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Research
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Root-knot nematode *Meloidogyne arenaria* infecting Swiss Chard (*Beta vulgaris* subsp. *cicla*)

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Abstract

Swiss Chard (*Beta vulgaris* subsp. *cicla*) is a vegetable crops growing in different regions of the world for variying purposes. Root-knot nematodes (*Meloidogyne* spp.) seem the potantial pest group on Swiss Chard according to the reported species from other countries. In this investigation, the root knot nematode (RKN) extracted from the root of Swiss Chard was identified as *M. arenaria* by using different primer sets (TRNAH-MRH106 and MORF-MTHIS) targeting the large sub-unit ribosomal DNA (lrDNA) and the intergenic spacer (IGS) of rDNA of mitochondrial (mtDNA). Furthermore, the restriction enzymes (*Hinf*I and *Mnl*I) for the fragments given by TRNAH-MRH106 primer set was also used for restriction and digestion. Finally, species-specific SCAR primers (Far/Rar) were performed to validation of the identification results. *M. arenaria* is considered as an important species among the major root knot nematodes in the world. In this context, the Swiss Chard as an intercroped, small-scale produced or randomly dispersed commodity must be observed as the potantial host for the nematode. This is especially important in case of any change for long term professional production planning in the same area for more valuable agricultural commodity.

Key words: *Beta vulgaris* subsp *cicla***,** Root knot nematodes, *Meloidogyne arenaria*, Swiss chard

Pazı (*Beta vulgaris* subsp. *cicla*) bitkisi zararlısı kök-ur nematodu *Meloidogyne arenaria*

Öz

Pazı (*Beta vulgaris* subsp. *cicla*), çeşitli amaçlar için dünyanın farklı bölgelerinde yetişen bir sebze türüdür. Bazı ülkelerde kök-ur nematodları (*Meloidogyne* spp.) pazı üzerinde potansiyel zararlı grubu olarak bildirilmiştir. Bu araştırmada, Ordu ilinde pazı köklerinden elde edilen kök-ur nematodu (RKN)'nun, mtDNA'nın bölgelerini (lrDNA ve IGS) hedefleyen farklı primer setleri (TRNAH-MRH106 ve MORF-MTHIS) kullanılarak teşhisi yapılmış ve *M. arenaria* olarak tanımlanmıştır. Ayrıca, TRNAH-MRH106 primer setinin kullanımı ile elde edilen DNA bant büyüklüklerini kesme işlemi için *Hinf*I ve *Mnl*I enzimleri de kullanılmıştır. Son olarak, teşhis sonuçları türe özgü SCAR primerleri (Far/Rar) kullanılarak doğrulanmıştır. *M. arenaria*, dünyadaki başlıca kök-ur nematodları arasında önemli bir tür olarak kabul edilmektedir. Bu bağlamda kök-ur nematodları, küçük ölçekli üretilmiş veya rastgele dağılmış bir ürün olarak pazının potansiyel konukçusu olarak gözlenmesi gerekir. Bu durum özellikle, aynı alanda daha değerli tarımsal ürünlerin uzun vadeli profesyonel üretim planlaması durumunda herhangi bir değişiklik olması durumunda önemlidir.

Anahtar kelimeler: *Beta vulgaris* subsp *cicla***,** kök-ur nematodları, *Meloidogyne arenaria*, pazı

Introduction

Swiss chard (*Beta vulgaris cicla*, BVc) is member a of the Chenopodiaceae (Ninfali and Angelino, 2013). *Beta vulgaris* subsp. *vulgaris* var. *cicla* is called by different names in worlwide as Chard, Swiss chard, beet greens, spinach beet (EPPO, 2020) and known the leaf beet group vegatable crop (Lange et al. 1999, McGratht, 2011,). The total cultivated area is not known exactly in worlwide, but it is consumed for its leaves in salads or in case of more ripening also cooked in the recipies as spinach done. In addition, the chard is used as pot and medicinal herb (Biancardi et. al., 2011). BVc became commercially important in 19th century in Europe (Norton and Esposito, 1994). The crop is also popular in Turkey. The planted area was 6.278 da and the production was 9.631 tons in total for 2018 (TUİK, 2019). Even it is commercially available in the markets, especially produced in the gardens for small scale consumption by families. Ordu is one of the province revealing traditional vegetable production aspects such as home garden practices for local family consumption and also small scale production by traditional methods for markets. The chard is mainly the intercrop among other vegetables in small gardens, but also planted 37 da area and produced 45 tons in 2018 in Ordu, Turkey (TÜİK, 2019).

