Identification of *Armillaria* species on different hosts from Iran

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Abstract. Thirty five isolates of *Armillaria* were obtained from 15 different host species in fruit orchards and forest regions of Iran. In order to identify species, diploid and haploid cultures were paired with two or three known haploid tester isolates from each intersterile group. Sexual compatibility was evaluated after 6 to 8 weeks based on changes in morphology of haploid colonies from white, with aerial mycelium (fluffy) to brownish, without aerial mycelium (crustose). *Citrus aurantium* and *Abies alba* were identified as new hosts of *Armillaria mellea; Carpinus betulus* was identified as a new host of *Armillaria gallica* in Iran, which previously has been reported from Serbia and Montenegro. *Armillaria* spp. isolated from *Diospyros lotus, Carpinus betulus,* and *Alnus subcordata,* were not compatible with any tester strains. *Diospyros lotus, Citrus aurantium,* and *Abies alba* were new hosts of *Armillaria* spp. from Iran.

Key words: *Armillaria,* compatibility test, Iran, isolates, hosts

Introduction

Armillaria species were divided into two sections distinguished by the presence or absence of a veil: annulate and exannulate respectively (Singer 1986). These species are found in most natural and exotic forests, in orchards and parks, throughout the world, from north temperate coniferous to tropical forests. The genus includes at least 36 species (Singer 1978; Watling *et al.* 1991; Volk & Burdsall 1995). They cause an important disease known as Armillaria root rot. The disease is well known to plant pathologists due to substantial damage that it can cause in natural forests, commercial forests plantation, and horticultural crops and in agriculture where specifically cash crop plantations are damaged (Hood *et al.* 1991; Kile *et al.* 1991).

Pairing tests based on sexual behaviour and *in vitro* somatic compatibility of isolates of *Armillaria* have been widely used to assess interspecific incompatibility in the genus *Armillaria.* Haploid monosporous cultures exhibit white, fluffy colony morphology. After compatible mating, the culture morphology becomes crustose, which is characteristic of diploid mycelium. Pairing tests have distinguished biological species in Europe, America, and Australia (Korhonen 1978; Anderson & Ullrich 1979; Kile & Walting 1983; Guillaumin *et al.* 1993). In Japan, Terashita & Chuman (1989) recognized five biological species of *Armillaria* from *Galeola septentrionalis,* an achlorophyllous orchid. Six intersterility groups were recognized as distinct by Matsushita *et al.* (1996) using isozyme analysis in annulated *Armillaria* spp., of which 5 species occur in Europe, 9 in North America, and 5 in Australia. In exannulate *Armillaria* spp., 2 species have been reported in Europe and 1 in North America (Guillaumin *et al.* 1991). *A. mellea* was reported on *Corylus* in some regions of Turkey (Sesli 2007).

In Iran, *Armillaria* is widely distributed throughout the country and is a well known causal agent of root rot (Saber 1974). Armillaria root rot was reported in association with many cultivated and forest tree species (Ershad 1995). Six intersterility groups were recognized including *A. mellea, A. cepistipes, A. gallica, A. borealis,* and *Armillaria* spp. as IISG5 and IISG6 (Asef *et al.* 2003).

The objective of this study was to determine the species of *Armillaria* found on trees of fruit orchards and forest regions in Iran.

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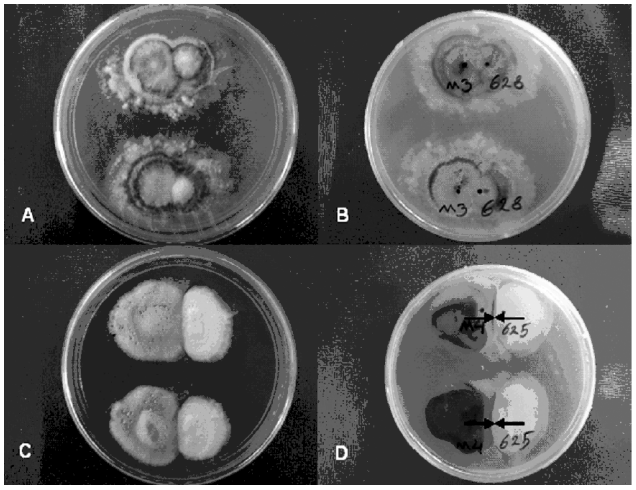

