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IDENTIFICATION OF IMPORTANT SPECIES OF THE GENUS *ARMILLARIA* IN HORTICULTURAL AND FOREST REGIONS OF IRAN BY CLASSIC MATING-TESTS

Forty isolates were obtained from different plant-hosts in fruit orchards and forest regions of Iran. In order to identify species of *Armillaria* diploid and haploid cultures were paired with two or three known haploid tester isolates from each intersterile group. Sexual compatibility was evaluated, based on the differences in morphology of haploid colonies from white and with fluffy to brownish, without aerial mycelium (crustose). In this study 19 plant species were introduced as hosts for *Armillaria* species. Twenty nine isolates of *Armillaria mellea* (72,5%) were obtained from following plant hosts: *Armeniaca vulgaris, Juglans regia, Amygdalus communis, Pyrus communis, Malus domestica, Populus nigra, Platanus orientalis, Citrus aurantium*, *Abies alba*, *Carpinus betulus* and *Parrotia persica. Quercus castaneifolia, Diospyros lotus* and *Fagus orientalis* were identified as the hosts of *Armillaria galica. Armillaria* spp. isolates from *Picea abies*, *Crataegus pentagyna*, *Acer* sp., *Alnus subcordata* and *Carpinus betulus* were not compatible with any of tester strains, representing different species. *Gleditsia caspia* was the new host of *Armillaria* sp. from Iran.

The genus *Armillaria* has a world-wide distribution and includes some of the most important root pathogens of forest trees and fruit crops [1]. Armillaria root disease was first reported on *Castanea crenata* in 1903 [2]. The disease also has been recorded on various planted and natural hosts in South America and Indo-Malaysia [3]. Many *Armillaria* species linked to outbreaks of the disease in South America are thought to be restricted to this area [4].

Until the late 1970's *Armillaria mellea* (Vahl:Fr.) Kumm. was considered by most researchers to be a pleiomorphic species with a wide host range and distribution. Hintikka [5] developed a technique that allowed determination of mating types in *Armillaria* and showed that *Armillaria* has a bifactorial, sexual incompatibility system. Isolates of different species of *Armillaria* produce incomepatible matings due to intersterility barriers [1, 6]. Single spore isolates of *Armillaria* species are generally white and fluffy, but when fusion of compatible mating types occurs, the coalesced colonies become dark brown, appressed, crustose and sometimes produce rhizomorphs, depending on nutritional and cultural conditions.

If the single spore isolates are from different species, the colonies will not grow together and will remain white and fluffy [5].

In Europe there are seven intersterile groups or biological species [1, 7, 8]. They belong to exanulates species *Armillaria tabescens* (Scop.: Fr.) Emel and *A. ectypa* (Fr.) Lamoure and the other species with annulate basidiomes, *A. mellea* (Vahl: Fr.) Kumm., *A. gallica* Marxm.: Romagn. (syn.: *A. bulbosa* (Barla) Velen.); *A. cepistipes* Velen., *A. borealis* Marxm.: Romagn. and *A. ostoyae* (Romagn.) Herink.

Anderson and Ullrich [6] applied the techniques used by Korhonen for isolates collected from widely distributed locations in North America and demonstrated that what had been considered as *A. mellea* in North America was actually for 10 distinct biological species. Several species of *Armillaria* are known to occur in Australia and New Zealand and these ones have been reasonably well studied [9, 10].

The existence of 10 intersterile groups of *Armillaria* was determined by pairing haploid single spore isolates in a wide geographic distribution in Japan. Of the 10 intersterile groups, identified in Japan, 7 were authenticated as *A. gallica*, *A. nabsnona*, *A. ostoyae*, *A. cepistipes*, *A. mellea*, *A. sinapina* or *A. tabescens*. Three of the groups were not compatible with any of the tester species [11].

In Iran *A. mellea* is widely distributed throughout the country and is a well known causal agent of root rot diseases [12–14]. The disease was reported in association with many cultivated and forest tree species [15].

The objectives of this study were determination of the species of *Armillaria* occurring on the trees of fruit orchard and forest regions in Iran.

