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CHLORATE SENSITIVITY, TEMPERATURE RESPONSES AND PATHOGENICITY OF SOME IRANIAN ISOLATES OF *MACROPHOMINA PHASEOLINA* FROM OILSEED PLANTS

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The species *Macrophomina phaseolina* is the principle pathogen species of oilseed plants in Northern Iran. The result of infection is charcoal root or collar rot. Twenty-seven isolates of *M. phaseolina* were collected from plant tissues of soybean (*Glycine max* L.), sunflower (*Helianthus annuus* L.) and sesame (*Sesamum indicum* L.) growing in different areas of Northern Iran during 2008. The chlorate sensitivity of each isolate was determined after subculturing on minimal medium containing 120 mM potassium chlorate. In general, sesame isolates were chlorate-resistant, whereas soybean and sunflower isolates were chlorate sensitive. The pathogenicity was tested at the seedling stage on three plant species. None of the isolates were pathogenic on maize and all three were pathogenic on soybean and sunflower. The optimum temperature for growth was 35° for all North Iranian isolates.

Pathogenicity - Chlorate phenotype - Temperature responses – Macrophomina phaseolina - charcoal rot

Macrophomina phaseolina-ն՝ ածխային փտման հարուցիչը, բույսերի կարեվոր պաթոգեն է, հատկապես հյուսիսային Իրանում յուղատու բ*ույսերի համար: Սոյայի (Glycine max L.), արևածաղկի (Helianthus* annuus L.) և քնջութի (*Se-samum indicum* L.) հյուսվածքներից քսանյոթ իզոլյատները հավաքված են հյուսիսային Իրանի տարբեր շրջաններից 2008 թ.-ի ընթացքում: Յուրաքանչյուր իզոլյատի քլորատային զգայունությունը որոշված է 120 mM կալիումի քլորատ պարունակող մինիմալ միջավայրի վրա ինոկուլյացիայից հետո:

Սովորաբար քնջութից ստացված իզոլյատները դիմացկուն են քլորատների նկատմամբ, մինչդեռ սոյայից և արևածաղկից ստացված իզոլյատները զգայուն են: Պաթոգենության ստուգումը անց է կացվել երեք տեսակի բույ-սերի վրա՝ տնկիների փուլում: Իզոլյատներից ոչ մեկը պաթոգեն չէ եգիպտա-ցորենի համար և բոլորը պաթոգեն են սոյայի և արևածաղկի համար: 35^0 -ը օպտիմալ է բոլոր ուսումնասիրված իզոլյատների աճի համար:

Պաթոգենություն – քլորատային ֆենոտիպ – ջերմաստիճանի նկատմամբ ռեակցիա - Macrophomina phaseolina - ածխային փտում

Macrophomina phaseolina – возбудитель угольной гнили является важным патогеном растений, особенно на масличносемянных в северном Иране. Растения-хозяева собраны из различных областей северного Ирана в течение 2008 года. Было выделено 27 изолятов M. phaseolina с тканей сои (Gly-cine max L.), подсолнечника (*Helianthus annuus* L.) и кунжута (*Sesamum indicum* L.).

Хлоратная чувствительность каждого изолята определена после инокулирования на минимальной среде, содержащей 120 mM хлората калия. Обычно изоляты с кунжута устойчивы к хлоратам, тогда как изоляты с сои и подсолнечника чувствительны к хлоратам. Проверка патогенности проведена на стадии саженцев на трех видах растений. Ни один из изолятов не оказался патогенным для кукурузы, но все оказались патогенными для сои и подсолнечника. Для роста всех изученных изолятов оптимальной температурой является 35°.