Chard production was affected by different pest and disease agents and one of them as an important group is the genus *Meloidogyne*. The genus is capable to infect more than 5,500 plant species (Trudgill and Blok, 2001) and some species of the genus were also reported on chard. *Meloidogyne* spp. in Cyprus (Philis, 1983), *M. hapla* in Germany (Decker, 1989), *M. incognita* var. acrita in Africa (Martin, 1959), *M. arenaria* in Spain (Millán de Aguirre, 1991), *M. enterelobii* in Mexico (Bastidas et. al., 2019), *Meloidogyne* spp. in Iraq and Turkey (Kareem et. al., 2017) were the countries and root knot nematode species reported with Chard. In Turkey, some studies on identification and distribution of root-knot nematodes have been conducted on different vegetables by different research groups (Elekçioğlu et al., 1994; Mennan and Ecevit 1996; Kaşkavalcı and Öncüer 1999; Söğüt and Elekçioğlu, 2000; Özarslandan and Elekçioğlu, 2010; Çetintaş and Çakmak, 2016; Uysal et. al., 2017), but *M. arenaria* species have not been reported on swiss chard. As determined in previous investigations, *Meloidogyn*e species are in relation with chard and must be identified in case of the likelihood of encounter with a new *Meloidogyne* species on the crop. In this context, we found the crop infested in a small garden-field and identified the species in order to confirm the previous studies and also contribute to the crop in a different region as the host status for *Meloidogyne* species.

Material and Methods

Nematode sampling and extraction

The plant was encountered by chance during a visit in a small garden in 2018. When the infected chard roots were observed (Figure 1), the root samples were transferred into a polyethylene bag and then carried to the laboratory and stored at 4°C in refrigerator till the nematode extraction process. For the extraction, the infected swiss chard roots were dissected under a stereomicroscope (Leica, S8APO) at 10x magnification. Females were collected separately and used for molecular characterization.

DNA extraction and PCR amplification

For DNA extraction, a single mature female was transferred into 10 µl of extraction buffer (1 mM EDTA, 10 mM Tris, 0.1 mg/ml proteinase K and 0.1 % triton X) in a 1.5 ml tube, and the females were disrupted using a probe (Pagan et al., 2015). Samples were frozen at –20°C overnight in 0.2 ml PCR tubes. Then, samples were incubated at 56°C for 1 h followed by 95°C for 10 min, and used immediately for PCR or stored at –20°C.

The primer sets TRNAH and MRH106 or MORF/MTHIS developed by Stanton et al. 1997 were used to amplify the mitochondrial DNA fragments. The primer sequences are given in Table 1. Total reaction volume was 25 µl containing DreamTaq Green PCR Master Mix (2X) (Thermo Fisher Scientific), 1.5 µl of DNA, and 0.5 µM of each primer. The thermal cycling was performed in the Veriti Thermal Cycler. The thermo cycling reactions using the primers TRNAH/MRH106 and MORF/MTHIS (Stanton et al., 1997; Pagan et al., 2015) were as follows: 95°C for 3 min, followed by 40 cycles of 95°C for 30 sec., 50°C for 30 sec. 68°C for 1 min, and a final extension step of 68°C for 7 min. DNA fragments were separated by electrophoresis in 1x TAE (Tris-acetic acid- EDTA buffer) using 1% agarose gels containing Etidium Bromid for 25 min at 150 V and visualized under UV light using GEN-BOX imagER. Secondly, in order to determine the mitochondrial haplotype, the fragments amplified using the primer set, TRNAH and MRH106 were subjected to restriction digestion according to the

restriction enzymes *Hinf*I and *Mnl*I (New England Biolabs, Ipswich, MA) as recommended by the manufacturer. Finally, the identity of the sample was also confirmed by species specific SCAR primer set Far/Rar developed for *M. arenaria* (Zijlstra et al., 2000). The PCR conditions 95°C for 3 min, followed by 40 cycles of 95°C for 30 sec., 61°C for 30 sec. 68°C for 1 min, and a final extension step of 68°C for 7 min.

Table 1. Primer sets used for identification of root-knot nematode *Meloidogyne arenaria.*

Primer	Primer sequences	Reference
TRNAH	5' TGAATTTTTTATTGTGATTAA 3'	Stanton et al. 1997
MRH106	5' AATTTCTAAAGACTTTTCTTAGT 3'	Stanton et al. 1997
MORF	5' ATCGGGGTTTAATAATGGG 3'	Stanton et al. 1997
MTHIS	5' AAATTCAATTGAA ATTAATAGC 3'	Stanton et al. 1997
Far	5' TCGGCGATAGAGGTAAATGAC 3'	Zijlstra et al., 2000
Rar	5' TCGGCGATAGACACTACAACT 3'	Zijlstra et al., 2000

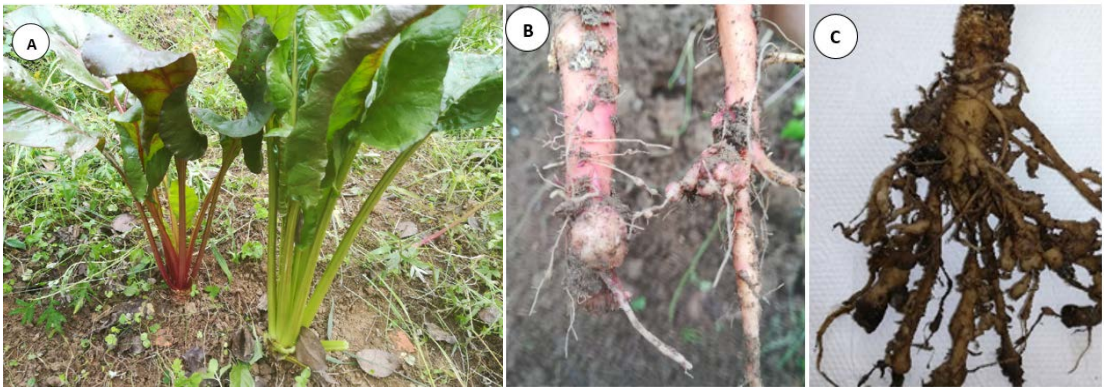

Figure 1. Appearance of swiss chard plant in the field (A) and roots with root-knot nematode gall symptoms (B,C) caused by *Meloidogyne arenaria.*

