

Phylogenetic Analysis of Georgian Populations of Potato Cyst Nematodes *Globodera rostochiensis*

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Abstract : Potato is one of the main agricultural crops in Georgia. Georgia produces early and late potato varieties in almost all regions. In traditional potato growing regions (Svaneti, Samckhet javakheti and Tsalka), the yield is higher than 30-35 t/ha. Among the plant pests that limit potato production and quality, the potato cyst nematodes (PCN) are harmful around the world. Yield losses caused by PCN are estimated up to 30%. Rout surveys conducted in two geographically distinct regions of Georgia producing potatoes - Samtskhe - Javakheti and Svaneti revealed potato cyst nematode *Globodera rostochiensis*. The aim of the study was the Phylogenetic analyses of *Globodera rostochiensis* revealed in Georgia by the amplification and sequencing of 28S gen in the D3 region and intergenic ITS1-15.8S-ITS2 region. Identification of all the samples from the two *Globodera* populations (Samtskhe - Javakheti and Svaneti), i.e., *G. rostochiensis* (20 isolates) were confirmed by conventional multiplex PCR with ITS 5 universal and PITSp4, PITSr3 specific primers of the cyst nematodes' (*G. pallida*, *G. rostochiensis*). The size of PCR fragment 434 bp confirms that PCN samples from two populations, Samtskhe- Javakheti and Svaneti, belong to *G. rostochiensis*. The ITS1-5.8S-ITS2 regions were amplified using prime pairs: rDNA1 (5' -TTGATTACGTCCCTGCCCTTT-3' and rDNA2(5' TTCACTCGCCGTTACTAAGG-3'), D3 expansion regions were amplified using primer pairs: D3A (5' GACCCCTCTTGAAACACGGA-3') and D3B (5'-TCGGAAGGAACCAGCTACTA-3'. PCR products of each region were cleaned up and sequenced using an ABI 3500xL Genetic Analyzer. Obtained sequencing results were analyzed by computer program BLASTN (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Phylogenetic analyses to resolve the relationships between the isolates were conducted in MEGA7 using both distance- and character-based methods. Based on analysis of *G.rostochiensis* isolate`s D3 expansion regions are grouped in three major clades (A, B and C) on the phylogenetic tree. Clade A is divided into three subclades; clade C is divided into two subclades. Isolates from the Samtckhet-javakheti population are in subclade 1 of clade A and isolates in subclade 1 of clade C. Isolates) from Svaneti populations are in subclade 2 of clade A and in clad B. In Clade C, subclade two is presented by three isolates from Svaneti and by one isolate (GL17) from Samckhet-Javakheti. . Based on analysis of *G.rostochiensis* isolate`s ITS1-5.8S-ITS2 regions are grouped in two main clades, the first contained 20 Georgian isolates of *Globodera rostochiensis* from Svaneti . The second clade contained 15 isolates of *Globodera rostochiensis* from Samckhet javakheti. Our investigation showed of high genetic variation of D3 and ITS1-5.8S-ITS2 region of rDNA of the isolates of *G. rostochiensis* from different geographic origins (Svameti, Samckhet-Javakheti) of Georgia. Acknowledgement: The research has been supported by the Shota Rustaveli National Scientific Foundation of Georgia : Project # FR17_235

Keywords : *globodera rostochiensis*, PCR, phylogenetic tree, sequencing

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