Патогенность – хлоратный фенотип – реакция на температуру - Macrophomina phaseolina – угольная гниль

Macrophomina phaseolina (Tassi) Goid. is pathogen of a wide number of cultivated and wild species in warm, temperate and tropical regions of the world [5]. The fungus is a soil born pathogen, infecting about 500 plant species in more than 100 families throughout the world [7]. *M. phaseolina* is classified as a *Deuteromycetes* which shows two asexual sub-phases: one a mycelial phase and the other - a pycnidial phase [11]. The charcoal root rot (CRR) is an economically important disease of many crop plants in Asia, Africa, North and South America and some parts of Europe. The fungus is most widely distributed on soybean, sorghum, bean, cotton, corn. During 1994 the soybean yield loss due to charcoal rot in Argentina, Brazil, Canada, India, Paraguay and United States was 1.23 million metric tons [12, 13]. Despite having a wide host range, *Macrophomina* is a monotypic genus, although only one species is recognized within the genus. Great variability in morphology has been observed among isolates from different hosts [8, 9]. Many researchers have also found great variability in pathogenicity and in morphological characteristic among isolates from the same host [2]. The great variability of the fungus reflects its heterokaryotic character. The studies on nuclei of *M. phaseolina* affirmed that during hyphal fusion heterokaryosis could occur after mitotic segregation and recombination. This may explain the occurrence of cultural types or physiological races reported. Efforts to divide *M. phaseolina* into sub-species were unsuccessful mainly due to the extreme intra-specific variations in morphology and pathogenicity [3]. A recent study suggested the use of chlorate phenotypes (colony morphologies on media supplemented with 120 mM potassium chlorate) as a marker for identifying host-specific isolates of *M. phaseolina* [1, 10].

The present paper focuses on the assessment of chlorate sensitivity, temperature responses, and pathogenicity of Iranian isolates of *M. phaseolina* causing charcoal rot in soybean, sunflower and sesame.

Materials and methods. Isolation of the fungus. The samples were collected from the stems and roots of soybean, sunflower and sesame plants infected with of *M. phaseolina* infection from Mazandaran Province in Northern Iran during vegetation season of 2008. Each root or stem was thoroughly washed and dried at room temperature. Infected tissues were sterilized in 0.8% NaOCl for 1 min and washed in sterile water for 2 min. The tissues were placed on potato dextrose agar (PDA). The Petri dishes were incubated at 28±1º in the darkness for 4 days. Pure cultures were developed by single microsclerotium culture and maintained on PDA at 28±1º. Twenty seven isolates were obtained.

Chlorate sensitivity test. The chlorate phenotype of *M. phaseolina* isolates was assessed on chlorate containing minimal medium described by Pearson et al. [8]. A 5 mm culture disc from 5-day old culture growing on PDA was placed in the center of 9 cm Petri dish containing minimal medium supplemented with 120 mM of potassium chlorate. One set of cultures was grown on minimal medium without potassium chlorate as control. The cultures were incubated at 30±1° for 6 days in the dark. Each isolate was replicated twice. The growth of each isolate was recorded for phenotype of colony.

Pathogenicity study. The pathogenicity of 27 isolates from the different areas was tested on three plant species: soybean (*Glycine max* L.) sunflower (*Helianthus annuus* L.) and maize (*Zea mays* L.), at the seedling stage. Seeds of commercial varieties were sterilized with 2% sodium hypochlorite for 4 min and rinsed twice in sterile tap water.

Seeds were placed on 6-day old colonies of each *Macrophomina* isolates, and were grown on PDA in 9 cm plates at 30° in the dark. Each treatment includes six seeds that arranged in two plates, it was replicated three times in a completely randomized block design. Plates were incubated at 28±1° in the dark, and 2 ml of sterile water was added to each plate to promote seed germination. Pathogenicity was recorded 6 days after seed inoculation using the severity assessment. The disease index was calculated by multiplying the number of seeds by the degree of disease severity [6].

Temperature responses. Growth rate (GR) of 27 isolates was recorded at 30°, 35° and 40°. Culture disks with 5 mm diameter cut from the edge of a 4-day old PDA culture of each isolates, grown at 28°, were transferred to the center of 9 cm Petri dishes with 10 ml of PDA and incubated in the dark at the three different temperatures. Each treatment was replicated three times in a completely randomize design [4]. The minor and major radii of the colonies were measured after 48 hrs.

