ONE OF THE SOUTHERN LIMITS OF GEOGRAPHICAL DISTRIBUTION OF SCLEROTIUM FORMING SNOW MOULD FUNGI: FIRST RECORDS OF *TYPHULA* SPECIES FROM IRAN

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Abstract

Three kinds of sclerotia of *Typhula* spp. from Ardabil, East Azerbaijan and Qazvin Provinces in the northwest of Iran were collected. Morphology and ITS sequences of rDNA suggested that the collected fungi belonged to *Typhula incarnata* Lasch, *Typhula phacorrhiza* (Reichardt) Fr. and *Typhula* sp. These three taxa are new records in the Middle East and the result of this research suggests that Iran is one of the southern limits of geographical distribution of sclerotium forming snow mould fungi.

Key words: Iran, snow mould fungi, *Typhula incarnata*, *T. phacorrhiza*

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Introduction

Snow mould fungi are psychrophilic and psychrotrophic fungal pathogens of perennial grasses and winter cereals in the Northern Hemisphere (HSIANG *et al.* 1999, SMITH 1986). They grow and attack dormant plants at low temperatures under snow cover. "Snow mould fungi" or "snow moulds" are terms used by phytopathologists rather than mycologists. Therefore, various fungi and fungus-like organisms (Oomycetes, Ascomycetes and Basidiomycetes) are included in snow mould fungi. The typical snow moulds of grasses, three species in *Pythium* (*P. iwayamae* S. Ito, *P. okanoganense* P.E. Lipps and *P. paddicum* Hirane), *Microdochium nivale* (Fr.) Samuels & I.C. Hallett [Teleomorph: *Monographella nivalis* (Schaffnit) E. Müll. var. *nivalis*], *Myriosclerotinia borealis* (Bubák & Vleugel) L.M. Kohn, *Typhula incarnata* Lasch and *Typhula ishikariensis* S. Imai, are important plant pathogens. However, our knowledge of geographical distribution of snow mould fungi is limited. There have been many reports of snow mould fungi in East Asia, Europe and North America (SMITH, 1986), but there are few reports of these fungi in central Asia, especially lower latitude area including the Middle East.

Two mountain ranges of Iran (Alburz and Zagros) have annual snow cover during winter, and winter wheat is one of the major crops in Iran. Two snow mould fungi, *M. nivale* in Gorgan (ERSHAD 1995) and *P. okanoganense* in Fars (MOSTFIZADEH-GHALAMFARSA & BANIHASHEMI 2001), have already been found in Iran. *Typhula idahoensis* Remsberg, a synonym of *T. ishikariensis*, was collected from twigs (of unknown grass) in Firuzabad, Fars Province of Iran in April 2003 (SABER & ESMAILI-TAHERI 2004). A specimen of this fungus is kept in the Mycology Collection of Herbarium Ministerii Iranici Agriculturae (IRAN) (IRAN 11904F). Sclerotium morphology of "*T. idahoensis*" from Iran is irregular in shapes and is very different from *T. ishikariensis* reported from other localities.

On the other hand, related saprophytic species, *Typhula* sp. and *Typhula ramealis* (Lib.) Speg., were collected from fallen logs and branches of *Ulmus* sp. in Mazandaran Province of Iran (SABER 1987, ERSHAD 1995). SABER (2000) reported *T. ramealis* from *Malus pumila* (Tehran), *Abutilon theophrasti* (Golestan Province) and rotten twig (Astara). AREFIPOR *et al*. (2004) reported *Typhula* sp.

from Golestan Province without mentioning the substrate (see also ABBASI *et al.* 2004). Snow mould fungus, *Typhula phacorrhiza* (Reichardt) Fr., that is weakly pathogenic on winter wheat in Canada (SCHNEIDER & SEAMAN 1988), was found near Iran, in Azerbaijan (POTATOSOVA 1960, PARMASTO 1965) and in Abkhazia Republic in Georgia (PARMASTO 1965). These records indicated the possibility of existence of sclerotium forming snow mould fungi in Iran. In February to March 2004, we surveyed northwest of Iran to collect sclerotium forming snow mould fungi. In this article two *Typhula* species and a *Typhula* sp. are reported for the first time from Iran and biological characteristics of sclerotium forming snow mould fungi is described.

