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Research Article

A Study on the Presence of Potato Cyst Nematodes: *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 and *Globodera pallida* Stone 1973 (Tylenchida: Heteroderidae) in Nevşehir Province, Turkey

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Abstract

Potato (*Solanum tuberosum* L.) cyst nematodes (*Globodera rostochiensis* (Wollenweber) and *Globodera pallida* (Stone) cause significant yield losses and have been listed as quarantine pests for many countries in the world including Turkey, and the European and Mediterranean Plant Protection Organization (EPPO, A2). In Turkey, the current potato production area is decreasing due to contamination by quarantine agents, and new fields are under threat of invasion by new quarantine organisms. In this study, samples were collected from Nevşehir province, Turkey, in potato growing fields during the spring growing season in 2014-2015. Soil samples were washed using Fenwick's can and cysts were extracted and identified using molecular markers. The species-specific molecular markers generated a 435 bp using PITSr3, PITSp4 and ITS5 primers. The potato golden cyst nematode, *G. rostochiensis* was identified in Nevşehir potato production areas and this is the first report for the Central Anatolia Region. In addition, G. rostochiensis was found in two samples of the same field in the surveys. The average cyst nematode density was determined as 60 cysts in 250 g soil.

Key words: Potato, cyst nematode, molecular identification, density.

Nevşehir İlinde Patates Kist Nematodlarının *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 ve *Globodera pallida* Stone 1973 (Tylenchida: Heteroderidae) Belirlenmesi

Özet

Patates (*Solanum tuberosum* L.) kist nematodları (*Globodera rostochiensis* (Wollenweber) ve *Globodera pallida* (Stone) önemli verim kayıplarına neden olmakta, Türkiye de dahil olmak üzere dünyadaki birçok ülke, Avrupa ve Akdeniz Bitki Koruma Örgütü'nde (EPPO, A2) karantina zararlıları olarak listelenmiştir. Türkiye'de mevcut patates üretim alanı karantina zararlısı olarak bulaşmaya bağlı olarak azalmakta ve yeni alanlar yeni karantina organizmaları tarafından istila tehdidi altında bulunmaktadır. Bu çalışmada, Türkiye, Nevşehir ilinden 2014-2015 yıllarında bahar mevsiminde patates yetiştirme alanlarında örnekler toplanmıştır. Toprak örnekleri Fenwick aletinde yıkanarak bulaşık örneklerdeki kistler elde edilmiş ve kist popülasyonlarının moleküler olarak teşhisleri yapılmıştır. Kist popülasyonlarının moleküler düzeyde türe özgü PITSr3, PITSp4 ve ITS5 primerleri kullanılarak yapılan teşhiste söz konusu primerlerin 435 bp de bant oluşturduğu tespit edilmiştir. Bu çalışma sonucunda Nevşehir ili patates ekiliş alanlarında patates kist nematodu *G. rostochiensis* saptanmış olup, bu sonuç İç Anadolu Bölgesi için ilk kayıt olma özelliğini taşımaktadır. Ayrıca, sürveylerde aynı tarlaya ait iki örnekte *G. rostochiensis'e* rastlanılmış, 250 g toprakta ortalama 60 kist yoğunluğunda olduğu tespit edilmiştir.

Anahtar kelimeler: Patates, kist nematodu, moleküler teşhis, yoğunluk.

Introduction

Potato (*Solanum tuberosum* L.) is one of the most important food and industrial crops. Due to its high adaptability in different ecological conditions, it is produced in many regions of the world and is a key component in the human diet as a rich source of carbohydrates, proteins, vitamins (C, B1, B3, B6, K) and minerals. For this reason, it is produced worldwide on 19 million ha at the global production of 368 million tons/year. It is one of the most important crops and its production rate comes only after maize, rice and wheat (FAOSTAT, 2013) all over the world. Turkey is among the leading potato-producing countries worldwide with a 125.030 ha production area, and a 3.948.000 kg production quantity. Around 60% of potato production in Turkey is supplied from Niğde, Nevşehir, İzmir, Bolu and Afyon provinces, respectively. Only the Nevşehir province supplies 320 000 tons/year (TUIK, 2012) but it has been reduced due to a severe infection by *Synchytrium endobioticum* (Schilbersky) Percival and root knot nematode (*Meloidogyne chitwoodi* Golden) which have extended in the Nevşehir province during the recent years (Özarslandan et al., 2009). In addition, potato production has started in other provinces; particularly, seed potato production is seen in the provinces of Konya, Kayseri and Sivas.

