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MOLECULAR AND MORPHOLOGICAL DETECTION OF GLOBODERA ROSTOCHIENSIS (NEMATODA: HETERODERIDAE) IN A SEED POTATO CROP

Violeta ORO[*], Rade STANISAVLJEVIĆ[*], Marijenka TABAKOVIĆ[**], Dragoslav ĐOKIĆ[***]
**Institute for Plant Protection and Environment, 11000 Belgrade, Teodora Drajzera 9, Serbia*
***Maize Research Institute „Zemun Polje“, 11185 Belgrade-Zemun, Slobodana Bajića 1, Serbia*
****University of Nis, Faculty of Agriculture in Krusevac, 37000 Kruševac, Kosančićeva 4, Serbia*
Contact: viooro@yahoo.com

Potato (Solanum tuberosum L.) is one of four major food crops in the world beside wheat, maize and rice. The plant originated in the highlands of Peru particularly the region around Lake Titicaca and it was first domesticated at least 7 000 years ago. The food security provided by potato and maize allowed the development and survival of civilizations such as, Huari and Inca for centuries. In the 16th century the Spanish conquistadores searching for the "treasure of the Andes" brought to Europe, beside gold, potato along with its parasites – the potato cyst nematodes (PCN): Globodera rostochiensis (Wollenweber) Behrens and G. pallida (Stone) Behrens, two nematode species that have quarantine status. The morphology of potato cyst nematodes was until recently almost the only way to identify these quarantine organisms. In the last two decades, molecular analyses as new trends in modern agriculture, contributed to faster and more efficient identification of these species and allowed insight into the genetic structure of those parts that were practically inaccessible by morphological studies. The nematodes are present in all European potato growing regions, especially in the Balkan (Helm) peninsula, either PCN or both are reported.

The collected specimens of cysts were found in soil originating from a seed potato crop in a village near Gornji Milanovac after the official phytosanitary control in 2022. Individual cysts were used for DNA extraction with a Dneasy blood & tissue kit. The PCR was done with primers for direct sequencing: TW81 and AB28. The ITS1-5.8S-ITS2 region of PCN is used for confirmation of species identity together with its morphological characterization. According to EPPO Standards, the morphological identification comprised larval and cyst characteristics, namely larval stylet length and stylet knob shape, cyst vulval basin diameter, distance between vulva and anus, Granek´s ratio, and number of cuticular ridges in perineal area.

Results confirmed the species identity. The morphology of our population of G. rostochiensis was similar to the previously reported domestic and foreign populations. The degree of similarity was expressed as a percentage of direct matching i.e. pairwise distances. Phylogenetic analyses indicated a possible ancestor of our PCN population showing evolutionary relationships among world populations of G. rostochiensis and a phylogenetic placement of the Serbian population.

Key words: *PCN, phylogeny, morphology*

MOLEKULARNA I MORFOLOŠKA DETEKCIJA GLOBODERA-E ROSTOCHIENSIS (NEMATODA: HETERODERIDAE) U USEVU SEMENSKOG KROMPIRA

Violeta ORO[*], Rade STANISAVLJEVIĆ[*], Marijenka TABAKOVIĆ[**], Dragoslav ĐOKIĆ[***]
**Institut za zaštitu bilja i životnu sredinu, 11000 Beograd, Teodora Drajzera 9, Srbija*
***Institut za kukuruz „Zemun Polje“, 11185 Beograd-Zemun, Slobodana Bajića 1, Srbija*
****Univerzitet u Nišu, Poljoprivredni fakultet u Kruševcu, 37000 Kruševac, Kosančićeva 4, Srbija*
Contact: viooro@yahoo.com

Krompir (*Solanum tuberosum* L.) je jedan od četiri glavna prehrambena useva u svetu pored pšenice, kukuruza i pirinča. Biljka potiče iz planinskog pojasa Perua, posebno iz regiona oko jezera Titikaka i prvi put je odomaćena pre najmanje 7 000 godina. Sigurnost u hrani koja je nastala gajenjem krompira i kukuruza je omogućila vekovni razvoj i opstanak civilizacija kao što su Huari i Inke. U 16. veku su španski osvajači tražeći "blago sa Anda" pored zlata, u Evropu doneli i krompir zajedno sa parazitnim nematodama-cistolikim nematodama krompira (CNK): *Globodera rostochiensis* (Wollenweber) Behrens i *G. pallida* (Stone) Behrens, dve nematodne vrste koje imaju karantinski status.
Morfologija cistolikih nematoda krompira je do skoro bila jedini način identifikacije ovih karantinskih organizama. U poslednje dve decenije, molekularne analize kao novi trendovi u modernoj poljoprivredi, su doprineli bržoj i efikasnijoj identifikaciji ovih vrsta, omogućavajaći uvid u genetičku strukturu onih delova koji su praktično bili nedostupni morfološkim studijama. Nematode su prisutne u svim evropskim regionima gajenja krompira, posebno na Balkanskom (Humskom) poluostrvu, pojedinačno ili obe zajedno.
Prikupljeni uzorci cista su pronađeni u zemlji poreklom iz useva semenskog krompira u selu pored Gornjeg Milanovca, posle zvanične fitosanitarne kontrole. Pojedinačne ciste su korišćene za ekstrakciju DNK sa Dneasy blood & tissue kitom. PCR je urađen sa prajmerima za direktno sekvenciranje: TW81 and AB28. ITS1-5.8S-ITS2 regioni CNK su korišćeni za potvrdu identiteta vrste, zajedno sa morfološkom karakterizacijom. Prema EPPO Standardu, morfološka identifikacija obuhvata karakteristike larvi i cisti, zapravo dužinu i oblik stileta larvi, prečnik vulvalnog bazena cisti, distancu između vulve i anusa, Granekov odnos i broj kutikularnih nabora u perianalnoj oblasti.
Rezultati su potvrdili identitet vrste. Morfologija naše populacije *G. rostochiensis* je bila slična prethodno opisanim domaćim i stranim populacijama. Stepen sličnosti je predstavljen i kao procenat direktnog sparivanja, tj. parne distance. Filogenetske analize su ukazale na mogućeg pretka naše populacije CNK, predstavljajući evolutivne odnose svetskih populacija *G. rostochiensis* i filogenetsko mesto srpske populacije.
Ključne reči: *CNK, filogenija, morfologija*