Materials and Methods.

Sampling and fungal isolation. The roots, barks, and woods of the trees with suspicious symptom of *Armillaria* infection, with mycelial fans or rhizomorph signs or basidiocarps, were collected from various regions of Mazandaran, East Azerbaijan and Esfahan, the main forestry and horticultural provinces in Iran. Forty samples were made from the 19 different host species.

Table 1

Species, geographic location and source of European haploid testers of Armillaria spp.

Taxonomic Name	Isolate Code	Collectors Name	Determined By	Geographic Location
Armillaria borealis Marxm. & Korhonen	99 68/4	K. Korhonen	K. Korhonen	Finland
A. borealis Marxm. & Korhonen	n, 2n	M.R. Asef	M.R. Asef	Iran
A. cepistipes Velen.	MB 79.23.1	J.J. Guillaumin	K. Korhonen	Finland
A. cepistipes Velen.	MB 79.24.1	J.J. Guillaumin	K. Korhonen	Finland
A. gallica Marmx. & Romagn.	ME 70.1.2	J.J. Guillaumin	J.J.Guillaumin	France
A. gallica Marmx. & Romagn.	n, 2n	M.R. Asef	M.R. Asef	Iran
A. mellea (Vahl) P. Kumm.	87 085/10	K. Korhonen	Grillo	Italy
A. mellea (Vahl) P. Kumm.	90 254/3	K. Korhonen	Grillo	Italy
A. mellea (Vahl) P. Kumm.	90 260/1	K. Korhonen	Munda	Yugoslavia
A. ostoyae (Romagn.) Herink	99 088/3	K. Korhonen	K. Korhonen	Finland
A. ostoyae (Romagn.) Herink	MC 79.27.1	J.J. Guillaumin	K. Korhonen	Finland
A. sinapina Bérubé & Dessur.	96–7–1	Yuko Ota	Yuko Ota	Japan
A. sinapina Bérubé & Dessur.	96–7–2	Yuko Ota	Yuko Ota	Japan
A. tabescens (Scop.) Emel	NT 1-9	Yuko Ota	Yuko Ota	Japan
A. tabescens (Scop.) Emel	NT 1-10	Yuko Ota	Yuko Ota	Japan

The infected tissues or basidiocarpes were sterilized in ethanol 96 % for 1 *min*, and small pieces from parts of the tissues were excised and placed on the Petri dishes, including 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. The Petri dishes were incubated at 22±1(^0C) [16].

Identification of Armillaria mellea by compatibility tests. Compatibility tests of the Iranian diploid and haploid isolates were done by Korhonen [1] method, using the 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. Haploid tester strains from the species of *Armillaria,* and the geographic sources are indicated in Table 1.

Results and Discussion.

Distribution of the genus Armillaria. Forty isolates were obtained from the 19 different hosts in East Azerbaijan, Esfahan and Mazandaran provinces. In East Azerbaijan the fungus was presented in Khosro Shahr, Osko, Gokan, Azarshahr and Milan on the different species of horticultural plants, such as *Armeniaca vulgaris, Juglans regia, Amygdalus communis, Pyrus communis, Malus domestica* and *Populus nigra*.

In Esfahan province different samples were surveyed in Khomeni Shahr, Asghar Abad, Sahraie Koshek, Ghaleh Sefid, Zarin Shahr and Cham Hidar. The fungi were isolated from *Amygdalus communis, Juglans regia* and *Platanus orientalis* in Cham Hidar, Khomeni shahr and Sahraie Koshek regions.

In Mazandaran province *Fagus orientalis*, *Parrotia persica*, *Citrus aurantium*, *Abies alba*, *Carpinus betulus*, *Diospyros lotus, Alnus subcordata*, *Acer* sp., *Picea abies*, *Gleditsia caspia, Quercus castaneifolia* and *Crataegus pentagyna* were reported as the hosts species for the genus *Armillaria*. The fungi were collected from forest regions of Behshar and Neka in east regions, Sari and Chamestan, in the center and west regions of Mazandaran respectively.

