J. King Saud Univ., Vol. 9, *Agric. Sci.* (2), pp. 337-340, Riyadh (1417/1997)

Identification of Anastomosis Groups of *Rhizoctonia solani* Kuhn Causing Alfalfa Root Canker in Riyadh Area of the Kingdom of Saudi Arabia

S. M. Al-Kherb*, A. A. Al-Doss and S. El-Hussieni***

** Departement of Plant Protection, College of Agriculture, King Saud University, Riyadh*

*** Department of Plant Production, College of Agriculture, King Saud University, Riyadh,, Saudi Arabia*

(Received 17/12/1416; accepted for publication / /1417H)

Abstract. This is the first report on Anastomosis Group (AG) of *Rhizoctonia solani* Kuhn on alfalfa in the Kingdom of Saudi Arabia. Thirteen isolates collected from alfalfa plants showing symptoms of root, crown and stem canker in alfalfa commercial fields in Riyadh area, were identified as *R. solani* and tested for AG type with known tester isolates (AG1-AG9). Ten isolates were classified as AG4 isolates. Remaining three isolates did not anastomose with any of the AG tester isolates. The obtained results indicated the importance of AG4 type of *R. solani* in Saudi Arabia.

Introduction

Rhizoctonia solani Kuhn is a soil-born fungal pathogen that causes diseases in alfalfa (*Medicago sativa* L.) and many other crops which includes damping off, root canker, crown and bud rot, stem blight and foliage blight [1, p. 30-32]. *Rhizoctonia*-caused diseases have been reported from all alfalfa growing areas of the world with most damage during periods of high temperature and high soil moisture [2,1, p. 30-32]. *Rhizoctonia* root canker is common in irrigated alfalfa fields in southwestern U.S.A., Mexico, Australia and the Middle East [1, p. 30-32,3,4,5]. In the Kingdom of Saudi Arabia, *R. solani* was reported to cause damping-off, root rot and crown rot on alfalfa in several regions [6,7]. It was identified as the most common pathogen isolated from alfalfa and also considered as a major cause of yield loss and stand decline in alfalfa [7].

R. solani is currently composed of several anastomosis groups (AGs) distinguished by hyphal anastomosis. These groups differ morphologically, physiologically, and

serologically in host range, virulence and distribution in nature [8,9]. To date, isolates of *R. solani* have been assigned to 11 anastomosis groups [10]. Vincell and Herr [5] observed that two different diseases of alfalfa are caused by *R. solani* in Kentucky, U.S.A. They found that isolates from plants with symptoms of web blight and stem canker were identified as *R. solani* AG1 and AG4 respectively. Pathogenicity was confirmed for both diseases [5].

The objective of this study was to identify the anastomosis groups (AGs) of *R. solani* associated with crown rot in commercially grown alfalfa in Riyadh area.

Material and Methods

Alfalfa plants showing symptoms of root, crown and stems cankers were collected from alfalfa commercial fields in Riyadh area beginning in April, 1995. Small pieces of tissue from the margins of lesions on stem crowns and roots were surface disinfected in 1% sodium hypochlorite for 3 min, plated on potato dextrose agar (PDA) and incubated at 27°C. Hyphal tipped isolates identified as *Rhizoctonia* spp. were transferred to PDA slants and stored at 20°C.

Thirteen isolates of *R. solani* from alfalfa plants were used for the determination of Anastomosis Groups (AG) by using agar disks (8 mm diameter) containing mycelium of *R.* solani from the margins of an unknown isolate and appropriate tester strain of known AGs (AG1 - AG9 kindly provided by Dr. Ogoshi, A. at Hakkaido University, Japan). Anastomosis group identities were determined according to the modified method of Parameter et al. [8]. Isolates were paired on 3 X 1.5-cm rectangles of cellophane placed on 1.5% water agar in petri dishes. Cellophane rectangles had been dipped in soft (13 g/L) PDA before being placed on water agar. Disks of mycelium and agar (8mm diameter) from the margins of unknown isolate and an appropriate tester strain were placed on the cellophane rectangle approximately 2cm apart. Mycelial disks of tester and field isolates were produced on PDA. Anastomosis plates were incubated at room temperature in the dark until hyphae overlapped, usually 48-72 hr. The area of cellophane upon which hyphae overlapped was then removed from the agar, placed on a slide, stained with safranin -o-, and examined microscopically (400X) for hyphal anastomosis [11]. The fusion of cell wall and plasmalemma accompanied by death of anastomosing and adjoining cells (K reaction) was sought and at least five fusion sites were required for each positive anastomosis reading.

Results and Discussion

The cultural characteristics of the isolates were typical of *R. solani* Kuhn.[12,13,10] and all were multinucleate which is a characteristic of *R. solani*. Hyphal anastomosis of ten isolates from the thirteen isolates obtained from infected roots and stem crowns of

diseased alfalfa plants in Riyadh area were anastomosed with the known isolate AG4. Three isolates did not anastomose with any of the AGs tester isolates.

Symptoms of stem canker of alfalfa cultivars were characterized as *R. solani* AG4. Typical cortical and crown rot lesions developed on plants inoculated with *R. solani* AG4. Symptoms on inoculated plants resembled those observed in the field. Typical cortical decay and brown sunken canker or girdling of the root were observed. Infection occurs at the points of emerging secondary root and extend below the soil surface.