Potato reproduction is made by mainly as vegetative propagation, thus it facilitates the soil borne plant pests (insects and nematodes), pathogens (bacteria, viruses and fungi) and weeds can spread out into new areas. The most important plant-parasitic nematodes in potato, such as potato rot nematode (*Ditylenchus destructor* Thorne), potato cyst nematodes (*Globodera rostochiensis* (Wollenweber), *Globodera pallida* (Stone)), root-knot nematodes (*Meloidogyne chitwoodi*), the stem and bulb eelworm (*Ditylenchus dipsaci* Filipjev), false eelworm (*Neotylenchus vigissi* (Skarbilovich) Goodey), false root-knot nematode (*Nacobbus aberrans* (Thorne) and root lesion nematodes (*Pratylenchus* spp.) have also been reported by previous researchers (Winslow and Willis, 1972; Jatala, 1978; Jensen, 1978; Mendoza and Jatala, 1978; Evans and Trudgill, 1992).

Potato Cyst Nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are important species causing huge damage to potato crop (Winslow and Willis, 1972; Jatala, 1978; Jensen, 1978; Mendoza and Jalata, 1978; Evans and Trudgill, 1992). The PCN causes to 9% crop losses of UK potato production and cause noteworthy damage to potatoes in Scandinavia (Holgado and Magnusson, 2012). The potato cyst nematodes were firstly detected in the Andes, then they were reported in Europa, Asia (Israel and India), North Africa, Canary Islands, and western America (Winslow and Willis, 1972). Nowadays, potato cyst nematodes have been placed in the quarantine list for many countries including Turkey by European and Mediterranean Plant Protection Organisation (EPPO). Cyst nematodes cause weakness, wilting, yellowing, stunting and drying of the potato plant (Turner and Evans, 1998; EPPO, 2004). The decline in production rate caused by PCN is estimated to be more than 12% per year (Bates et al., 2002). The yield losses can reach up to 70% in Mexico in some years (Tovar et al., 2006).

The morphological identification of potato cyst nematodes is difficult because species characteristics are very similar to each other (EPPO, 2004). For this reason, molecular identification techniques have been developed and species-specific primers have been used (Bulman and Marshall, 1997; Fullaondo et al., 1999; Vejl et al., 2002; Powers, 2004; Madani et al., 2008). The species-specific primers were also used in Turkey to identify the *G. rostochiensis* (Ulutas et al., 2012).

This study was conducted on Nevşehir province, which is one of the most important potato production areas in Turkey. In this study, we carried out the molecular identification of potato cyst nematodes and estimated the nematode population density in soil.

Material and Methods

The survey of Globodera spp. and collection of cysts

Quarantine surveys were conducted in 2014-2015 with the help of Provincial Directorate for Food Agriculture and Livestock, Nevşehir, Turkey. Sampling was performed according to Erdogan et al (2011). The soil samples from 50 different fields (locations) were collected in Nevşehir province. The 60 different samples (60 samples/ha) were collected from each field and they were combined and mixed. The 250 g soil was taken from the combined mix into polyethylene bags and total 50 samples were brought to the nematology laboratory. Samples were washed in Fenwick cans to collect cysts, then extracted cysts were counted under the binocular microscope. This process was conducted with 4 repetitions and average values were calculated. Molecular identification of *Globodera* spp.

DNA isolation

The molecular identification was performed from collected cysts and 13 repetitions for each sample were made. DNA isolation was achieved from a single cyst according to the description of

Waeyenberge et al. (2000). In this method, cysts were crashed, then, the cysts were placed in the double distilled H_2O (0.2 mL) PCR tube and 25 µL Worm Lysis Buffer [WLB] (950 µL Lysis Buffer WLB (-) +10 µL beta-mercaptoethanol + 40 µl (20mg/ml Proteinase K, Sigma) was added. The lysis buffer was incubated at 65ºC an hour and proteinase K inactivation was performed at 95ºC in10 minutes and finally DNA samples were stored at –20ºC until use.

