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Molecular-genetic diagnostics of potato cyst-creating nematodes which are spread in zones of Polisya, Forest-Steppe and Ukrainian Carpathians

Aim. To define specific composition of quarantine organisms — potato Cyst-forming eelworms, widespread in the zone of Polesye, Forest-steppe and Carpathians of Ukraine. Methods. Field (rout inspections), laboratory (extraction of eelworms is from the ground tests; authentication of eelworms is for the use of the multiplexed polymerase chainreaction with specific primersComplementary to the ribosomal gene 18S and internal Transcribed to the spacerof ITS1). Results. For the use of the multiplexed polymerase chain reaction (PCR) with specific primersdistribution of Globodera of rostochiensis is set within the limits of Ternopil (general area of the infected lands — 375,02 hectare), Zakarpattia (16,75 hectare) Chernivtsi (0,61 hectare) areas of Ukraine. A location of quarantine hearths in swingeing majority is on homestead lands of population. Conclusions. Expediency of further Validationmethod is witnessed for the use of more wide panel of standards of cyst-formingeelworms from the different regions of Ukraine. The got results are fixed in basis of recommendations in relation to realization of phytosanitarycontrol within the limits of the infected territories for effective localization and liquidation of found out quarantine hearths.

Keywords: potato цистоутворювальні eelworms, molecular diagnostics, multiplexed полімеразно-ланцюгова reaction, distribution of eelworms.

Diagnosticating of eelworms on morphological and morphometric signs is a labour intensive enough process through the microscopic sizes of these organisms and morphological similarity of family kinds. Therefore in the quarantine laboratories of the world during realization of Phytogelminthologicaanalysis all anymore use the methods of molecular-genetic diagnostics [13]. It gives an opportunity to increase diagnostic possibility of quarantine laboratories and in time to make decision in relation to the input of corresponding measures of
phytosanitary control [3].

On the initial stage of introduction of molecular-genetic methods in nematology practice current was a selection of genome DNA of eelworms, treatment and hybridization her restriction enzymes , with certain probes. Because of it got the excellent profiles of
Restrictive fragments, after that carried out diagnostics. This method required the far of DNA of material, great while on his realization, use of radioactive mark, and also characterized by subzero specificity and was based on not high polymorphism of genetic material [2]. In course of time wider in all during realization of Phytogelminthologicaanalysis in the quarantine laboratories of the world began to apply such varieties of Polymerase chainreaction, in particular «classic» Polymerase chain reaction with the

use of species-specific праймерів (B. xylophilus [5], Globodera of rostochiensis, Globodera of pallida [4], Meloidogyne of chitwoodi, Meloidogyne of fallax, Meloidogyne of enterlobii [14], Nacobbus of aberrans [1], Ditylenchus of dipsaci [12]; Ditylenchus of destructor [8], B. xylophilus [10], H. glycines, H. avenae [11]).

Aim of researches — to define specific composition of potato cyst-formingeelworms, widespread in the zone of Polesye, Forest-steppe and Carpathians of Ukraine for the use of multiplexed PCR with specific Primers are complementaryto the ribosomal gene 18S and internal Transcribed to the spacerof ITS1.

Materials and methods of researches. The standards of soil, selected during the rout inspections of agricultural lands and homestead lands on territory of 3th areas, were used: Ternopil (Ternopil, Berezhany, Zbarazh, Buchatsky, Zborivsky and Pidgaitsey districts), Chernivtsi (Putilsky) and Zakarpattia (Intermountain, Rakhiv, Velyky Bereznyanskydistricts).

For a further analysis cysts of eelworms distinguished from soil with the use of the improved method of Fenwick[6]. Extraction of DNA was conducted from 10-25 cystsfor drawing on the set of reagents of Diatom™ DNA Prep100 (NeoGene) in accordance with the protocol worked out by a company-producer.

Multiplexed PCR carried out for the use of reagents of GenPak® PCR Core (NeoGene) and PrimersComplementaryto the ribosomal gene 18S and internal transcribed to the spacerof ITS1 : direct universal праймер 18S — UNI [7] but reverse species-specific Primersof PITSp4 (G. pallida) and PITSr3 (G. rostochiensis)[4].