Materials and Methods

Fungal isolation from overwintering grass leaves: Fungal sclerotia were collected from decayed leaves or stems of various plants from Ardabil, East Azerbaijan and Qazvin Provinces during February 27 - March 7, 2004. Collected sclerotia were packed in paper envelopes and were dried at room temperature during transportation. Fungal sclerotia were surface-sterilized in 70% (v/v) ethanol and 0.5% (as active chlorine) sodium hypochlorite solution and thoroughly washed with sterilized distilled water. They were then cut with sterilized razor blades, placed on potato dextrose agar (PDA; Difco) and incubated at 4˚C. Mycelia from growing margins of colonies were transferred to new plates containing PDA. All isolates were maintained on PDA slant cultures at 0˚C.

Growth temperature of mycelia: Mycelial discs of 5 mm in diameter were cut from the margin of actively growing colonies, transferred to the centre of PDA plates, and incubated at 5 different temperatures from 0 to 25˚C, in duplicates. After 1, 2 and 3 weeks of incubation, the colony diameters were measured. The linear mycelial growth rate per week was calculated after the initial lag period.

DNA sequence analysis: Fungal isolates were cultured for 1 month at 10˚C on PDA. Sclerotia were harvested and DNA was extracted by the protocol of DNeasy Plant MiniPrep (QIAGEN GmbH, Germany). ITS regions including the 5.8S gene of

genomic rDNA were amplified using primer pair P_{ITS1} (5'-TCCGTAGGTGAACCTGCGG) and P_{ITS4} (5'-TCCTCCGCTTATTGATATGC), as described by HSIANG & WU (2000). PCR products were purified using a QIAquick PCR Purification Kit (QIAGEN GmbH, Germany) and sequenced in one direction on ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, USA) using the P_{ITS1} primer. Multiple alignment of the ITS sequences was performed, and the nucleotide substitution rate (*Knuc* value) was calculated in CLUSTAL W (THOMPSON *et al.* 1994). A phylogenetic tree was constructed by neighbor-joining method (KIMURA 1980, SAITOU & NEI 1987) using the program CLUSTAL W.

Results and Discussion

Three species of *Typhula*, *T. incarnata* in Ardabil and East Azerbaijan, *T. phacorrhiza* in East Azerbaijan, and *Typhula* sp. in Ardabil, East Azerbaijan and Qazvin, were found. *Typhula incarnata* and *T. phacorrhiza* are new records to the mycoflora of Iran. Other psychrophilic snow mould fungi such as *T. ishikariensis* and *M. borealis* could not be found in Iran during this survey.

Symptoms of *T. incarnata* and *Typhula* sp. in winter wheat are shown in Figs 1 and 3. Host plants of these fungi had been only slightly damaged by fungal infections, suggesting that snow mould diseases caused by *Typhula* spp. do not have a great impact on overwintering grasses in Iran.

***Typhula incarnata* Lasch, Epicr. syst. mycol. (Upsaliae): 585, 1838**

Clavaria elegantula P. Karst., Bidr. Känn. Finl. Nat. Folk 37: 179, 1882
Pistillaria elegantula (P. Karst.) Corner, Ann. Bot. Mem. 1: 480, 1950
Typhula elegantula P. Karst., (1871) in Syll. fung. VI: 748, 1888
Typhula graminum P. Karst., Mycoth. fenn. (Helsinki) 3: 340, 1876 (non *senso* Karsten et Corner)
T. itoana S. Imai, Trans. Sapporo nat. Hist. Soc. 11: 42, 1930 (1929)

Fungal sclerotia that were rarely found in stubble of winter wheat in northern Iran were collected in Nir (Ardabil) and Seyvan (East Azerbaijan) (Fig. 1A). Voucher specimens are preserved in the Mycology Collection of Herbarium Ministerii Iranici Agriculture (IRAN) (IRAN 19960F) and AIST Hokkaido (Japan)

(AIST-H-I1). A living culture is deposited at Iranian Fungal Culture Collection at RZ address (IRAN 820C). Sclerotia were orange or apricot (look like young sclerotia) based on colour identification chart (Flora of British Fungi, Royal Botanical Garden, Edinburgh) and globose or oval in shape, and they were formed on the inside of overlapped leaves in winter wheat stubble (Fig. 1B). Sclerotia were 0.2-0.4 × 0.3-0.8 mm in diameter and sclerotium size was smaller than those in other localities (0.5-4.5 × 0.5-2 mm: ITO 1995 from Japan, 0.5-2 × 1.5-4 mm: EMSBERG 1940 from Finland).