Fig. 1. Compatible (A, B) and incompatible (C, D) reactions and formation of black line in contact site of two incompatible colonies of *Armillaria mellea*

Materials and Methods

Sampling and fungal isolation

The roots, bark, and wood of trees with mycelial fans, rhizomorphs or basidiocarps of *Armillaria,* were collected from various regions of Mazandaran, Golestan, East Azerbaijan, and Esfahan, which are the main forestry and horticultural provinces in Iran. Samples were taken from 15 different host species. The infected tissues were sterilized in ethanol 96 % for 1 min, and small pieces from parts of the tissues were excised and placed on plates of malt extract agar (20 g/l Malt extract, 16 g/l agar) amended with benomyl WP 50 (4 µg a.i./ml) and streptomycin sulfate (100 µg/ml) added after autoclaving, and incubated at 22±1 °C (Worrall 1991).

Compatibility tests

Compatibility tests for the Iranian diploid and haploid isolates followed the Korhonen (1978) method, using known haploid tester strains of *Armillaria* spp. Seven biological species were used. Each isolate was paired with two or three different tester strains of the known biological species. The haploid tester strains of *Armillaria,* their hosts, and the geographic sources are listed in Table 1.

Inoculum consisted of undifferentiated mycelium without crusts or rhizomorphs. Mycelial plugs (3 mm diam) were derived from the margin of a growing culture. Plugs of two different pairs of isolates were placed side by side in each Petri dish and each pairing was repeated twice. The plates with diploid-haploid or haploid-haploid pairings were incubated at 22±1 °C and the evaluation was made after 6-8 weeks.

Table 1. Species, hosts, geographic origin, and sources of haploid tester strains of *Armillaria* spp. used in compatibility tests

Species	Isolate Number	Substrate/host	Geographic origin	Original strain number	Source
A. borealis	617	*Picea abies*	Loppi / Finland	99 68\4	Korhonen
A. borealis	659	*Quercus castaneaefolia*	Mazandaran / Iran	n, 2n	Asef
A. cepistipes	624	*Alnus* sp.	Tampere / Finland	MB 79.23.1	Guillaumin
A. cepistipes	625	*Acer platanus*	Helsinki / Finland	MB 79.24.1	Guillaumin
A. gallica	639	*Rubus fruticosus*	France	ME 70.1.2	Guillaumin
A. gallica	660	Stump	Mazandaran / Iran	n, 2n	Asef
A. mellea	627	*Opuntia s*p.	Sicilia / Italy	87 085\10	Korhonen
A. mellea	628	unknown	Sicilia / Italy	90 254\3	Korhonen
A. mellea	629	*Mallus* sp.	Slovenia	90 260\1	Korhonen
A. ostoyae	634	*Pinus sylvestris*	Lohja / Finland	99 088\3	Korhonen
A. ostoyae	636	*Pinus sylvestris*	Nurmijurvi /Fi nland	MC 79.27.1	Guillaumin
A. sinapina	642	*Larix kaempferi*	Hokkaido / Japan	96-7-1	Ota
A. sinapina	643	*Larix kaempferi*	Hokkaido / Japan	96-7-2	Ota
A. tabescens	647	*Quercus robur*	France	MT 85.68.1	Guillaumin
A. tabescens	649	*Quercus myrsinaefolia*	Ibaraki / Japan	NT 1-9	Ota

Table 2. Characteristics of identified *Armillaria* isolates from Iran, based on pairing tests

