## COMPARISON OF MORPHOLOGICAL AND MOLECULAR CHARACTERS OF DIFFERENT POPULATIONS OF *Heterodera schachtii*\*

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## Abstract

Heterodera schachtii is one of the most economically important pests of sugar beet worldwide. It is also widespread in most sugar beet producing regions in Iran and causes serious yield reduction and decreasing sugar content of sugar beet in infested fields. Populations of H. schachtii show differences in morphological characters. Traditional identification based on morphological and morphmetrical characters of cysts and J2s is time consuming and demands careful study. In recent years, the DNA technique based on ITS-PCR-RFLP has been a useful tool for separating of H. schachtii from the similar species. 120 populations of H. schachtii were collected from different sugar beet fields in Khorasan Razavi province. The populations were studied based on morphological and morphometrical characters. 88 populations with high variation in morphological characters were selected for further studies. DNA was extracted from full cyst and the ITS-rDNA regions were amplified with using of universal primers. The PCR product of each sample was digested with MvaI. All populations showed similar ITS-PCR-RFLP profiles, which were in agreement with the published data. Clustering of these populations based on morphometrics features separated the south regions populations from the northern populations, however, there was not logical separation among the smaller geographic areas. Four morphological types with different underbridge and bullae in vulval cones were revealed. It was demonstrated in this study that ITS-PCR-RFLP could be used as a helpful tool for identifying different populations of H. schachtii with various morphological characters.

**Keywords:**Clustering, Sugar beet cyst nematode, Khorasan Razavi, Morphology, ITS-PCR-RFLP, *Heterodera schachtii*.

See Persian text for figures and tables (Pages ۴.۵–۴۱۸).

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## References

- AKHIANI, A., DAMADZADE, M. and AHMADI, A. 1996. Distribution of sugar beet nematode in sugar beet fields of Esfahan Province. **Iran. J. Plant Pathol.** 32: 104-105(In Farsi With English Summary).
- AMIRI, S., SUBBOTIN, S. and MOENS, M 2001. An effective method for identification of the *Heterodera* schachtii sensu stricto group using PCR with specific primers. **Nematology Mediterranea** 29: 241-246.
- AMIRI, S., SUBBOTIN, S. and MOENS, M. 2002. Identification of beet cyst nematode *Heterodera* schachtii by PCR. **Eur. J. Plant Pathol.** 108: 497-506.
- AMIRI, S., SUBBOTIN, S. and MOENS, M, 2003. Comparative morphometrics and RAPD studies of *Heterodera schachtii* and *H. betae* populations. **Russian J. Nematol.** 11: 91-99
- BALDWIN, J.G and MUNDO-OCAMPO, M, 1991. Heteroderinae, cyst and non cyst forming nematodes. Pp. 275-362. *In*: W.R Nickel (Ed.), **Manual of Agricultural Nematology**. Marcel Dekker Inc., New York.
- DE GRISSE, A. 1969. Redescription ou modifications de quelques techniques utilisées dans letude des nematodes phytoparasitaires. **Mededelingen Rijksfaculteit der Landbouwwetenschappen Gent.** 34: 351-369.
- DUNN, R.A. 1969. Extraction of cysts of *Heterodera* species from soils by centrifugation in high density solutions. **J. Nematol.** 1: 7.
- ESMAILPOUR, M.H. and SCHAFER, R. 1970. Occurrence of sugar beet nematode *Heterodera schachtii* in Iran. **Entomologie et Phytopathologie Appliquées** 29: 6-7.
- EVANS, K. and ROWE, J.A. 1998. Distribution and economic importance. Pp. 1-30. *In*: S. B. Sharma(ed.), **The Cyst Nematodes.** Klower Academic Pub., London, UK.
- JOYCE, S.A., REID, A., DRIVER, F. and CURRAN, J. 1994. Application of polymerase chain reaction (PCR) methods to identification of entomopathogenic nematodes. Pp. 178-187. *In*: Burnell, A.M., Ehlers, R.U. and Masson, J.P. (Eds). Cost 812 Biotechnology: Genetics of entomopathogenic nematode-bacterium complexes. Proceedings of Symposium & Workshop, St. Patrick's College, Maynooth, Co. Kildare, Ireland, Luxembourg, European Commission, DG XII.
- KAREGAR, A. 2006. Identification of plant-parasitic nematodes associated wit sugar beet fields and their distribution in Hamadan province, Iran. **Iran. J. Plant Pathol.** 42: 159-178. (In Farsi With English Summary).
- KEIVANLOO, E., KARIMI, J., SADEGHI, H. and MOKARAM, A. 2010. DNA barcode as a tool for identification of *Tetranychus urticae*. 8<sup>th</sup> Iran. Boil. Cong., Mashhad, Iran.
- KHEZRINEJAD, N., NIKNAM, GH. and GHOSTA, Y. 2006. Record of plant parasitic nematodes from sugar beet fields in West Azarbaijan province. **Proc. 17<sup>th</sup> Iran. Plant Protec. Cong., Karaj, Iran.** 111 (AbSt).
- MAHDIKHANI MOGHADAM, E. and JAFARPOUR, B, 2008. Identification and distribution of *Heterodera* species in sugar beet fields in Mashhad region. **J. Agric. Sci.** 22(1): 3-17.
- MAHDIKHANI MOGHADAM, E. and KHEIRI, A. 1995. Some plant parasitic nematodes fauna of sugar beet fields in Mashhad region. **Iran. J. Plant Pathol.** 31: 58-68.
- MAHDIKHANI MOGHADAM, E., KHEIRI, A. and OKHOVAT, M. 1996. Morphological and morphometrical study of three endoparasitic nematodes of sugar beet in Mashhad. **Iran. J. Plant Pathol.** 32: 1-8. (In Farsi With English Summary).
- MOKARAM HESAR, A., MAHDIKHANI MOGHADAM, E., TANHA MAAFI, Z. 2010. Comparison of different populations of *Heterodera filipjevi* by morphological characters, PCR-RFLP and sequencing of rDNA-ITS in sugar beet fields of Khorasan Razavi province. **Proc. 19**<sup>th</sup> **Iran. Plant Protec. Cong., Tehran, Iran.** 650 (Abst)
- MULLER, J. 1999. The economic importance of *Heterodera schachtii* in Europe. **Helminthologia** 36: 205–213.
- PLANTARD, O. and PORTE, C. 2003. Isolation and characterization of microsatellite loci in the sugar beet cyst nematode *Heterodera schachtii*. **Mol. Ecol. Notes** 3: 139-141.