Results and Discussion. Mycelial growth of *M. phaseolina* on chlorate medium was classified into three categories. Table 1 shows the growth response of *M. phaseolina* isolated from soybean, sesame and sunflower in Petri dishes on a defined medium containing 120 mM potassium chlorate. The rating scale used was to compare with growth on medium lacking chlorate. Three various growth patterns (feathery spreading growth, restricted growth and dense growth) were observed when the isolates were grown on the minimal medium containing 120 mM potassium chlorate: feathery and restricted of soybean and sunflower isolates, feathery and dense growth of sesame isolates (Table 1).

Table 1. Chlorate phenotypes of *Macrophomina phaseolina* isolates, obtained from different hosts.

Isolates code	Source	Collection site	Reaction to chlorate	Phenotype
Se. 1	Sesame	Behshar	Resistant	Dense
Se. 2	Sesame	Galoga	Sensitive	Feathery
Se. 3	Sesame	Neka	Resistant	Dense
Se. 4	Sesame	Sari	Sensitive	Feathery
Se. 5	Sesame	Neka	Sensitive	Feathery
Se. 6	Sesame	Sari	Sensitive	Feathery
So. 1	Soybean	Behshar	Sensitive	Feathery
So. 2	Soybean	Behshar	Sensitive	Feathery
So. 3	Soybean	Behshar	Sensitive	Feathery
So. 4	Soybean	Galoga	Sensitive	Feathery
So. 5	Soybean	Galoga	Sensitive	Feathery
So. 6	Soybean	Galoga	Sensitive	Feathery
So. 7	Soybean	Galoga	Sensitive	Restricted
So. 8	Soybean	Ghamemshar	Sensitive	Feathery
So. 9	Soybean	Ghamemshar	Sensitive	Feathery
So. 10	Soybean	Ghamemshar	Sensitive	Feathery
So. 11	Soybean	Goybar	Sensitive	Feathery
So. 12	Soybean	Goybar	Sensitive	Feathery
So. 13	Soybean	Goybar	Sensitive	Feathery
So. 14	Soybean	Neka	Sensitive	Feathery
So. 15	Soybean	Neka	Sensitive	Feathery
So. 16	Soybean	Sari	Sensitive	Feathery
So. 17	Soybean	Sari	Sensitive	Feathery
So. 18	Soybean	Sari	Sensitive	Feathery
So. 19	Soybean	Sari	Sensitive	Feathery
So. 20	Soybean	Sari	Sensitive	Feathery
Su. 1	Sunflower	Neka	Sensitive	Feathery

The restricted and feathery isolates were sensitive to chlorate, whereas dense isolates were resistant to chlorate. The host had significant effects on chlorate phenotype of *M. phaseolina* isolates. Among soybean isolates, feathery isolates were much more abundant than either restricted or dense isolates

whereas dense isolates predominated in sesame roots. Sclerotia production on chlorate medium by the sensitive isolates was very low when compared to resistant ones. Generally, isolates from sesame grew more rapidly on the medium containing chlorate than isolates from soybean or sunflower. All isolates had dense growth when they were grown on the minimal medium without chlorate and could not be differentiated.

In our investigation all soybean and sunflower isolates were not pathogenic on maize. Sunflower and soybean isolates were pathogenic on the both of them. The pathogenicity of isolates on soybean and sunflower shows that *M. phaseolina* from soybean and sunflower may be a potential pathogen on other crops in Northern Iran, especially under water stressed conditions. The pathogenicity test showed that soybean and sunflower plants are susceptible while maize plant is resistant to *Macrophomina* (Table 2).

Table 2. Pathogenicity of 24 isolates *M. phaseolina* on three plant species

Species	Name of Cultivars	Average
Glycine max	Willyams	28.56 A
Helianthus annuus	Shafagh	22.48 B
Zea mays	307	0 C

Moreover, repeatedly cultivation of susceptible plants such as soybean, sunflower and sesame are increased the density of sclerotium in the soil. Cultivation of resistant species such as maize and other cereals in crop rotation in northern Iran, enable to reduce soil infection and incidence of diseases. A temperature of 35° was optimal for all Mazandaranian isolates and no isolates growth at 40°.

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