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Isolation and Some Pathologic Properties of *Rhizoctonia zeae* from Cultural Soils of Golestan and Mazandaran Provinces, Iran

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Abstract: *Rhizoctonia* is a soil inhabitant fungus with many species and wide host range. As a facultative parasite, baiting is a shortcut for its isolation from soil. In current research, some soil samples were prepared from soils of cultivated fields and gardens of Golestan and Mazandaran provinces. Some sheath blight bearing symptom plant materials were subjected to sampling. Many *Rhizoctonia* isolates were isolated then cultures with orange to red soft sclerotes and orange mycelium were identified as *R. zeae*. Fifty seven from total of seventy had successful anastomosis with *R.zeae* CBS 384.34 as standard. Pathogenic ability of some isolates were tested on young seedling in petri plates on corn, wheat, cucumber, squash and radish. Pathogenicity tests had positive results as rot symptoms on emerged roots. This is the first report for pathogenicity of *R.zeae* on wheat sheath, cucumber, squash and radish from Golestan and Mazandaran provinces. Baiting is an easy and useful method on preliminary ecological and pathological studies of *Rhizoctonia* spp.

Key words: *Rhizoctonia zeae* · Corn · Wheat · Cucumber · Squash · Sheath blight

INTRODUCTION

The genus *Rhizoctonia* is an imperfect fungus and has many soil inhabitant species. Some species are facultative parasite, some are saprophyte and few symbiont [1]. *Rhizoctonia* is an important plant pathogen with a wide host range [2]. Different *Rhizoctonia* species are distinguishable by morphological cultural, molecular structural properties [3]. First description of *Rhizoctonia* was introduced by De Candolle in 1815, later Kuehn in 1858 named fungus on potato tubers by black sclerotia *Rhizoctonia zeae*. Complete definition of *Rhizoctonia* species took place by Ogoshi (1984) after introducing *Waitea*, *Thanatephorus* and *Ceratobasidium* and their nuclear status [4]. *Rhizoctonia zeae* have more than two nucelui with *Waitea* teleomorph. *Waitea circinata* has two anastomosis group named WAG-O and WAG-Z related to *R. oryzae* and *R. zeae* respectively [5]. Based on morphological characters of the colony three varieties were introduced by Gunnell (1986): *W.circinata* var. *circinata* with more than 2 mm orange to pale brown sclerotia, *W. circinata* var. *oryzae* with irregular sclerotes and *W. circinata* var. *zeae* with more than 1mm irregular sclerotes. Gunnel based on Warcup & Talbot, description didn't introduce any anamorphic name for *Waitea circinata*. Until Leiner & Carling [6] for complexity of anamorph and teleomorph names and rarity of teleomorph used *Rhizoctonia* again. Their discussions on *Waitea* anamorph-teleomorph complexity facilitates suggestion of a new grouping system similar of *R.solani*.In current research an easy method for *R.zeae* isolation and pathogenicity tests were improved to study different properties of the isolated fungi in Golestan and Mazandaran provinces.

MATERIALS AND METHODS

Sampling, Isolation and Purification: Random selected soil samples from 10-15cm depth of fields and gardens of Golestan and Mazandaran provinces were transferred to

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laboratory then 9 cm petri plates filled with soil samples. After adding water at field capacity, boiled sugar beet seeds and tooth picks were covered with soil as baits. After two days, the baits were transferred on 1.5% water agar medium (WA). Two days later the appeared fungal colonies were transferred to PDA slants by hyphal tip method. The isolates were stored at 4°C for next applications [7].

Hyphal Diameter and Nucleus Counting: Some five mm diameter agar disks from fresh 2-3 days culture were transferred on sterile microscopic slides on petri plates. Three days later the agar was removed then a drop of Safranin-O plus one drop of 3% KOH covered the grown mycelia on slide. Then number of nuclei per cells and hyphal diameter were determined by light microscope [8].

Morphological Properties of Colony and Growth Temperatures: Morphological view of mycelia growth on petri plates, colour, sclerotial formation and morphology were determined based on Kim *et al.* [9] proposed method. Sclerotial dimensions were assayed by Sairan stero microscope and ocular micrometer.

Anastomosis Group (AG) Determination: The type species of *R. zeae* (CBS 384.34) were subjected as standard isolate of *R. zeae* for AG determination experiments. Crossing between standard and field isolates were tested based on Kornland & Stanghellini [10] and Dhingra &Sinclair [11].

Pathogenicity Tests: Fresh seeds of corn, wheat, cucumber, radish and squash were surface sterilized with 2% chlrorax for ten minutes. The seeds were transferred to sterile petri plates on wet filter paper. A five mm agar disk containing fresh cultures of testing isolates were close contacted to young seedling of selected host plants. A check plant inoculated just with agar was selected for treatments. Inoculated treatments were stored at 28°C up to appearing probable infection symptoms.