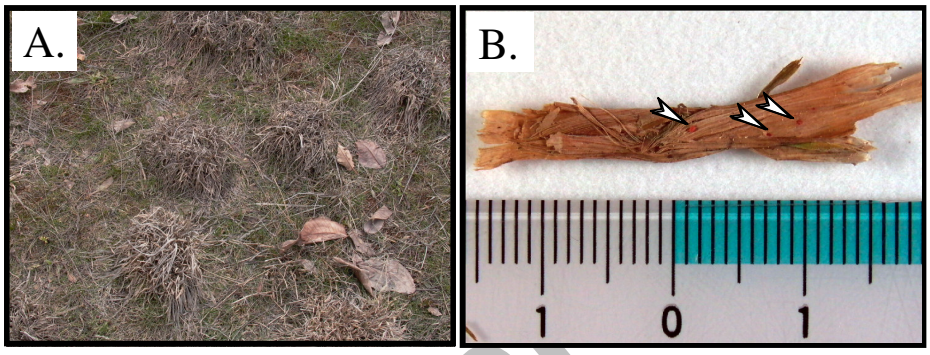

Fig. 1. *Typhula incarnata*: A. symptoms in winter wheat stubble, B. sclerotia on winter wheat under natural conditions.

HOSHINO *et al.* (2004) reported *T. incarnata* from Poland that produced large amount of small-sized sclerotia (< 1 mm diam.) on PDA plates at 0˚C. This phenomenon is probably related to adaptation to a short snow cover period in Poland. Mycelial growth of isolates from Iran occurred at -5 to 20˚C on PDA, with optimum growth temperature of 10˚C, and Iranian isolates formed normal-sized sclerotia on PDA at 0˚C. The physiological characteristics of isolates from Iran are similar to those of previously reported isolates from Nordic countries, Canada and Japan (SMITH 1986).

***Typhula phacorrhiza* (Reichardt) Fr., Observ. mycol. (Havniae) 2: 298, 1818**

Clavaria phacorrhiza Reuchardt, Schr. naturf. Fr. Berlin 1: 315, 1780
Sclerotium scutellatum Alb. & Schwein., Consp. fung. lusat., in Lusatiae Superioris Agro Niskiensi Crescentium e Methodo Persooniana (Leipzig): 74, 1805

Sclerotia rarely found on dead leaves of deciduous trees in northern Iran, collected from Seyvan, East Azerbaijan (Fig. 2). Voucher specimens are preserved in the Mycology Collection of Herbarium Ministerii Iranici Agriculture (IRAN) (IRAN 11961F) and AIST Hokkaido (Japan) (AIST-H-I2). A living culture is preserved at the Iranian Fungal Culture Collection at RZ address (IRAN 818C). Sclerotia were fulvous (wet) to umber (dry) based on a colour identification chart (Flora of British Fungi, Royal Botanical Garden, Edinburgh) and flattened in shape, and they were formed on dead leaves of deciduous trees. Sclerotia were 0.5-2.2 × 1.6-3.1 mm in diameter. Mycelial growth of isolates from Iran occurred at -5 to 25˚C on PDA, with optimum growth temperature of 15˚C.

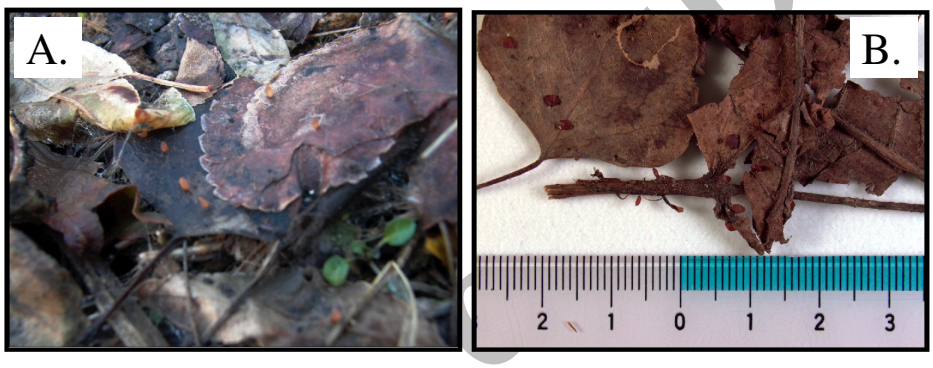

Fig. 2. *Typhula phacorrhiza*: A. wet sclerotia on dead leaves of deciduous trees in natural condition, B. dry sclerotia under natural conditions.

