UDC 601.4:577.21:635.21

THE DEVELOPMENT OF MOLECULAR BIOLOGICAL SYSTEM BASED ON POLYMERASE CHAIN REACTION AND OPTIMIZATION FOR DIAGNOSTIC OF POTATO SPINDLE TUBER VIROID

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Polymerase chain reaction (PCR) diagnostic test systems for the identification of potato spindle tuber viroid has been developed. Laboratory research of created PCR diagnostic system has been carried out and the conditions of amplification reaction realization have been optimized.

Diagnostic system, PCR, potato spindle tuber viroid

Viral diseases of potatoes lead to a dramatic reduction in yield and quality of products. Potato virus among the first to have been identified as pathogens of major economic importance [1]. Because now there is no remedy against viral diseases The issue of monitoring and identification to prevent their proliferation [2]. Veretenovydnosti viroids potato (VVBK) was first described in 1922 by WH Martin in the United States. In Ukraine, first identified in 1932. VVBK belongs to the Pospiviroidae, kind Pospiviroid. Viroids presented single-circuit closed circular RNA molecule length of 356-375 nucleotides. Replication occurs in the nucleus [9].

Symptoms depend on the type of plant varieties and environmental conditions [3, 8]. On potato plants are as follows: for

aboveground plant parts are typical thin erect stems and stalks, are longer than healthy plants; smaller share leaf with wavy edges and a tendency to bend inwards; sharper angles between stems and cuttings; leaves near the ground noticeably shorter and erect, contrasting with healthy plants, leaves lying on the ground, have a dark plate [7].

For diagnosis and identification VVBK need to use a set of highly specific methods. Serological methods are not applicable for lack of viroids protein coat, or any

specific proteins and low antigenic properties of RNA [4, 9]. Using electron microscopy also is not a very effective method for diagnosing VVBK [5].

To diagnose VVBK effective methods are those where used as a target gene viroids, one of which is a method using polymerase chain reaction (PCR), thus increasing the number of even small concentrations of specific nucleic acid fragments.

The purpose of research - development kits for diagnosis of viroids veretenovydnosti potato PCR conditions and optimization of reaction amplification.

Materials and methods research. Material for analysis were leaves of potato plants with symptoms selected during the growing season.

For extraction of total RNA using commercial fish set-sorbet-B (AmpliSens, Russia). Reverse transcription reaction was carried out using a commercial set Reverta-L, (AmpliSens, Russia) according to the manufacturer's recommendations. PCR was performed in the reaction mixture volume of 15 ml. Terms PCR: initial denaturation 94 ° C - 5 min; 35 cycles of denaturation: 94 ° C - 30 s, annealing of primers 55 ° C - 30 C, the synthesis of 72 ° C - 30 C, the final synthesis of 72 ° C - 7 min. Electrophoresis was performed in 1.5% agarose gels, which were stained with ethidium bromovym concentration of 0.5 mg / ml, was investigated in the rays of ultraviolet light.

Conclusions

Developed its own test system for the diagnosis and identification veretenovydnosti viroids potato-based PCR, optimized PCR conditions and set optimal performance components and reaction temperature annealing of primers for PCR analysis.

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