RESULTS AND DISCUSSION

A total of 604 samples from Golestan and Mazandaran cultural and garden soils were transferred to lab. The baiting was a so suitable method for *Rhizoctonia* isolation and could isolate it from many soil samples. Some isolates were harvested from blighted sheath and leaves of corn and sorghum (Figure 1). More than seventy isolate of *Rhizoctonia zeae* were harvested in result of

Fig. 1: Symptoms on corn plant (*Zea mays*) caused sheath blight by *Rhizoctonia zeae* (left picture). Start stem rot (right p.)

isolation process. Among them 57 were determined as WAG-Z (*Waitea* Anastomosis Group-*Zeae*). The fungus were isolated from all geographical regions from west of Mazandaran to east of Golestan provinces. The WAG-Z was reported as a first report from Iran by Aghajani [12] isolated from Gramineous host plants in control regions of Mazandaran. The first description of *R. zeae* was introduced by Voorhees [13] as causal of corn sclerotial rot. Fungal colony on PDA white changing to orange after seven days inverse color of colonies was orange too (Figure 2). Sclerotes formation were observed 5-12 days after inoculation on petri plates, primarily were white to yellow, finally orange to pale red. Their diameters were measured as 0.06-0.06 × 0.81- 1.35 mm, irregular, globose to ellipsoid, mostly single rarely in two- three pairs. The nuclear numbers in cells were counted between 3-12, hyphal diameter 3-12µm and optimum growth temperature was determined in 32°C.

Morphological features of surveyed isolates had complete coincidence with Aghajanies descriptions [14]. Orang to pale red color of colony and sclerotes distinguish separated the isolates from *R. solani*. In pathogenicity tests, the first symptoms of infection were appeared on corn and wheat seedlings four days after inoculation (Figure 3) without any symptoms in check plants, so cucumber, squash and radish seedling were showed symptom six day after inoculation too. Green house test on flag sheath of wheat (Figure 4) had similar symptoms close to eye spot of wheat reported by Rahimian [15]. Li *at al*. [16] were introduced *R. solani* AG-1-IA, *R. solani* AG-1-IB, AG-4, AG-5 and *R.zeae* and binucleate AG-K as causal agents of corn sheath blight. Except *R.zeae* and *R. solani* AG-1-IA other agents

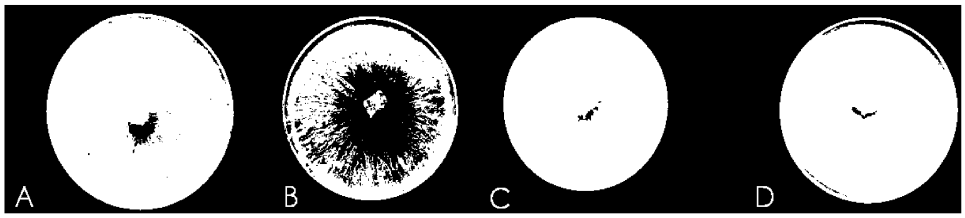

Fig. 2: Anastomosis group *Rhizoctonia zeae* grown on PDA after 12 dayes. A-112 (Nezam Abad). B-520 (Noshahr). C- 448 (Gonbad). D- *Rhizoctonia zeae* Voorhees CBS.

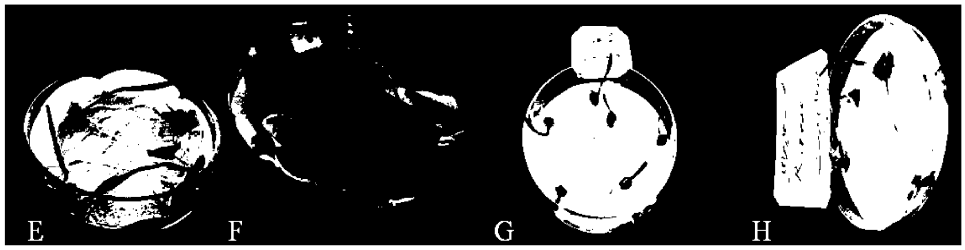

Fig. 3: Pathogenicity test on corn and wheat. E, F- respectively wheat control seedling and symptoms caused by isolate 400 on wheat sheath. H, G - Corn control seedling and symptom caused by isolate 448 on corn sheath.

Fig. 4: Pathogenicity on flag sheaths of adult wheat. Isolate 448 (right), check (center), Blight on corn sheath by isolate 400 (left).

have weak pathogenicity on corn and winter wheat have a few probability to infect with current agents for temperatures below 30°C [17]. There is a report on *R.zeae* pathogenicity on corn, wheat, soybean, creeping bentgrass and cotton from Mississippi [18].

Some tested host plants are new for hosts of *R. zeae* in Iran. Prior complete survey of *R. zeae* and introducing new hosts were presented by Aghajani [14]. This research introduces wheat sheath blight, radish, cucumber and squash root rot as new disease caused by *R. zeae* from Golestan and Mazandaran provinces of Iran.

CONCLUSION

Rhizoctonia zeae as a facultative parasite is present in soil of gardens and cultivated fields of Golestan & Mazandaran and have potential for pathogenicity on many vegetables and cultivated plants. The baiting is an easy and very successful method for trapping *R. zeae* from soil. Many cellulosic materials can be used as bait for its isolation. These results are very useful for ecological and pathological studies of *Rhizoctonia* and *R. zeae* in different levels as morphological or molecular.

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