***Typhula* sp.**

Sclerotia that were widely spread in western Iran were found on dead leaves of grasses and deciduous trees in Nir (Ardabil), Azarshahr, Hashtrud, Seyvan, Tabriz (East Azerbaijan) and Qazvin (Qazvin) (Fig. 3). Voucher specimens are preserved in the Mycology Collection of Herbarium Ministerii Iranici Agriculture (IRAN) (IRAN 11962F, 11963F) and AIST Hokkaido (Japan) (AIST-H-I3, I4, I5, I6, I7). Two living cultures are deposited at Iranian Fungal Culture Collection at RZ address under IRAN 819C and IRAN 821C. Typical symptoms of winter wheat were found just after snow melt that were probably caused by this fungus (Fig. 3A). Presumably this species has the ability to infect winter wheat leaves under snow cover. Sclerotia

were fuscous black to olivaceous black (based on a colour identification chart, Flora of British Fungi, Royal Botanical Garden, Edinburgh) and globose or oval in shape, and they were produced on dead leaves of grasses (Fig. 3B) and deciduous trees (Fig. 3C) and they were not firmly attached to plant tissues. The rind cell pattern of this fungus was like the pieces in an interlocking jigsaw puzzle (Fig. 3D). Sclerotia were 0.2-1.5 × 0.6-2.2 mm in diameter. Mycelial growth of isolates from Iran occurred at -5 to 25˚C on PDA, with optimum growth temperature of 15˚C. Morphology (scleotium) of *Typhula* sp. is very close to *Typhula variabilis*, but they significantly differ in ITS sequences (similarity is less than 97%). According to Berthier (1976), which is still the most recent monograph on *Typhula*, there is no similar species to this and therefore we decide to describe this species when more material is available.

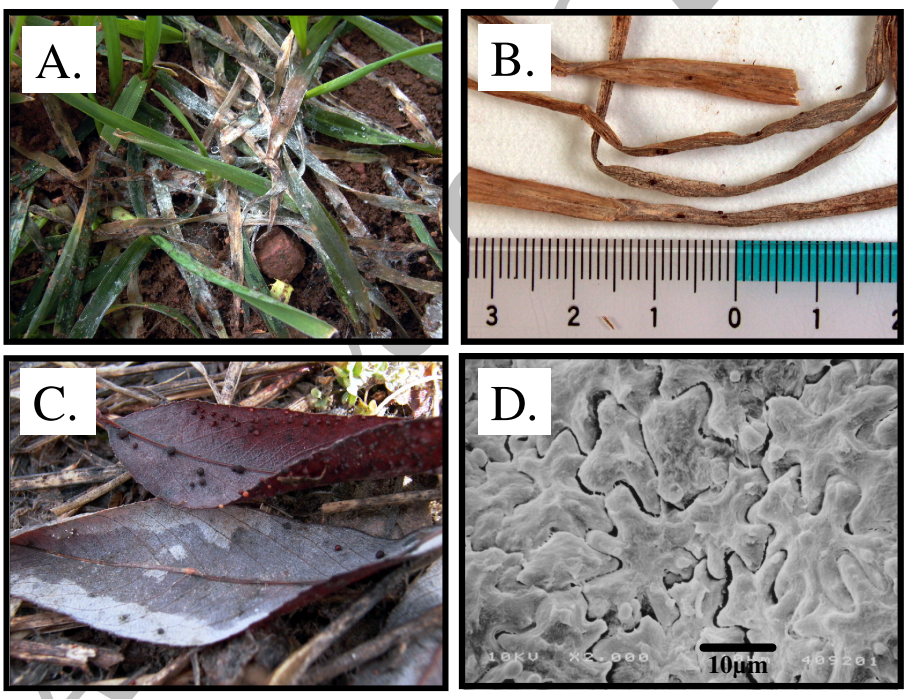

Fig. 3. *Typhula* sp.: A. symptoms in winter wheat, B. sclerotia on winter wheat under natural conditions, C. sclerotia on dead leaves of deciduous trees in natural conditions, D. the rind cell pattern in cultivated mature sclerotia.

Phylogenic analysis of Iranian isolates

Typhula idahoensis Remsberg in Iran (IRAN 11904F) has very different morphological characteristics from those of known sclerotia of *T. ishikariensis* from other localities (data not shown). ITS sequence of IRAN 11904F [AB267390] showed the highest homology to *Marasmius* sp. [AY21476] (91.6% homology) from GenBank. These facts suggest that the specimen of "*T. idahoensis*" from Fars Province of Iran was not correctly identified.