The general volume of reactionary mixture presented 10 mkl; PCR was a reaction carried out on amplifier 2720 GeneAMP System after such protocol: initial denaturation (94-C) — 3 min, 35 cycles of amplification : denaturation 94°C — 30 c, annealing of праймерів 55°C — 30 c, lengthening 72°C — 30 c; eventual lengthening — 72°C — 5 хв; table of contents — 4°C.

Foods of amplification divided by means of electrophoresis in 2% th agarosegael in a 1X TBE- Buffer with addition to the етидій bromide. Visualization of fragments was conducted in the ultraviolet light. For determination lengths of fragments used the markers (Set of 100 bp + 1,5 Kb DNA Ladder with of stain company SibEnzyme) of DNA.

Positive control eelworms served as from the populations of G. rostochiensis of Ro1 of collection of laboratory of nematologyInstitute of defence of plants of NAAS. The result of PCR was interpreted as positive, if in an analysable standard found out Ampliconof G. by rostochiensis length of 423 п.н. and G. by pallida length of 254 п.н.

Results of researches. For the use of multiplexed ПЛР in tests from the 3th areas of country (Ternopil, Zakarpattia, Chernivtsi) discovered the амплікон size of 423 п.н., characteristic for G. rostochiensis

Presence in a Agarosegael on occasion Loopa fragments of DNA (see drawing, line 8) testifies about expedience of further Validationof method with the use of more wide panel of standards of Cyst-formingeelworms from the different regions of Ukraine. It will give an opportunity more precisely to define the degree of analytical specificity of method that represents him quality side, in particular possibility to distinguish in standards the credible closely-related types of eelworms, polymorphism of that can substantially differ after a select genetic marker.

By previous researches efficiency of the use of method was already well-proven for authentication of 60 populations of potato cyst-formingeelworms from the 13 areas of country [9], other types of cyst-formingeelworms to the chart of researches are not entered.

With the aim of validationmethod it is expedient to carry out also further researches with the use of other types of cyst-forming eelworms.

The conducted researches confirmed the fact of distribution of G. rostochiensis within the limits of the Ternopil, Zakarpattia and Chernivtsi areas, that answers data of Government veterinary and Phytosanitaryservice of Ukraine.

Presently from the marked areas most area of the lands populated by a yellow-green potato eelworm, register in the Ternopil area — 375,02 hectare (SA the «Ternopil regional phytosanitary laboratory»). Within the limits of 2th other areas of hearth of G. rostochiensis it was discovered on homestead lands on areas 16,75 hectares (Zakarpattia) and 0,61 hectare (Chernivtsi to the area).

The got results were fixed in basis of recommendations in relation to realization of phytosanitarycontrol within the limits of the infected territories for effective localization and liquidation of found out quarantine hearths.

Conclusions

For the use of the multiplexed polymerase chain reactionwith specific primers are complementaryto the ribosomal gene 18S and internal transcribed to the spacerof ITS1, distribution of G is set. rostochiensis within the limits of the Ternopil, Chernivtsi and Zakarpattia areas. A location of quarantine hearths in swingeing majority is on homestead lands of population. Expediency of further validationmethod is confirmed for the use of more wide panel of standards of cyst-formingeelworms from the different regions of Ukraine.

Results of multiplexed species-specific PCR from authentication of potato cyst-forming eelworms : 1. Marker of the molecular masses — Set of of 100 bp + 1,5 Kb DNA Ladder with of stain company SibEnzyme. 2. G. rostochiensis of Ro 1, positive control. 3. Negative control; 4. G. rostochiensis, Chernivtsi region, Putilsky district, v. Porculaine . 5. G. rostochiensis, Zakarpattia region, Intermountain area, v. Maidan . 6. G. rostochiensis, Zakarpattia region, Rakhivskyare, v. Surupi. 7. G. rostochiensis, Zakarpattia region, Rakhivsky are, v. of Yasinya . 8. G. rostochiensis, Zakarpattia region, Veliko-Berezny district, v. of Zhornava.

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