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New Anastomose Group of Binucleate *Rhizoctonia* Causing Leaf Wilt Disease of *Erica* sp

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Abstract. Wilt leaves of *Erica* sp. was observed at a flower cultivation base in JiYang county of Shandong Province in China. The disease of *Erica* sp. was determined according to Koch's postulation. The pathogen was isolated and purified from diseased leaves, and was identified as binucleate *Rhizoctonia* sp. according to its morphological characteristics and DNA sequencing of the internal transcribed spacer (ITS). All hyphal anastomosis reactions using representative isolates revealed that they were belonged to anastomosis group U(AG-*Erica*). Based on the results of hyphal anastomosis and sequence analysis of an rDNA-ITS region and the phylogenetic tree, the AG-*Erica* is considered as a new anastomose of *Rhizoctonia* spp. This is the first report that AG-*Erica* was found in the *Erica* sp. in China.

Introduction

Erica is belong to Ericaceae and its leaves maintain all the year round. There are 860 species of *Erica* in the world, mainly distribute in South Africa and Europe. *Erica* sp. is chosen as national flower of Norway[1].

Rhizoctonia is a kind of fungus that widespread distribution in the nature, which is existed widely in cultivated soils and non-cultivated soils all over the world, and it is easily isolated from the infected plants and soils[2]. *Rhizoctonia* has a large number of host plants and it can infect many varieties of plants. This fungus mainly caused the damping-off disease in plant seedling, and *Rhizoctonia solani* Kuhn can cause the *Thanatephorus cucumeris* (Frank) Donk of cereal crop[3]. So many plant pathologists and fungal taxonomists are interested in research *Rhizoctonia*[4-6].

Root rot of *Erica* is the only disease which has been found in the *Erica* sp.. The disease occurred in the root, and started infection from the root tip. Root tip became tawny in the early days, and then gradually turn into dark brown, exodermis of root tip decayed and fell off, disease spread upward to the part of the roots and finally caused the whole roots dark brown rot. Serious disease resulted in branches and leaves dry and shriveled[7].

On survey of the plant disease in Shandong Province, a special diseased of *Erica* sp. was found in a commercial greenhouse in JiYang county of Shandong province. The typical symptom of diseased plant is that leaves and stems are wilt and die quickly（Figure 1A-C). A research was carried out on the pathogen types of this kind of disease.

Materials and Methods

Fungal Isolates and Culture

Infected leaves and stems were collected and brought to the laboratory, and washed thoroughly in running tap H_2O to remove the dust, air dried, and cut several 5mm tissue blocks from disease and health junction. Leaves were surface disinfected in 75% alcohol for 15 to 20 seconds, then in $HgCl_2$ 10 to 20 seconds and rinsed three times in sterile distilled H_2O. Pieces of leaves were dried on sterilized filter papers and placed on petri dishes (five segments per plate) containing PDA medium. Cultures were incubated for 3 to 4 days at 28℃[8,9].

Determination of Pathogenicity

According to Koch's postulation, a healthy plant was rinsed by water and then the leaves and stems of the healthy plant were broken using a sterilized blade, and inoculated with purified fungus which have already obtained from the diseased leaves and stems. The inoculated plant was placed in the constant temperature incubator at 28℃ in the dark and regularly sprayed with distilled water during the cultivation.

rDNA-ITS Analysis

Isolates were grown in petri dishes containing 20ml of potato dextrose agar (PDA) at 28℃ for 3 to 5 days depending on their growth rate. After diameter of 0.3cm fungus cake were put into flakes contained PD medium and were cultivated and shook for three days in shaking incubator. Mycelium was collected by the machine of vacuum suction and was ground three times with liquid nitrogen. The total DNA was extracted by method of CTAB. The universal oligonucleotide primers ITS1 and ITS4 were used to amplify the ITS regions. The reaction mixture contained 5μl of 10× polymerase chain reaction (PCR) buffer, 1μl of Taq polymerase, 2μl of dNTP mixture, 2μl of $MgCl_2$, 30μl of ddH_2O, 4μl of ITS1 and 4μl of ITS4, and 2μl of fungal DNA. Amplification was carried out at 94℃ for 3min; followed by 30 cycles at 94℃ for 1 min, 55℃ for 1 min, and at 72℃ for 2 min; with the final extension at 72℃ for 10 min. Amplification products were electrophoretically separated through a 1.5% agarose gel and visualized under UV light after staining with ethidium bromide[10]. A marker of DM2000 was used as reference in each stripe. The total DNA was sent to HuaDa Sequencing Company.