Mating analysis. Pairing tests based on sexual behavior and *in vitro* somatic compatibility of isolates of *Armillaria* have been widely used to assess the interspecific incompatibility in the genus *Armillaria*. In the experiment after 6–8 weeks the pairing of the haploid tester strains with the haploid and diploid Iranian isolates were surveyed. At first the haploid monosporous cultures exhibited white and fluffy colony morphology. After compatible mating the culture morphology changed to crustose, which is characteristic of diploid mycelium.

To identify the isolates, the total of 2400 pairings was performed, using a set of two or three testers from each of the seven *Armillaria* species. The majority of isolates were paired with the known tester strain, and some of isolates showed the incompatible reaction. The results from the pairing test method allowed the identification of thirty two isolates, and the remaining isolates have shown ambiguous reactions (Table 2).

In East Azerbaijan 11 isolates of *A. mellea* were obtained from the different orchard plant species. *Armeniaca vulgaris, Juglans regia, Amygdalus communis, Pyrus communis, Malus domestica* and *Populus nigra* were identified as the hosts

for *A. mellea*. The results have shown that *A. mellea* is very important, and it is present in some horticultural regions of East Azerbaijan.

Table 2

Armillaria isolates from Iran: source tissues, host trees, origin, ploidy of cultures, identifications based on pairing-test

Isolate №	Derivation	Host	Localities	Ploidy	RFLP results
A1	Wood fragment	*Armeniaca vulgaris* Lam.	East Azerbaijan (Azar Shahr)	D	*A.mellea*
A2	Wood fragment	*Amygdalus communis* L.	East Azerbaijan (Azar Shahr)	D	*A.mellea*
A3	Wood fragment	*Juglans regia* L.	East Azerbaijan (Azar Shahr)	D	*A.mellea*
A4	Wood fragment	*Pyrus communis* L.	East Azerbaijan (Azar Shahr)	D	*A.mellea*
A5	Wood fragment	*Armeniaca vulgaris* Lam.	East Azerbaijan (Kandovan)	D	*A.mellea*
A6	Mycelium	*Juglans regia* L.	East Azerbaijan (Azar Shahr)	D	*A.mellea*
A7	Wood fragment	*Juglans regia* L.	East Azerbaijan(Khosroshar)	D	*A.mellea*
A8	Wood fragment	*Juglans regia* L.	East Azerbaijan(Khosroshar)	D	*A.mellea*
A9	Wood fragment	*Armeniaca vulgaris* Lam.	East Azerbaijan (Oskoh)	D	*A.mellea*
A10	Wood fragment	*Malus domestica* Borkh.	East Azerbaijan (Kandovan)	D	*A.mellea*
A11	Wood fragment	*Populus nigra* L.	East Azerbaijan (Oskoh)	D	*A.mellea*
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	*Platanus orientalis* L.	Esfahan(Zarin Shahr)	D	*A.mellea*
E6	Wood fragment	*Amygdalus communis* L.	Esfahan(Khomeini Shahr)	D	*A.mellea*
E7	Wood fragment	*Amygdalus communis* L.	Esfahan(Khomeini Shahr)	D	*A.mellea*
M1	Basidiocarp	*Citrus aurantium* L.	Mazandaran(Tonekabon)	N	*A.mellea*
M2	Wood fragment	*Abies alba* Mill.	Mazandaran(Neka)	D	*A.mellea*
M3	Basidiocarp	*Parrotia persica* C.A. Mey.	Mazandaran(Neka)	N	*A.mellea*
M4	Basidiocarp	*Parrotia persica* C.A. Mey.	Mazandaran(Neka)	N	*A.mellea*
M5	Basidiocarp	*Parrotia persica* C.A. Mey.	Mazandaran(Neka)	N	*A.mellea*
M6	Basidiocarp	*Parrotia persica* C.A. Mey.	Mazandaran(Neka)	N	*A.mellea*
M7	Basidiocarp	*Picea abies* (L.) Karst.	Mazandaran(Sangedeh)	D	*Armillaria sp.*
M8	Basidiocarp	*Citrus aurantium* L.	Mazandaran(Tonekabon)	N	*A.mellea*
M9	Basidiocarp	*Citrus aurantium* L.	Mazandaran(Tonekabon)	N	*A.mellea*
M10	Wood fragment	*Carpinus betulus* L.	Mazandaran(Behshar)	D	*Armillaria sp.*
M11	Wood fragment	*Crataegus pentagyna* Waldst. et Kit.	Mazandaran(Behshar)	D	*Armillaria sp.*
M12	Basidiocarp	*Alnus subcordata* C.A. Mey.	Mazandaran(Chamestan)	D	*Armillaria sp.*
M13	Basidiocarp	*Carpinus betulus* L.	Mazandaran(Sangedeh)	D	*Armillaria sp.*
M14	Wood fragment	*Fagus orientalis* Lipsky	Mazandaran(Behshar)	N	*A.mellea*
M15	Wood fragment	*Carpinus betulus* L.	Mazandaran(Behshar)	D	*A.mellea*
M16	Basidiocarp	*Carpinus betulus* L	Mazandaran(Sangedeh)	D	*A.mellea*
M17	Wood fragment	*Carpinus betulus* L.	Mazandaran(Chamestan)	D	*Armillaria sp.*
M18	Rhizomorph	*Fagus orientalis* Lipsky	Mazandaran(Neka)	D	*A.gallica*
M19	Rhizomorph	*Diospyros lotus* L.	Mazandaran(Neka)	D	*A.gallica*
M20	Rhizomorph	*Quercus castaneifolia* C.A.Mey.	Mazandaran(Sari)	D	*A.gallica*
M21	Wood fragment	*Gleditsia caspia* Desf.	Mazandaran(Chamestan)	D	*Armillaria sp.*
M22	Wood fragment	*Acer* sp.	Mazandaran(Chamestan)	D	*Armillaria sp.*