The obtained results indicated the importance of AG4 isolates in Saudi Arabia. As three isolates did not anastomose with any of the tester isolates, this indicated the possibilty of the presence of a new types on alfalfa in Saudi Arabia. Further work is needed to clarify the picture of anastomosis groups on alfalfa in the Kingdom of Saudi Arabia.

References

[1] Graham, J. H., Frosheiser, F. I., Stuteville, D. L., and Erwin, D. C. *A Compendium of Alfalfa Diseases.* American Phytopathological Society Press, 1987.

[2] Graham, J. H., Kreitlow, K. W. and Faulkner, L. R. "*Alfalfa Diseases.*" p. 497-526. In: C. H. Hanson (Ed.) "*Alfalfa Science and Technology*" *Agrononmy monograph* .No. 15 (1972). Madison, Wisconsin: American Society of Agronomy, Inc., U.S.A. 509-514.

[3] Roberts, D.A. "Important Diseases of Alfalfa in North Florida." *Proceedings of the Thirty-first American alfalfa Improvement Conference,* U.S.A., 1988, p. 10.

[4] Lomeli, O.C., Leyva, M.G. and Castro, A. L. "Root Diseases of Alfalfa Transmission." *Revista-exicana-de Fitopatologia,* 9, No. 1 (1991), 8-11.

[5] Vincell, P.C. and Herr, L.J. "Two Diseases of Alfalfa Caused by *Rhizoctonia solani* AG1 and AG4." *Plant Disease,* 76, No. 12 (1992), 1283.

[6] Abul-Hayja, Z.M., Al-Hazmi, A.S. and Trabulsi, I.Y. "A Preliminary Survey of Plant Diseases in Al-Kharj Region, Saudi Arabia." *Phytopath. Medit.,* 22, No. 1 (1983), 65-70.

[7] Kassim, M.Y., Bokhary, H. A. and Abou-Heilah, N. A. "General Survey of Plant Disease and Pathogenic Organisms in Saudi Arabia." *Saudi Biological Society,* No. 4 (1987). 20-21.

[8] Parameter, J.R., Jr., Sherwood, R.T. and Platt, W.D. "Anastomosis Grouping Among Isolates of *Thanatephorus cucumeris.*" *Phytopathology,* 59, No. 9 (1969), 1270-1278.

[9] Adams, G.C., and Bulter, E.E. "Serological Relationships Among Anastomosis Groups of *Rhizoctonia solani.*" *Phytopathology,* 69, No. 5(1979), 629-633.

[10] Sneh, B., Burpee, L. and Ogoshi, A. Identification of *Rhizoctonia* Species. St. Paul. MN: *American Phytopathological Society.*, (1991), p.133.

[11] Carling, D. E., and Leiner, R. H. "Isolation and Characterization of *Rhizoctonia solani* and Binucleate *R. solani*-like Fungi from Aerial Stems and Subterranean Organs of Potato Plants". *Phytopathology*, 76, No. 7 (1986), 725-729.

[12] Dillard, H. R. "Characterization of Isolates of *Rhizoctonia solani* from Lima Beans Grown in New York State." *Phytopathology,* 77, No. 5 (1987), 748-751.

[13] Ogoshi, A. "Ecology and Pathogenicity of Anastomosis and Intraspecific Group of *Rhizoctonia solani* Kuhn." *Annual Review of Phytopathology,* 25 (1987), 125-143.

تعريف مجموعات اتحاد الخيوط الفطرية للفطر رايزوكتونيا سولاني المسبب لتقرح جذور البرسيم في منطقة الرياض في المملكة العربية السعودية

سليمان محمد الخرب*، عبدالله عبدالعزيز الدوس، وصلاح الدين الحسيني***

** قسم وقاية النبات، جامعة الملك سعود، الرياض** قسم الانتاج النباتي، جامعة الملك سعود، الرياض*
المملكة العربية السعودية

(قدم للنشر في ١٤١٦/١٢/١٧هـ وقبل للنشر في ١٤١٧/٨/٢٠هـ)

ملخص البحث: هذا هو اول تقرير عن مجموعة اتحاد الخيوط الفطرية (AG) للفطر رايزوكتونيا سولاني *Rhizoctonia solani* Kuhn على البرسيم في المملكة العربية السعودية. عرفت ثلاثة عشر عزلة على انها الفطر رايزوكتونيا سولاني كان قد تم جمعها من حقول برسيم تجارية في منطقة الرياض ظهرت عليها اعراض تقرح الجذور والتيجان والساق وتم اختبار مجموعة اتحاد خيوطها الفطرية مع عزلات اختبار معرفة (AG1-AG9). وقد اظهرت النتائج ان عشرا من العزلات التي تم اختبارها تنتمي الى مجموعة AG4 اما العزلات الثلاث الباقية فلم تتحد خيوطها الفطرية مع الخيوط الفطرية لاي من عزلات الاختبار. دلت النتائج المتحصل عليها على اهمية عزلات الفطر رايزوكتونيا سولاني التابعة للمجموعة AG4 في المملكة العربية السعودية.