The Establishment of the Phylogenetic Tree

rDNA-ITS sequences of pathogen from *Erica* were obtained from HuaDa Sequencing Company. And some similar genetic sequences to those of *Erica* were downloaded from the NCBI (National Center of Biotechnology Information) web site, and then all the genetic sequences were put together so that phylogenetic tree were established by MEGA6 software[11].

Results

Fungal Isolation and Culture

In all, 20 isolates were obtained from diseased plant of *Erica* sp. that was collected from the field. The 20 isolates were purified and cultured, and were confirmed belonging to the same cultural types (Figure 2A).

Morphological Characteristics of Colony and Mycelium

Purified isolates expressed white, loose and villous colonies on cultural medium, and mycelia covered the culture petri dish five days later. The hyphae were hyaline, thin, and were easily picked. There are a few sclerotia in the colonies of all isolates under the dissecting microscope (Figure 2B). The young hyphae are colorless but turn brown with age, the diameter of hypha is 6~11μm and its thickness is uniform. Hyphal branches are at 90 degree angles to the parent hypha and there is a cross-wall and a

constriction of the cell at the base of each branch (Figure 2C). All isolates have two nuclei in each cell of hyphae (Figure 2D).

Determination of Pathogenicity

The fungus have been inoculated on healthy plant for five days, the leaves wilt began to appear and the hyphae rapidly infect the leaves, and then stems were infected and turned wilt with the development of plant disease. Ten days later, the whole plant became yellow and died back. In addition, hyphae grew from leaves and stems. The symptoms on experimental *Erica* sp. plant identified as being identical to the original symptoms on diseased plants in field (Fig 1D, but there is no symptoms on control plants.

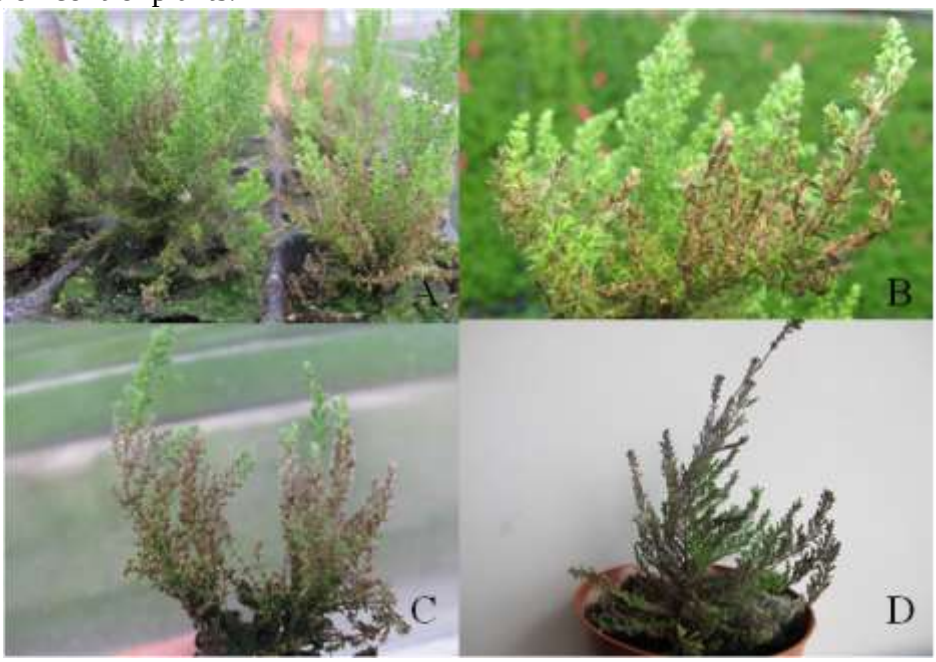

Figure 1. The disease damage and symptom of leave wilt disease of *Erica* sp.