We also obtained ITS sequences from *T. incarnata* [AB267391], *T. phacorrhiza* [AB267392] and *Typhula* sp. on winter wheat [AB267393] in Iran. The results of phylogenetic analysis of our isolates and previous ITS sequences of *Typhula* spp. from Iran are shown in Fig. 4. DNA sequence data also indicated that our identification of *T. incarnata* and *T. phacorrhiza* was correct and *Typhula* sp. on winter wheat in Iran is possibly a new species in this genus. In the case of [AB267392] (*T. phacorrhiza*) and [AB267391] (*T. incarnata*), the ITS sequences of American strains of *T. phacorrhiza* and *T. incarnata* are only found in GenBank and the material could not be obtained for examination. No other strains were found from other localities such as Asia and Europe. Therefore, this is very difficult to discuss the relationship between genetic variability of *Typhula* spp. and geographical distribution.

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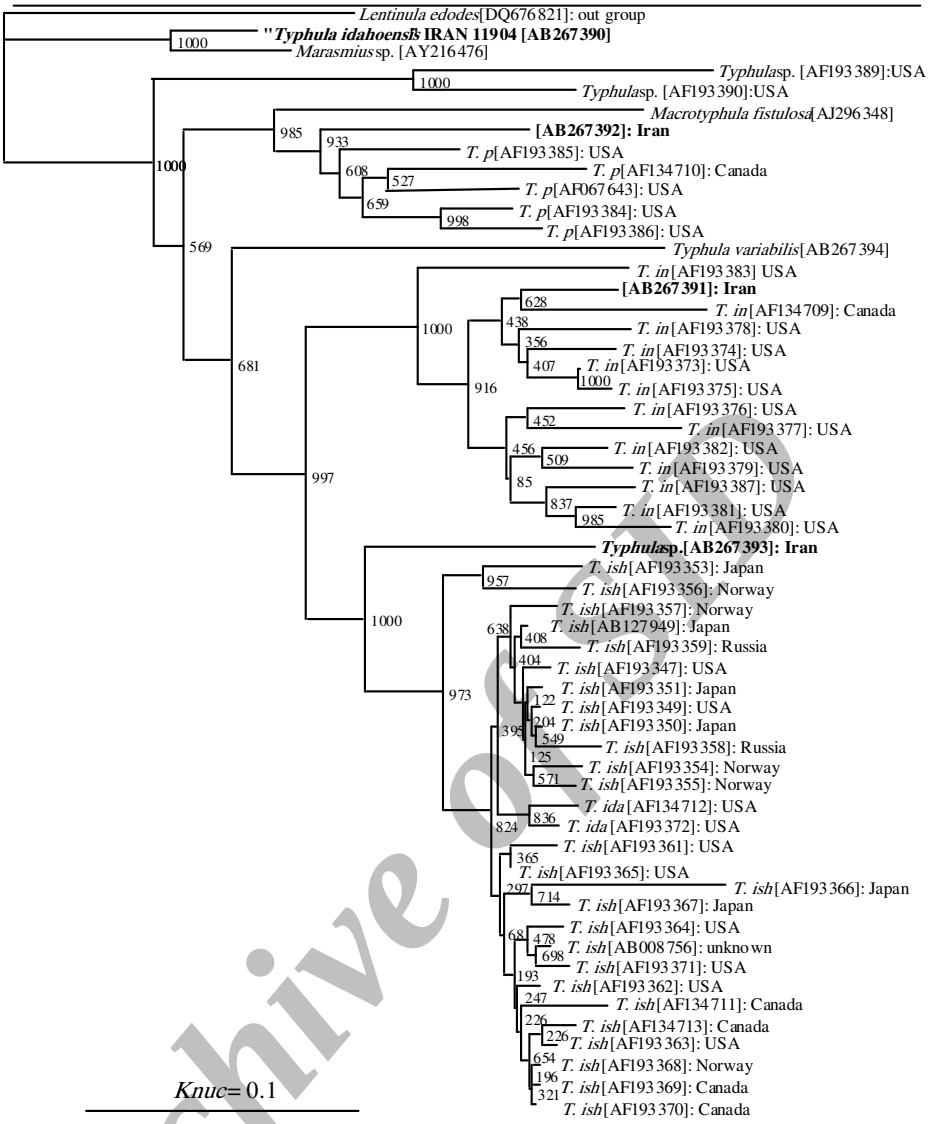

Fig. 4. Phylogenetic tree based on ITS1-5.8S-ITS2 region of *Typhula* spp. from Iran and other localities using the program Clustal W with Neighbor-Joining method. Numbers are bootstrap values (1000 replicates). *T. ida*: *Typhula idahoensis*, *T. in*: *Typhula incarnata*, *T. ish*: *Typhula ishikariensis*, *T. p*: *Typhula phacorrhiza*.

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