Seven isolates were collected from Esfahan. *Amygdalus communis, Juglans regia* and *Platanus orientalis* were identified as hosts for *A. mellea* in Esfahan. The fungus was present in some parts of the orchard regions, and it seems in some locations that the other root rot pathogens are more important than the genus *Armillaria*.

Twenty two isolates were obtained from Mazandaran. About 63,6% of isolates of the provinces were identified. Eleven isolates of *A. mellea* were recovered from *Fagus orientalis*, *Abies alba*, *Carpinus betulus*, *Citrus aurantium* and *Parrotia persica,* and three isolates of *A.gallica* were obtained on *Fagus orientalis*, *Diospyros lotus* and *Quercus castaneifolia*.

In this study *Armillaria mellea* (Vahl:Fr.) P. Kumm. was the most commonly found species, representing 72,5% of the collections. *A. mellea* is the causal agent of root rots of numerous perennial woody in Italy [17]. Asef *et al*. [12] reported 50% of the collections identified *A. mellea*, which was the most common, comprising the collections. *A. mellea* was reported on *Corylus* sp. in some regions of Turkey [18]. *A. mellea* was present in coniferous and broad-leaved forests in most of the areas except the high altitudes of the mountains of north Greece. It was found to cause significant damage in fir forests as well as in fruit orchards and vineyards [19].

Armillaria gallica was the second most commonly collected species, constituting 7,5 % of the collections. *A. gallica* was only isolated from the forest in Mazandaran. *A. gallica* has been reported from Iran [12], Europe [1], North America [6] and Japan [11]. It was the second most commonly collected species in Wisconsin that was found on Angiosperms [20]. *A. gallica* was common in coniferous and broad-leaved forests in the high altitudes of central and northern Greece, predominating in the beech forests. The fungus was a weak parasite or a saprophyte of forest trees and was occasionally found on cultivated plants [19].

Eight isolates from six hosts: *Picea abies*, *Crataegus pentagyna*, *Acer* sp., *Alnus subcordata, Gleditsia caspia* and *Carpinus betulus* were not compatible with any of tester strains. Therefore, DNA-based tools are necessary in order to obtain definitive identification the genus *Armillaria* in Iran. *Gleditsia caspia* is reported as a host for the first time in Iran.