İdentification

The species-specific primers (PITSr3 5'AGCGCAGACATGCCGCAA-3', PITSp4 5'ACAACAGCAATCGTCGAG-3') were used to identify the *Globodera* spp. (Bulman and Marshall, 1997) and ITS5 5' GGAAGTAAAAGTCGTAACAAGG-3 (White et al., 1990) combinations were used to distinguish *G. rostochiensis* and *G. pallida*. Polymerase Chain Reaction (PCR) reagents consisted of: 5 ng μL^{-1} DNA, 2.5 µL PCR buffer solution, 2 mM $MgCl_2$, 200 µM dNTP, 0.4 µM 2 µl primer (ITS5 and PITSp4 *G. pallida* specific, ITS5 and PITSr3 *G. rostochiensis* specific), and 1 unit *Taq* DNA polymerase and ddH_2O and total volume 25 µl. Due to lacking of positive control, no negative control was performed.

PCR reactions were performed as follows: 94ºC 3 min, followed by 35 cycles of 94ºC 1 min, 55ºC 1 min, 72ºC 1 min. After completion of PCR products, they were analyzed by electrophoresis on agarose (1.5%) gels. Visual analysis was made after the gels were stained with ethidium bromide on a UV transilluminator.

Results and Discussion

Determining the population density of Globodera rostochiensis

The golden cyst nematode *G. rostochiensis* cysts were identified from two samples in Nevşehir. The presence of this species is very important for potato growing areas in this region. Potato cyst nematodes can easily spread to other areas by seed tubers, tillage tools and various other spreading factors. Two locations were found to be infested with cyst nematodes and cyst population density was determined as 60 cysts by 250 g of soil (Figure 1). Potato cyst nematodes cause economic losses to potato crop with the presence of 10 eggs/gram soil (Phillips et al., 1991). It has been reported that the viable cyst number reached up to 63 cysts/100 g soil during 4 years of consecutive potato production and at the fifth year the cyst number reached 180 cysts/100 g soil (3000 cysts/plant) and a 72% crop losses ensue (Zawislak et al., 1981). In this study, the cyst number was determined as 60 cysts/250 g soil, this number is very much higher than the economic losses threshold (8 eggs/g soil). Hence, the applying of pest management strategies is necessary in nematode infested fields.

Figure 1. Potato cyst nematode cysts (a and c) and second stage juveniles (b). Arrow indicates the higher magnification of a female potato cyst nematode.

Molecular identification of Globodera spp.

Polymerase chain reaction (PCR) reaction showed that species specific primers gave a 435 bp DNA band, which represents *G. rostochiensis* (Figure 2).

White et al. (1990) and Bulman and Marshall (1997) reported that the molecular identification of *G. rostochiensis* and *G. pallida* with PITSp4 and PITSr3 specific primers was improved by ITS primer combination that gives the DNA band at 435 bp for *G. rostochiensis*. Different researchers also confirmed that the band on 435 bp corresponded to *G. rostochiensis* (Fullaondo et al., 1999; Pylypenko et al., 2005; Skantar et al., 2007, Madani et al., 2008). Similar results in Ukraine where the prevalence of *G. pallida* was 2-5% but the occurrence of *G. rostochiensis* is 95-98% (Pylypenko et al., 2005). *G. rostochiensis* was also determined using the same primer combinations in İzmir – Ödemiş, Turkey. The result of our present study shared similarities with previous studies and the

same set of primers in Izmir- Ödemiş, Turkey (Ulutas et al., 2012).

The nematode damage on potato is directly correlated with nematode density. In this regard, cyst nematodes give damage on economic threshold at 10 eggs/g soil (Phillips et al., 1991). Potato cyst nematodes cause 2 ton/ha potato loss with the presence of 20 nematode eggs/g of soil (Brown, 1969). During the continuing of potato cultivation (i.e. without crop rotation), potato cyst nematodes cause to 72% crop losses after the 4th year (Zawislak et al., 1981).

