyxa betae, принадлежащий к отряду Plasmodiophorales. Болезнь проявляет повышенную вирулентность в условиях высокой температуры и влажности почвы, тогда как относительно сухая почва смягчает ее воздействие. Губительное действие ризомании проявляется в значительном уменьшении массы корней: зараженные корни весят в 10–15 раз меньше здоровых. Кроме того, в корнях, зараженных ризоманией, снижается сахаристость и выход сахара, что ухудшает технологическое качество сырья. Для предотвращения распространения заболевания необходимо соблюдать строгие карантинные меры при ввозе, вывозе, транспортировке и хранении посадочного материала, содержащего почву. Кроме того, для борьбы с заболеванием необходимо внедрение устойчивых к ризомании гибридов сахарной свеклы. Таким образом, понимание взаимодействия между вирусными штаммами и механизмами устойчивости хозяина имеет решающее значение для разработки эффективных стратегий борьбы и поддержания урожайности сахарной свеклы перед лицом этого грозного заболевания.

В данном исследовании мы подтвердили наличие вируса некротической желтой жилки свеклы (BNYVV) в растениях свеклы (сорта Виорика и Ардан), отобранных с полей в Жетысуском районе. Жетысуйский район признан крупным центром выращивания сахарной свеклы в Казахстане. Вирус был обнаружен с использованием коммерческого набора для ПЦР в реальном времени (Letgen), который идентифицировал патотипы A, B и P. Было подтверждено наличие только патотипа A.

ТҮЙІН

Ризомания – қант қызылшасы дақылдарына әсер ететін, тамырлардың ісінуі, некроз және тамырлардың сарғаюымен сипатталатын жойқын ауру. Қант қызылшасында ризоманияны қоздыратын негізгі қоздырғыш – Plasmodiophorales отрядына жататын бір жасушалы паразиттік Polymyxa betae организмі тарататын қызылша некротикалық сары тамыр вирусы (BNYVV). Ауру топырақтың жоғары температурасы мен ылғалдылығы жағдайында жоғары вируленттілікті көрсетеді, ал салыстырмалы түрде құрғақ топырақ оның әсерін азайтады. Ризоманияның деструктивті әсері тамырлардың салмағының айтарлықтай төмендеуімен көрінеді: жұқтырған тамырлардың салмағы сауға қарағанда 10–15 есе аз. Сонымен қатар, ризоманиямен зақымданған тамырларда қант мөлшері мен қант шығымы төмендейді, бұл шикізаттың технологиялық сапасын нашарлатады. Аурудың таралуын болдырмау үшін құрамында топырақ бар отырғызу материалын әкелу, әкету, тасымалдау және сақтау кезінде қатаң карантиндік шараларды сақтау қажет. Сонымен қатар, аурумен күресу үшін ризоманияға төзімді қант қызылшасының будандарын енгізу қажет. Осылайша, вирустық штамдар мен иесінің төзімділік механизмдерінің өзара әрекеттесуін түсіну осы ауыр ауру жағдайында қант қызылшасының өнімділігін бақылау және сақтаудың тиімді стратегияларын әзірлеу үшін өте маңызды.

Бұл зерттеуде Жетісу өңіріндегі егістік алқаптарынан жиналған қызылша өсімдіктерінде (Виорика және Ардан сорттары) қызылша Некротикалық Сары Вена Вирусының (BNYVV) болуын растадық. Жетісу ауданы Қазақстандағы қант қызылшасын өсіретін ірі орталық ретінде танылды. Вирус A, B және P патотиптерін анықтайтын коммерциялық нақты уақыт режиміндегі ПТР жинағы (Letgen) арқылы анықталды. Тек A патотипі бар екендігі расталды.

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