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PCR DIAGNOSTIC OF THE BEAN YELLOW MOSAIC VIRUS

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Nucleotide sequences of various isolates of the bean yellow mosaic virus (BYMV) analysed. It is showing conserved nucleotide sequence of the part of gene encoding the coat protein and developed design primers for the BYMV identification. Developed a polymerase chain reaction (PCR) diagnostic test-system for BYMV detection.

Bean yellow mosaic virus, polymerase chain reaction, diagnostic.

Bean yellow mosaic virus is a member of the *Potyviridae* genera, genus *Potyvirus.* Virions filamentous, not enveloped, usually flexuous with a clear modal length of 750 nm and 12-15 nm wide [5].

Symptoms of BYMV on plants are very diverse and are largely dependent on the variety and environmental conditions. The main feature is the mosaic, green and pale green spots on leaves [6, 9].

BYMV is distributed worldwide and causes disease of many legumes and ornamentals [4]. Today the virus is believed to be distributed worldwide. BYMV is known to infect *Vicia faba*, several species of clover (*Trifolium hybridum*, *T. vesiculosum*, *T. incarnatum*, *T. pratense*, *T. repens*, *T. subterraneum*), alfalfa (*Medicago sativa*), vetch (*Vicia sativa*), lupine (*Lupinus luteus*). Compared to other viruses of the genera *Potyviridae*, BYMV has a wide range of host plants. Transmission of the virus from plant to plant occurs in different ways: through insects, seeds, grafting and contact [7, 8].

For early detection of viral diseases and for developing the protective measures is necessary to determine the causes of their illness, identify factors that affect the plant. The studies of this problem are very limited and require further development. It is important to develop diagnostic methods to identify of BYMV.

The aim of the study is to develop a diagnostic test-system for the detection of bean yellow mosaic virus with polymerase chain reaction (PCR).

Research materials and methods. To search for nucleotide sequences of various isolates of the bean yellow mosaic virus was used a database of NCBI (National Center for Biotechnological Information) [3]. Alignment of

nucleotide sequences was performed using the software «MultAline» (Multiple sequence alignment) [2]. Design of primers developed using software «Primer3» [1].

Extraction of RNA was performed using a commercial kit "RIBO-sorb» (AmpliSens, Russia), reverse transcription reaction was performed using a commercial kit "Reverta-L-100» (AmpliSens, Russia), according to manufacturer's recommendations.

The reaction mixture for the polymerase chain reaction, a volume of 15 µl containing: 1 × PCR buffer with 1.5 mM $MgCl_2$ (Amplisens, Russia), 0.2 mM deoxynucleoside triphosphates (dNTPs) (Amplisens, Rossio), 10-40 ng of cDNA, 0,5 units *Taq* polymerase (Amplisens, Russia). All primers were used at final concentration of 1 pmol per 15 µl in PCR reaction. Cycling conditions consisted of an initial denaturation step at 94 C^0 for 10 min, followed by 30 cycles at 94 C^0 for 30 s, 60 C^0 for 30 s and 72 C^0 for 30 s followed by a final elongation step at 72 C^0 for 7

min in an Thermocyclers "Tertsyk" TP4-PCR-01. PCR products were separated by electrophoresis in 1.5% agarose gels and visualized under an ultraviolet light after staining with ethidium bromide.

The research results. The nucleotide sequence of the BYMV genome for analysis were collected using genetic database of NCBI. It was carried out an alignment of the nucleotide sequences of parts of the gene encoding coat protein BYMV. Based on the analysis, consensus nucleotide sequence was constructed (Figure 1). It is showing conservative (uppercase letters) and polymorphic (small letters) fragments of the gene encoding a virus coat protein. Strictly conserved fragments were selected for further primers design. Polymorphic sites of the genome were excluded from the analysis.