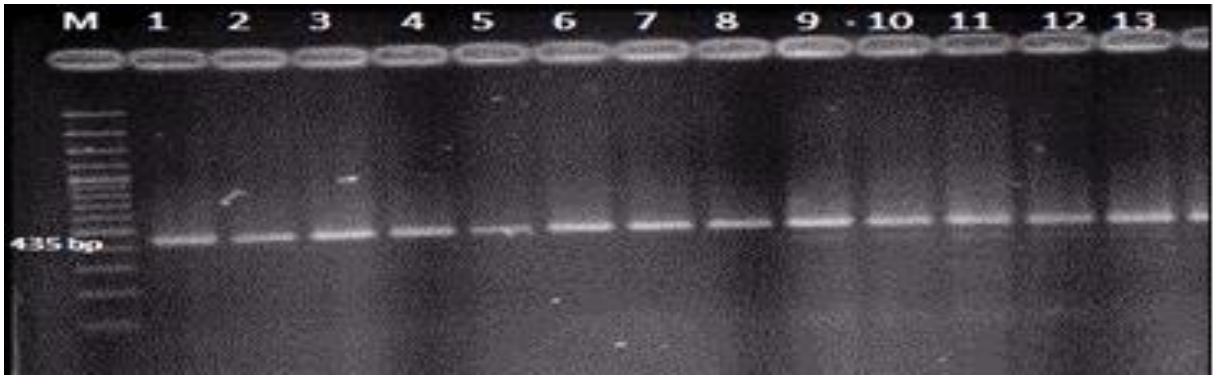

Figure 2. Molecular identification of *Globodera rostochiensis* DNA by using multiplex PCR with species-specific primers. Markers (M), 100 bp (Fermentas –Thermo, Lithuania), numbers indicate 13 repetitions in 2 locations of infested area.

As the potato cyst nematodes *G. pallida* and *G. rostochiensis* are considered important quarantine organisms for European and Mediterranean Plant Protection Organisation (EPPO, 2004). Turkey must take measures to avoid the dissemination of PCN because does not have enough seed potato production, so it has to import them. The imported seed potato amount has dramatically increased in recent years. For this reason, very strict quarantine checks should be made in terms of excluding potato cyst nematodes from nematode free area. Seed potato is the main cost for potato production, and the health status of seed potato is crucially important for productivity. Reproduction of potato with tubers (vegetative way) causes easy contamination by pest and disease. Potato cyst nematodes can simply move to new areas. Hence, the use of certified seeds and checking production areas are important management activities to protect nematode free areas.

To conclude; *G. rostochiensis*, was molecularly identified for the first time in samples from the central Anatolia region, the most important potato production area of Turkey. *G. pallida* was not detected in the samples using molecular protocols. The 60 cysts in the 250 g soil were determined as the PCN density in Nevşehir province of Turkey.

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References

Bates, J.A., Taylor, E.J., Gans, P.T., Thomas, J.E. 2002. Determination of relative proportions of *Globodera* species in mixed populations of potato cyst nematodes using PCR product melting peak analysis. Molecular Plant Pathology, 3: 153-161.

Brown, E.B. 1969. Assessment of the damage caused to potatoes by potato cyst eelworm, *Heterodera rostochiensis* Woll. Annals of Applied Biology, 63: 493-502.

Bulman, S.R., Marshall, J.W. 1997. Differentiation of Australian potato cyst nematode (PCN) populations using the polymerase chain reaction (PCR). New Zealand Journal of Crop and Horticultural Science, 25: 123-129.

Erdogan, P., Kilic T., Ulutas E., Kepenekci, I., Toktay, H., Cakir, E., Aydin, M.H., Karahan, A., Ustun, N., Ozdemir, S., Akbas, B., Yurtmen, M., Basaran, S., Elibuyuk, E.A., Velioglu, A.S., Erdogan, C., Kodan, M., Sabahoglu, Y., Aydar, A., Akbas, R., Hantas, C. 2011. Editors: Atlamaz A., Gokce A.Y. Patates Entegre Mücadele Teknik Talimatı. Gıda Tarım ve Hayvancılık Bakanlığı, Tarımsal Araştırmalar ve Politikalar Genel Müdürlüğü, Bitki Sağlığı Araştırmaları Daire Başkanlığı. Ankara, p. 119.

EPPO, 2004. Diagnostic protocols for regulated pests. *Globodera rostochiensis* and *Globodera pallida*. EPPO Bulletin, 34: 309-314.

Evans, K., Trudgill. D.L. 1992. Pest aspects of potato production Part 1. The nematode pests of

potatoes. Ed: P.Harris. "Potato Crop" Chapman, Hill London, pp. 438-475.

FAO, 2013. Food and Agricultural Organization of United Nations. http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor.

Fenwick, D.W. 1940. Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. Journal of Helminthology, 18: 155-172.

Fullaondo, A., Barrena, E., Viribay, M., Barrena, I., Salazar, A., Ritter, E. 1999. Identification of potato cyst nematode species *Globodera rostochiensis* and *G. pallida* by PCR using specific primer combinations. Nematology, 2: 157-163.

