

PCR diagnostics and identification of beet mosaic virus

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As a result of destruction of sugar beet plants by various kinds of viruses is a decrease of sugar content and reduced yield seeds and roots. A large number of viral diseases in sugar beet crops appear with different symptoms and different composition of pathogens and their harmfulness.

The selection of plant material was carried out by external signs of injury. The material for research were puff plates of hybrids of sugar beet plants Carmelita, Aljona, Nastya, Georgina, Lavina, Leopard selected in agroecosystems NUBiP of Ukraine «Agronomy Research Station» (Str., Pp. Wheat) with symptoms of beet mosaic virus. The symptoms of illness on plants show up in brightening of veins, mosaic in young leaves. At the sharp forms of disease leaves are deformed and twisted. There is a delay of height which leads to dwarfism plants.

Food and biological safety largely depends on accurate and continuous monitoring of phytosanitary condition of crops of sugar beets. One of the main methods of such control is highly specific and effective diagnostics and identification of phytopathogens. Thus, there was a necessity of application of molecular-biological methods of diagnostics, such as polymerase chain reaction (PCR). PCR can detect the presence of a specific nucleotide sequence of the genome in samples of plant sugar beets. Standard PCR method involves the preparation of samples, RNA extraction, reverse transcription, amplification and electrophoresis of the reaction products.

In the process of research is conducted the bioinformatic analysis of nucleotide sequences of genes that encode the protein of virus shell. Specific primers is worked out for identification of beet mosaic virus (BtMV) by the PCR method. Analyzed nucleotide sequences of the gene of membrane protein of isolates VMB which are available in international bank and synthesized primers complementary to the most conservative areas of the gene. Approbation of primers is carried out with the use of RNA, extracted from sugar beet plants with symptoms of BtMV. It is shown that application of these primers for the indication RNA virus of BtMV in samples provides the synthesis of fragments of DNA of the expected size in the conditions of RT-PCR. Conditions of amplification is chosen. After The PCR was finished, electrophoretic separation of the amplification products was made by 1.5% agarose gel. The specificity of the amplified DNA fragment was determined by its size relative to the standard marker fragments. Visualization of amplification products was performed in UV light.

In samples hybrids Nastya, Aliona, Carmelita and Georgina size amplification product (239 bp) indicates the presence of Beet mosaic virus (BtMV). The presence of an appropriately sized products of amplification indicates the efficacy of diagnostic test kits.