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Development of molecular-biological system to identify RNA-4 of Ukrainian isolate of the virus of necrotizing yellowing of veins of beet

Goal. To develop a system for the identification of RNA-4 Ukrainian isolates of the necrotic yellowing virus of beetroot veins (VNPZHB) on the basis of the polymerase chain reaction. Methods. Molecular-biological. Results A bioinformative analysis of nucleotide sequences of RNK-4 isolates of VNPZHB was carried out. Conservative sequences of the gene encoding protein R31 VNPZHB were established, and primer design was developed for the identification of RNA-4. The PLR-system of identification of RNA-4 has been optimized for the temperature indices of the annealing of primers. Conclusions The developed diagnostic system allows timely identification of VNPZHB to prevent its spread.

Key words: virus necrotic yellowing of beetroot veins, protein, RNA.

The virus of necrotic yellowing of the veins of a buroc causing a disease of rhizomania, which significantly reduces the yield of beet sugar, losses can reach 80%. The best way to control the use of hybrid-resistant rhizomes and timely diagnosis to prevent the spread of the virus [1]. The genome of VNPZHB is multiparty, represented by RNA-1, RNA-2, RNA-3, RNA-4, in some isolates, there is RNA-5 [7]. RNA-4 in length 1431 nucleotides contains protein P31, which is a suppressor of the function of silencing genes [2]. RNA-4 is not important for replication of the virus in VNPZHB in plants of Nicotiana benthamiana, but plays an important role in the formation of symptoms. In the presence of RNA-4 in the HPV genome, Jetragonia expansa leaves form bright chlorotic lesions, in the absence of RNA-4, weak necroses [5, 7, 9, 11]. Protein R31 is not required for the transmission of HBVB with Polymyxa betae vector [10]. RNA-4 also encodes protein P6.5, whose functions are not defined [6]. The purpose of the research is to develop a system for the identification of RNA-4 VNPZHB based on a polymerase chain reaction. Research methodology. The NCBI (National Center for Biotechnological Information) [4] database was used to find the nucleotide sequences of RNA-4 and conduct a bioinformation analysis. Bioinformation analysis of genomes was carried out using the software "MultAline" (Multiple sequence alignment) [3]. The design of primers was developed using the software "Primer3" [8]. RNA extraction was performed using the commercial kit "RIBO-Sorb" (AmpliSens, Russia), the reverse transcription reaction using the commercial kit "Reverta-L-IOO" (AmpliSens, Russia) according to the manufacturer's recommendations. The reaction mixture for polymerase chain reaction (PCR) in a volume of 15 l contained: 1x PCR buffer with 1.5 mM MgCl2 (AmpliSens, Russia), 0.2 mM deoxynucleoside triphosphate (dNTF) (AmpliSens, Russia), 5 pmol each of the oligonucleotide primers, 10 -40 ng cDNA, 0.5 U Taq polymerase (AmpliSens, Russia). The amplification reaction was carried out in a DNA polymerase "Tercyk" TP4-PCR-01. After amplification, PCR products were separated by horizontal electrophoresis in a 1.5% agarose gel, which was prepared using a TBE buffer at a concentration of 0.5 mg / ml of ethidium bromide. The results of the PCR were visualized in transilluminator UV rays (T-312-C), photographed with a digital camera "Sony" (DSLR-A500).

Fig. 1. Localization of sites of hybridization of primers on DNA of the matrix of the consensus sequence of the gene R31 VNPZHB

Research results. To create the design of primers specific for the nu-kloloid sequences of the genomic VNPZHB, they conducted a bioinformation analysis, the first stage of which was the screening of the conservative sequences of the P31 gene using data from the genetic database (GenBank). On the

basis of generalized data on known nucleotide sequences of genomes, specific conserved nucleotide sequences of the P31 gene were found, which subsequently were used as templates for oligonucleotide digestion in the process of synthesis of a virus-specific fragment (Fig. 1). On the matrix of the consensus sequence of the P31 gene, the design of prime masters with optimal characteristics was created. For synthesis, primers with optimal GC parameters and close annealing temperature of both primers were taken: Forward 5'-GCTAGGATGGTGCAGAAACG-3 ', Reverse 5'-ATCACAAAACCTTCGCCACC-3' with the names respectively P31-F and P31-R . The calculated optimum annealing temperature was 58.99 ° C for the Forward primer, 59.04 ° C for the Reverse primer and 55% for the Forward primer for the Forward primer, and the Reverse primer for the Forward primer, the percentage of deoxyguanosine-5'-phosphate and deoxycidine-5'- 50%. Polymerase chain reaction was carried out using cDNA of Ukrainian insulator VNPZHB under the following conditions: initial denaturation of 5 min-94 ° C; 30 cycles: denaturation 30 s - 94 ° C, annealing primers 30 s - 60 ° C, elongation 30 s - 72 ° C, final synthesis 72 ° C - 7 min. Negative control was used to monitor the quality of the PCR analysis. As a result of the rendering of PCR products of amplification, fragments of 693 pairs of nucleotides were identified. The presence on the electrophoregram of the expected product of the reaction indicates that the developed system of identification of RNA-4 VNPZHB is effective (Fig. 2). At the next stage of the study, it was necessary to select the operating temperatures of the annealing of the primers. We conducted a series of reactions with different temperature regimes of annealing primers from 50 ° C to 64 ° C. PCR was performed under the following conditions: initial denaturation of 5 minutes - 94 ° C; 20 cycles: denaturation 30 s - 94 ° C, annealing primers 30 s - 50- 64 ° C, elongation 30 s - 72 ° C, final synthesis 72 ° C - 7 min. Visually, the difference between the release of the products of the amplification reaction at temperatures between 50 and 62 ° C was not observed. Under these conditions, nonspecific amplification products were not noted, and the number of amplicons was sufficient for a clear visualization in an agarose gel (Fig. 3).

Fig. 2. Electrophoregram of product of PCR analysis of determination of RNA-4 VNPZHB: 1 - product of amplification of gene P31; 2 - negative control over the conduct of PCR analysis; M (GeneRuler 10 bp DNA Lader 0241) - marker of lengths of fragments (pairs of nucleotides) Fig. 3. Electrophoregram of temperature optimization of the annealing of primers for identification of P31 gene: 1 - 50 ° C; 2 - 52oC ;, 3 - 54oC; 4 - 56oC; 5 - 58oC; 6 - 60 ° C; 7 - 62oC; 8 - 64oC; M - GeneRuler 10 bp DNA Lader 0241 (Nucleoside pairs) marker lengths

Conclusions

A bioinformatical analysis of the nucleotide sequences of RNA-4 isotypes of VNPZHB was carried out. Showing conservative gene sequence encoding a protein R31 VNPZHB and made design primers to identify RNA VNPZHB 4: Forward 5'-GCTAGGATGGTGCAGAAACG-3', Reverse 5'-ATCACAAAACCTTCGCCACC-3'. Recommended to conduct PCR analysis to identify RNA-4 VNPZHB me¬zhah temperature of 50 62oS annealing Praia mayors.

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