Holgado, R., Magnusson, C. 2012. Nematodes as a Limiting Factor in Potato Production in Scandinavia. Potato Research, 55: 269-278.

Jatala, P. 1978. Parasitic Nematodes of potatoes CIP technical Information Bulletin. 8: 17.

Jensen, H.J. 1978. Nematode pests of vegetable and related crops Ed:Webster "Economic nematology" Academic Press., London and New York, pp. 377-408.

Madani, M., Ward, L.J., De Boer, S.H. 2008. Multiplex real-time polymerase chain reaction for identifying potato cyst nematodes, *Globodera pallida* and *Globodera rostochiensis*, and the tobacco cyst nematode, *Globodera tabacum*. Canadian Journal of Plant Pathology, 30: 554-564.

Mendoza, H.A., Jatala, P. 1978. Breeding for resistance to the root-knot nematode. International Potato, Center Report of the planning conference on the developments in the control of nematode pest of potatoes II. International Potato center, Lima, Peru, pp. 111-116.

Ozarslandan, A., Devran, Z., Mutlu, N., Elekcioglu, I.H. 2009. First Report of Columbia Root-Knot Nematode (*Meloidogyne chitwoodi*) in Potato in Turkey. Plant Disease, 93: 316.

Phillips, M.S., Hackett, C.A. and Trudgill, D.L. 1991. The relationship between the initial and final population densities of the potato cyst nematode *Globodera pallida* for partially resistant potatoes. Journal of Applied Ecology, 28: 109-119.

Powers, T. 2004. Nematode molecular diagnostics: from bands to barcodes. Annual Review of Phytopathology, 42: 367-383.

CABI, 2013. *Globodera pallida*. Crop Protection Compendium. Accessed September 23, 2013 from: www.cabi.org/cpc

Pylypenko, L.A., Uehara, T., Phillips, M.S., Sigareva, D.D., Blok, V.C. 2005. Identification of *Globodera rostochiensis* and *G. pallida* in the Ukraine by PCR. European Journal of Plant Pathology, 111: 39-46.

Skantar, A.M., Handoo, Z.A., Carta, L.K., Chitwood, D.J. 2007. Morphological and molecular identification of *Globodera pallida* associated with potato in Idaho. Journal of Nematology, 39: 133-144.

Tovar, A., Cid Del Prado, I., Sandoval, J., Martínez, A., Nicol, J., Evans, K. 2006. Los nematodos formadores de quistes en México. Revista Mexicana de Fitopatología, 24:145-151.

TUIK, 2012. T.C. Başbakanlık Türkiye İstatistik Kurmu, Ankara (www.tuik.gov.tr, Access date: June, 2014).

Turner, S.J., Evans, K. 1998. The origins, global distribution and biology of potato cyst nematodes (*Globodera rostochiensis* (Woll.) and *Globodera pallida* Stone). pp. 7-26. *In*: R.J. Marks and B.B. Brodie (Eds.). Potato Cyst Nematodes. Biology, distribution and control. CAB International, Wallingford, UK.

Ulutas E, Özarslandan, A., Kaşkavalcı, G., Elekcioğlu, İ.H. 2012. Molecular diagnosis of *Globodera rostochiensis* Wollenweber (Tylenchida: Heteroderidae) in the potato growing areas of Aegean Region, Turkey. Turkish Journal of Entomology, 36(1): 155-160.

Vejl, P., Skupinová, S., Sedlak, P., Domkarova, J. 2002. Identification of PCN species (*Globodera rostochiensis*, *G. pallida*) by using of ITS-1 region´s polymorphism. Rostlinna výroba 48: 486-489.

Waeyenberge, L., Ryss, A., Moens, M., Pinochet, J., Vrain. T.C. 2000. Molecular characterization of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. Nematology, 2: 135-142.

White, T. J., Bruns, T., Lee, S., Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics *In*: M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White (Eds.). PCR Protocols: A guide to methods and applications. Academic Press, San Diego, California, USA, pp. 315-322.

Winslow, R.D., Willis, R.J. 1972. Nematode Diseases of Potatoes. Economic Nematology J.M. Webster, Academic Press London, pp: 17-48.

Zawislak, K., Niewiadomski, W., Gronowicz, H. 1981. Continuous cultivation of potatoes and the golden nematode problem. Zeszyty Naukowe Akademii Rolniczo-Technicznej Olsztyn, Rolnictwo, 29: 259-270.