- POWERS, TO., TODD, TC., BURNELL, AM., MURRAY, PCB, FLEMING, CC., SZALANSKII, AL., ADAMS, BA. and HARRIS, TS. 1997. The rDNA internal transcribed spacer region as a taxonomic marker for nematodes. J. Nematol. 29: 441-450.
- SEINHORST, J.W, 1964. Methods for extraction of *Heterodera* cysts from not previously dried soil samples. **Nematologica** 10: 87–94
- SHIQI, O., DELIANG, P., XUEMIN, L., YU, L. and MOENS, M. 2008. Identification of *Heterodera glycines* using PCR with sequence characterized amplified region (SCAR) primers. **Nematology** 10: 397-403.
- STEELE, A.E. 1965. The host range of the sugar beet nematode. *Heterodera schachtii* Schmidt. **J. Amer. Soc. of Sugar Beet Technol.** 13: 573–603
- SUBBOTIN, S.A., VIERSTRAETE, A., DE LEY, P., ROWE, J., WAEYENBERGE, L., MOENS, M. and VANFLETEREN, J.R. 2001. Phylogenetic relationships within the cyst-forming nematodes (Nematoda, Heteroderidae) based on analysis of sequences from the ITS region of ribosomal DNA. **Mol. Phylogen. and Evol.** 21: 1-16.
- TALACHIAN, P., AKHIANI, A., GRAYELI, Z., SHAMOHAMADI, M. and TEYMORI, F. 1976. Survey on cyst forming nematodes in Iran and their importance. **Iran. j. Plant Pathol.** 12: 73-78. (In Farsi With English Summary).
- TANHA MAAFI, Z., SUBBOTIN, S.A. and MOENS, M. 2003. Molecular identification of cyst-forming nematodes (Heteroderidae) from Iran and a phylogeny based on ITS-rDNA sequences. **Nematology** 5: 99–111.