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ԴԱՍԱԿԱՆ ՋՈՒԳԱՎՈՐՄԱՆ ՄԵԹՈԴՈՎ *ARMILLARIA* ՑԵՂԻ ԿԱՐԵՎՈՐ ՏԵՍԱԿՆԵՐԻ ՈՐՈՇՈՒՄԸ ԻՐԱՆԻ ԱՆՏԱՌԱՅԻՆ ՇՐՋԱՆՆԵՐՈՒՄ ԵՎ ԱՅԳԻՆԵՐՈՒՄ

Ամփոփում

Ստացվել է քառասուն իզոլյատ Իրանի անտառային շրջանների և այգիների տարբեր տեր-բույսերից: *Armillaria* ցեղի տեսակների որոշման համար դիպլոիդ և հապլոիդ կուլտուրաները զուգավորվել են յուրաքանչյուր ինտերստերիլ խմբի երկու-երեք հապլոիդ տեստերի հայտնի իզոլյատների հետ: Գնահատվել է սեռական համատեղելիությունը՝ հիմնված հապլոիդ գաղութների մորֆոլոգիական տարբերությունների վրա: Այս հետազոտության մեջ ներկայացված են 19 տեսակ տեր-բույսեր *Armillaria* ցեղի սնկերի համար: Ստացվել են *Armillaria mellea* սնկի 29 իզոլյատներ (72,5%) հետևյալ տեր-բույսերից. *Armeniaca vulgaris, Juglans regia, Amygdalus communis, Pyrus communis, Malus domestica, Populus nigra, Platanus orientalis, Citrus aurantium*, *Abies alba*, *Carpinus betulus* և *Parrotia persica*: *Quercus castaneifolia*-ն, *Diospyros lotus*-ը և *Fagus orientalis*-ը որոշված են որպես *Armillaria galica*-ի տեր-բույսեր: *Picea abies*, *Crataegus pentagyna*, *Acer* sp., *Alnus subcordata* և *Carpinus betulus* տեր-բույսերից ստացված *Armillaria* spp.-ի իզոլյատները չէին համապատասխանում տարբեր տեսակներ ներկայացնող տեստերային շտամներից ոչ մեկին: *Gleditsia caspia*-ն նոր տեր-բույս է հանդիսանում *Armillaria* spp.-ի համար Իրանում:

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ОПРЕДЕЛЕНИЕ ВАЖНЫХ ВИДОВ РОДА *ARMILLARIA* В ЛЕСНЫХ ОБЛАСТЯХ И САДАХ ИРАНА МЕТОДОМ КЛАССИЧЕСКОГО СПАРИВАНИЯ

Резюме

Выделено сорок изолятов с разных растений-хозяев из фруктовых садов и лесных областей Ирана. Для определения видов *Armillaria* диплоидные и гаплоидные культуры спарены с двумя-тремя известными изолятами гаплоидного тестера из каждой интерстерильной группы. Оценена сексуальная совместимость, основанная на морфологических различиях гаплоидных колоний. В настоящем исследовании представлено 19 видов растений в качестве хозяев для видов *Armillaria*. Получено 29 изолятов (72,5%) *Armillaria mellea* со следующих растений-хозяев: *Armeniaca vulgaris, Juglans regia, Amygdalus communis, Pyrus communis, Malus domestica, Populus nigra, Platanus orientalis, Citrus aurantium*, *Abies alba*, *Carpinus betulus* и *Parrotia persica*. *Quercus castaneifolia, Diospyros lotus* и *Fagus orientalis* определены как хозяева *Armillaria galica*. Изоляты *Armillaria* spp. с *Picea abies*, *Crataegus pentagyna*, *Acer* sp., *Alnus subcordata* и *Carpinus betulus* не соответствовали ни одному из тестерных штаммов, представляющих различные виды. *Gleditsia caspia* является новым растением-хозяином для *Armillaria* sp. из Ирана.