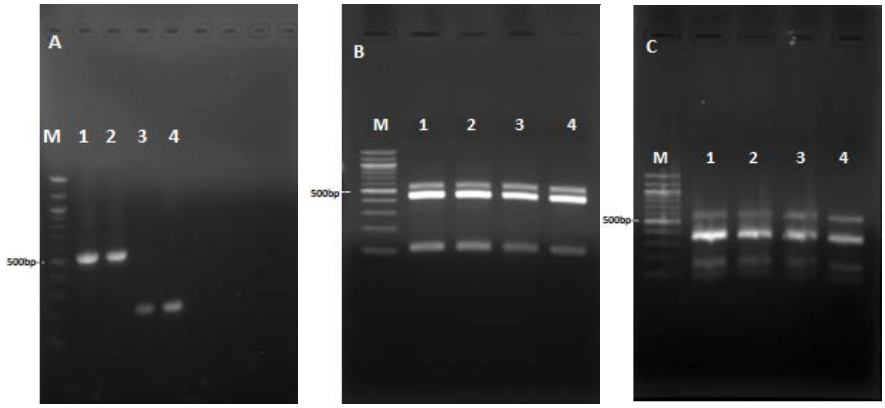

Figure 2. 557 bp fragments of TRNAH/MRH106 primers (Line;1,2) and 214 bp fragments of MORF/MTHIS primers (Line;3,4) for *M.arenaria.* (A). The digestion assay of TRNAH/MRH106 using *Hinf*I showed 445 and 112 bp products (B) and *Mnl*I gave three digestion products of 340, 140 and 77 bp in *M. arenaria* (C). Lanes labeled M contain 100bp marker ladder (Fermentas), with the position of the 500bp band indicated by an arrow.

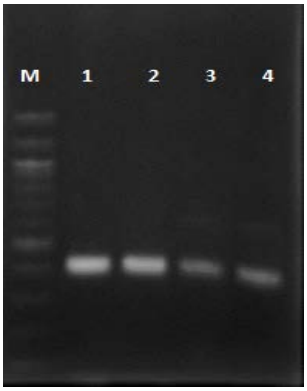

Figure 3. Amplification products of *Meloidogyne arenaria* with specific SCAR primer set Far/Rar (Line;1,2,3,4= 420 bp fragment; M: 100bp marker ladder).

Results

Root-knot nematode *M. arenaria* was detected on swiss chard plant. A PCR was conducted using primer set TRNAH/MRH106 and PCR amplification of the primers revealed fragments of 557 bp for *M. arenaria* (Figure 2A; Line 1, 2). For the primers MORF and MTHIS, a fragment was obtained in size of 214 bp (Figure 2A; Line 3, 4). The PCR product of TRNAH/MRH106 primers was processed for the digestion assay using *Hinf*I enzyme and this showed 445 and 112 bp products for *M. arenaria* (Figure 2B). The digestion assay of the enzyme *Mnl*I gave three digestion products of 340, 140 and 77 bp for *M. arenaria* (Figure 2C).

These results were also confirmed by using species-specific SCAR primer set Far/Rar showed 420 bp fragment for *M. arenaria* (Figure 3). This study results showed agreement with earlier studies Adam et al., (2007) and Zijlstra et al., (2000).

Conclusion

The production of the Swiss Chard is in small scale for Turkey and the production system is mainly based on family size production and traditional methods. It is easy to observe the intercrop status of this crop in Black Sea region and espacially in Ordu province. The production was also accompanied with the randomly dispersed production model of the crop in the fields or gardens and the lack of the modern approach results in non-management of pest or disease problems of the crop. This is also valid for root not nematodes. In this context, consideration of the crop as the host of *M. arenaria* is important, because yield losses are effected by the low population of nematodes in the soil. Swiss Chard may contribute this by increasing numbers of *M. arenaria* in the soil for the region. Especially, the nematode species is the one mentioned among the most encountered four species in worldwide (Taylor et. al. 1982) and identified different regions of Turkey as well (Devran and Söğüt, 2009, Akyazı et. al. 2012, Aydınlı ve Mennan, 2016). The previous researches are also the reported that major important root-knot nematodes prefer Swiss Chard as an host (Martin, 1959, Philis, 1983, Decker, 1989, Millán de Aguirre, 1991).

In conclusion, Swiss Chard is a potantial crop to spread and support the presence status of *M. arenaria* in the region and this is especially important in case of any change for long term professional production planing in the same area for more valuable agricultural commodity.

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