Isolate No.	Source	Host	Localities	Ploidity	Species
A1	Wood fragment	*Armeniaca vulgaris* Lam.	East-Azerbaijan (Azar Shahr)	D*	*A. mellea*
A2	Wood fragment	*Juglans regia* L.	East-Azerbaijan (Gogan)	D	*A. mellea*
A3	Wood fragment	*Amygdalus communis* L.	East-Azerbaijan (Azar Shahr)	D	*A. mellea*
A4	Wood fragment	*Juglans regia* L.	East-Azerbaijan (Azar Shahr)	D	*A. mellea*
A5	Wood fragment	*Pyrus communis* L.	East-Azerbaijan (Azar Shahr)	D	*A. mellea*
A6	Wood fragment	*Armeniaca vulgaris* Lam.	East-Azerbaijan (Kandovan)	D	*A. mellea*
A7	Mycelium	*Juglans regia* L.	East-Azerbaijan (Azar Shahr)	D	*A. mellea*
A8	Wood fragment	*Juglans regia* L.	East-Azerbaijan (Khosroshar)	D	*A. mellea*
A9	Wood fragment	*Juglans regia* L.	East-Azerbaijan (Oskoh)	D	*A. mellea*
A10	Wood fragment	*Juglans regia* L.	East-Azerbaijan (Khosroshar))	D	*A. mellea*
A11	Wood fragment	*Armeniaca vulgaris* Lam.	East-Azerbaijan (Oskoh)	D	*A. mellea*
A12	Wood fragment	*Malus domestica* Borkh.	East-Azerbaijan (Kandovan)	D	*A. mellea*
A13	Wood fragment	*Populus nigra* L.	East-Azerbaijan (Oskoh)	D	*A. mellea*
M1	Basidiocarp	*Fagus orientalis* Lipsky	Mazandaran (Behshar)	N**	*A. mellea*
M2	Basidiocarp	*Citrus aurantium* L.	Mazandaran (Tonekabon)	N	*A. mellea*
M3	Basidiocarp	*Citrus aurantium* L.	Mazandaran (Tonekabon)	N	*A. mellea*
M4	Basidiocarp	*Citrus aurantium* L.	Mazandaran (Tonekabon)	N	*A. mellea*
M5	Wood fragment	*Carpinus betulus* L.	Mazandaran (Sari)	D	*A. gallica*
M6	Rhizomorph	*Fagus orientalis* Lipsky	Mazandaran (Neka)	D	*A. gallica*
M7	Rhizomorph	*Diospyros lotus* L.	Mazandaran (Neka)	D	*Armillaria* sp.
M8	Rhizomorph	*Quercus castaneifolia* C.A. Mey.	Mazandaran (Sari)	D	*A. gallica*
M9	Wood fragment	*Abies alba* Mill.	Mazandaran (Neka)	D	*A. mellea*
M11	Basidiocarp	*Parrotia persica* C.A. Mey.	Mazandaran (Neka)	N	*A. mellea*
M12	Basidiocarp	*Parrotia persica* C.A. Mey.	Mazandaran (Neka)	N	*A. mellea*
M13	Basidiocarp	*Parrotia persica* C.A. Mey.	Mazandaran (Neka))	N	*A. mellea*
G1	Wood fragment	*Alnus subcordata* C.A. Mey.	Golestan (Gorgan)	D	*Armillaria* sp.
G2	Wood fragment	*Alnus subcordata* C.A. Mey.	Golestan (Gorgan)	D	*Armillaria* sp.
G3	Rhizomorph	*Carpinus betulus* L.	Golestan (Gorgan)	D	*Armillaria* sp.
E1	Wood fragment	*Platanus orientalis* L.	Esfahan (Khomeini Shahr)	D	*A. mellea*
E2	Wood fragment	*Amygdalus communis* L.	Esfahan (Khomeini Shahr)	D	*A. mellea*
E3	Wood fragment	*Amygdalus communis* L.	Esfahan (Khomeini Shahr)	D	*A. mellea*
E4	Wood fragment	*Juglans regia* L.	Esfahan (Zarin Shahr)	D	*A. mellea*
E5	Wood fragment	*Juglans regia* L.	Esfahan (Zarin Shahr)	D	*A. mellea*
E6	Wood fragment	*Platanus orientalis* L.	Esfahan (Zarin Shahr)	D	*A. mellea*