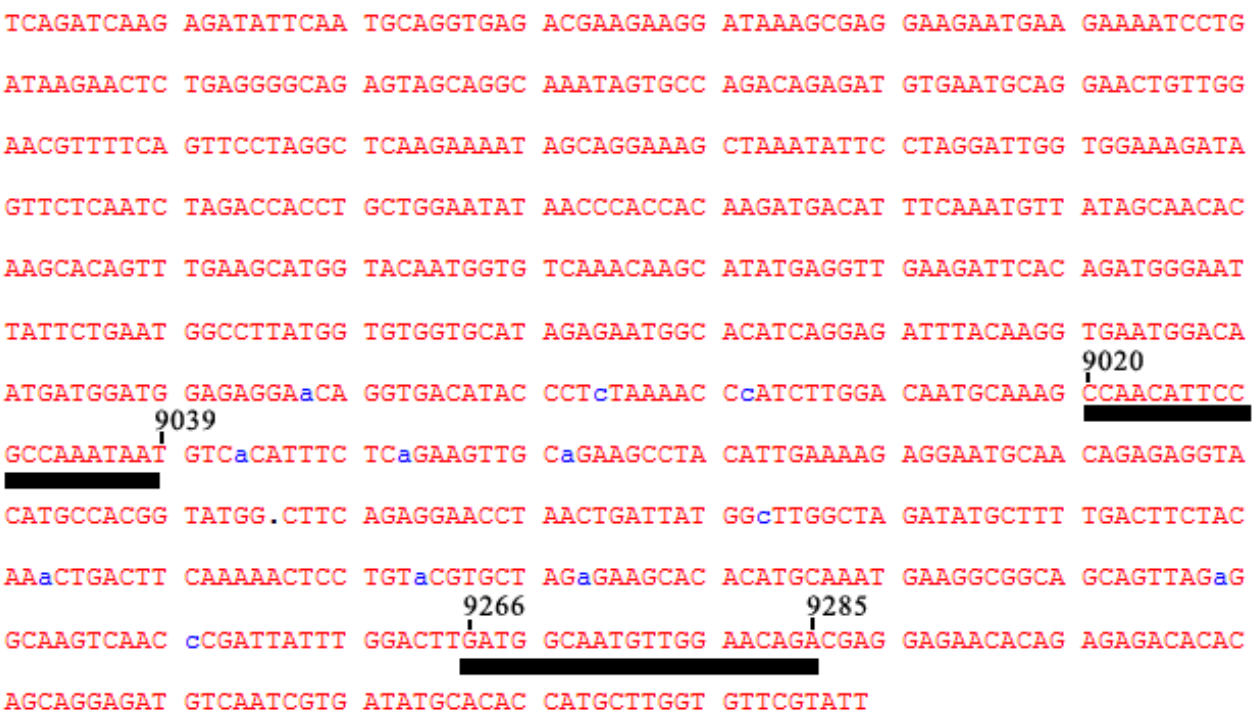

Figure 1. Location of BYMV primers hybridization sites to the DNA consensus of coat protein encoding sequence.

It was carried out a primers design with optimal performance. For synthesis was chosen primers: Forward 5'-CCAACATTCCGCCAAATAAT-3 'and Reverse 5'-TCTGTTCCAACATTGCCATC-3' with the names of BYMV-F and BYMV-R. The calculated optimum temperature for annealing is 59.66 ^{0}C forward primer and reverse primer for 59.50 ^{0}C. Percentage deoxyadenosine-5'-phosphate and deoxycytidine-5'-phosphate is 40% of forward primer and 45% of reverse primer. Selected primers did not form hairpin formation and was not self-annealing.

Molecular characterization of primers for BYMV identification

Position on the matrix	The nucleotide sequence of the 5'-3 '	Number of nucleotides	Annealing temperature, ^{0}C	GC-composition, %	Size of product, b.p.
Forward	ccaacattccgccaaataat	20	59,66	40,0	266
Reverse	tctgttccaacattgccatc	20	59,50	45,0	

Primer BYMV-F cDNA is annealing on the genome matrix in 9020-9039 n position and primer BYMV-R on 9266-9285n position respectively. The expected size of the amplification product was 266 base pairs.

Plants of beans (Phaséolus vulgáris) with BYMV symptoms was collected from Kiev region. Leafs of infected plants was used for RNA extraction. PCR was performed using cDNA Ukrainian isolate of BYMV (Figure 2).

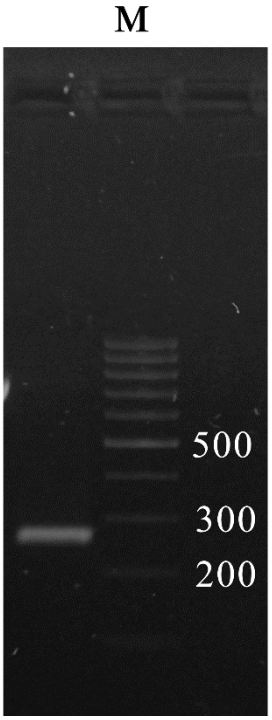

Figure 2. PCR analysis of Ukrainian isolate of BYMV (GeneRuler 10 bp DNA Lader 0241) - marker lengths of fragments (base pairs)

BYMV was detected in leafs with symptoms amplifying viral genome regions between nucleotides 9020-9285. It was showing amplifying PCR product with expected size (266 b.p.) on electrophoresis.

Conclusions

BYMV diagnostic and identification PCR test-system was developed. It was showing an effectiveness of developed test-system. It is proposed to use PCR as a highly sensitive method for laboratory BYMV diagnosis and identification.

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Проведено біоінформативний аналіз нуклеотидних послідовностей ізолятів віруса жовтої мозаїки квасолі (ВЖМК). Показано консервативні послідовності ділянки гену, що кодує білок оболонки та розроблено дизайн праймерів для ідентифікації ВЖМК. Розроблено діагностичну тест-систему на основі полімеразної ланцюгової реакції (ПЛР) для виявлення віруса жовтої мозаїки квасолі.

Вірус жовтої мозаїки квасолі, полімеразна ланцюгова реакція, діагностичні тест-системи.

Проведено биоинформативний анализ нуклеотидных последовательностей изолятов вируса желтой мозаики фасоли (ВЖМФ). Показано консервативные последовательности участка гена, кодирующего белок оболочки и разработан дизайн праймеров для идентификации ВЖМФ. Разработан диагностическую тест-систему на основе полимеразной цепной реакции (ПЦР) для выявления вируса желтой мозаики фасоли.

Вирус желтой мозаики фасоли, полимеразная цепная реакция, диагностические тест-системы.