*D - Diploid; **N - Haploid

Results and Discussion

Thirty five isolates were obtained from 15 different host species in East Azerbaijan (13 isolates), Esfahan (6), Golestan (3), and Mazandaran (13) provinces. The majority of isolates was paired with known tester strains and some of the isolates displayed incompatible reactions (Fig. 1). An evaluation of pairing tests and the results of pairing tests with known tester strains are shown in Table 2.

In East Azerbaijan, *Armeniaca vulgaris, Juglans regia, Amygdalus communis, Pyrus communis, Malus domestica,* and *Populus nigra* were identified as hosts of *A. mellea.* In Mazandaran, *Fagus orientalis* and *Parrotia persica* were identified as hosts of *A. mellea* and *Citrus aurantium* and *Abies alba* were identified as new hosts of *A. mellea. Carpinus betulus* and *Fagus orientalis* were identified as hosts of *A. gallica. Carpinus betulus* was reported as a new host plant for *A. gallica,* in Iran. The fungus has been reported on *C. betulus*

in Serbia and Montenegro (Keca *et al.* 2006). *Armillaria* sp. was collected from *Diospyros lotus* and it was not compatible with any of the tester strains. Therefore, it was reported as a new host for the genus *Armillaria* in Iran. In Esfahan, *Platanus orientalis, Amygdalus communis, Juglans regia,* and *Platanus orientalis* were identified as hosts for *A. mellea.* In Golestan, three isolates of *Armillaria* spp. were obtained from *Alnus subcordata* and *Carpinus betulus* which were not paired with any of the tester strains.

Armillaria mellea was one the most commonly found species, representing 77 % of all collections. The majority of isolates of *A. mellea* was obtained from *Armeniaca vulgaris* and *Juglans regia* in East Azerbaijan, *Citrus aurantium* and *Parrotia persica* in Mazandaran, and *Amygdalus communis, Juglans regia,* and *Platanus orientalis* in Esfahan. *Armillaria melea* was common in East Azerbaijan, Mazandaran, and Esfahan. These were the most widespread species.

Armillaria gallica was the second most commonly collected species, constituting about 10 % of the collections. *A. gallica* was the only isolate from the forest in Mazandaran. *A. gallica* has been reported in Iran (Asef *et al.* 2003), Europe (Korhonen 1978), North America (Anderson & Ulrich 1979), and Japan (Ota *et al.* 1998). This was the second most commonly collected species in Wisconsin found on angiosperms (Banik *et al.* 1995).

Four isolates from three hosts *Diospyros lotus, Alnus subcordata,* and *Carpinus betulus* were not compatible with any of the tester strains. Definitive identification of the genus *Armillaria* in Iran will require DNA-based tools.

References

Anderson, J.B. & Ullrich, R.C. 1979. Biological species of *Armillaria mellea* in North America. – Mycologia **71**: 402-414.

Asef, M.R., Goltahpeh, E. & Alizadeh, A. 2003. Identification of *Armillaria* biological species in Iran. – Fungal Diversity **14**: 51-60.

Banik, M.T., Paul, J.A. & Burdsall, H.H.Jr. 1995. Identification of *Armillaria* species from Wisconsin and adjacent areas. – Mycologia **87**: 707-712.

Ershad, D. 1995. Fungi of Iran. 2nd edn. Agricultural Research, Education and Extension Organization, Tehran.

Guillaumin, J.J., Anderson, J.B., & Korhonen, K. 1991. Life cycle, interfertility, and biological species. – In: C.G. Shaw & G.A. Kile [eds]. *Armillaria* root disease. Agriculture Handbook. No **691**. Pp. 10-20. USDA Forest Service, Washington.

Guillaumin, J.J., Mohammed, C., Anselmi, N., Courtecuisse, R., Gregory, S.C., Holdenrieder, O., Intini, M., Lung, B., Marxmuller, H., Morrison, D., Rishbeth, J., Termorshuizen, A.J., Tirro, A. & Van Dam, B. 1993. Geographical distribution and ecology of the *Armillaria* species in western Europe. – European Journal of Forest Pathology **23**: 321-341.

Hood, I.A., Redfern, D.B. & Kile, G.A. 1991. *Armillaria* in plant hosts. – In: C.G. Shaw & G.A. Kile [eds]. *Armillaria* root disease. Agriculture Handbook. No **691**. Pp. 122-149. USDA Forest Service, Washington.

Johnson, D.W. 1984. An assessment of root diseases in the Rocky Mountain Region. Technical Report R2-29. USDA Forest Service, Rocky Mountain Region, Lakewood, CO.

Keca, N., Bodles, W.J.A., Woodward, S., Karadzic, D. & Bojovic, S. 2006. Molecular-based identification and phylogeny of *Armillaria* species from Serbia and Montenegro. – Forest Pathology **36**: 41-57.

Kile, G.A. & Walting, R. 1983. *Armillaria* species from south-eastern Australia. – Transaction of the British Mycological Society **81**: 129-140.

Kile, G.A., McDonald, G.I. & Byler, J.W. 1991. Ecology and disease in natural forests. – In: C.G. Shaw & G.A. Kile [eds]. *Armillaria* root disease. Agriculture Handbook. No **691**. Pp. 102-121. USDA Forest Service, Washington.

Konhonen, K. 1978. Interferility and clonal size in the *Armillaria mellea* complex. – Karstenia **18**: 31-42.

Matsushita, N., Fukuda, K., Nagasawa, E., Terashita, T. & Suzuki, K. 1996. *Armillaria* species in Japan identified by isozyme patterns with special reference to the biological species of the Northern hemisphere. – Journal of Forestry Research **1**: 155-160.

Ota, Y., Matsushia, N., Nagasawa, E., Terashita, T., Fukuda, K. & Suzuki, K. 1998. Biological species of *Armillaria* in Japan. – Plant Disease **82**: 537-543.

Saber, M. 1974. *Armillaria* root rot. – Iranian Journal of Plant Pathology **9**: 54-62.

Sesli, E. 2007. Preliminary checklist of the macromycetes of the East and Middle Black Sea Regions of Turkey. – Mycotaxon **99**: 71-74.

Singer, R. 1978. Keys for the identification of the species of *Agaricales* I. – Sydowia **30**: 192-279.

Singer, R. 1986. The *Agaricales* in modern taxonomy. Koeltz Sci. Books, Koenigstein.

Terashita, T. & Chuman, S. 1989. Armillarias, isolated from the wild orchid, *Galeola septentrionalis.* – In: D.J. Morrison [ed.]. Proceedings of the Seventh International Conference on Root and Butt Rots. Pp. 364-371. Vernon and Victoria, Canada.

Volk, T.J. & Burdsall, H.H.Jr. 1995. A nomenclatural study of *Armillaria* and *Armillariella* species (*Basidiomycotina, Tricholomataceae*). – In: Synopsis Fungorum. Vol. **8**. Pp. 1-121. Fungiflora, Oslo, Norway.

Waltling, R., Kile, G.A. & Burdsall, H.H. 1991. Nomenclature, taxonomy, and identification. – In: C.G. Shaw & G.A. Kile [eds]. *Armillaria* root disease. Agriculture Handbook. No **691**. Pp. 1-9. USDA Forest Service, Washington.

Worrall, J.J. 1991. Media for selective isolation of hymenomycetes. – Mycologia **83**: 296-302.