A. Damage of leave wilt Disease of *Erica* sp. in field
B. Symptom of Leave wilt Disease of *Erica* sp. in field
C. The mycelia of pathogen on leave of *Erica* sp.in field
D. Symptom of artifical inculation indoors

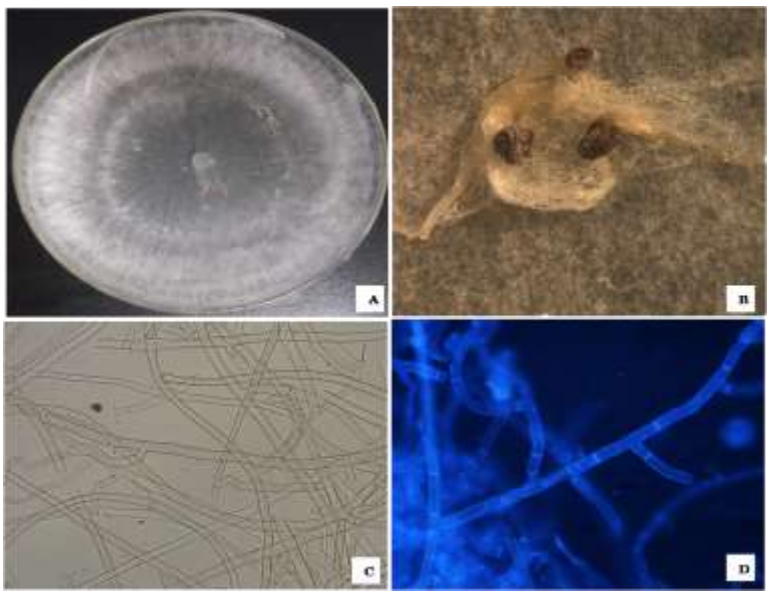

Figure 2. Anastomose group of binucleate *Rhizoctonia*.

A. Colony on PDA B. Sclerotia of AG-*Erica* C. Hyphae of AG-*Erica* D. Two nuclei in each cell of hyphae.

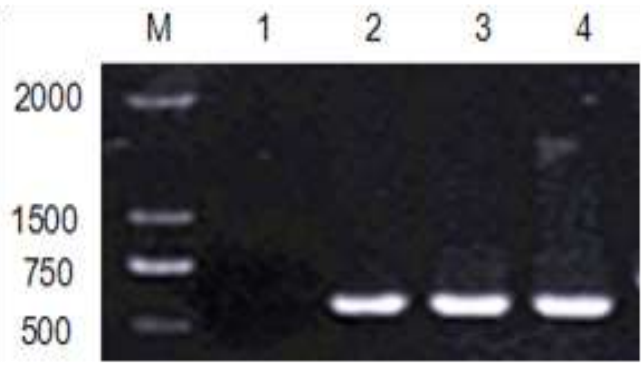

Figure 3. Gel electrophoretogram of PCR products amplified from rDNA ITS of *Rhizoctonia* strains.

rDNA-ITS Sequence Analysis

Amplification products for the respective gene regions were about 600-660 bp in size (Figure 3). The number of sequences bases is 692 bp by sequencing company of huada. The similarity between pathogens of *Erica* sp. and *Ceratobasidium* sp. is 99% through the NCBI (National Center of Biotechnology Information) sequence alignment. The ITS sequence of representative isolate (SDAU-*Erica* 01) was submitted to GenBank, and GenBank accession number (KX271051) was obtained.

The Establishment of the Phylogenetic Tree

The phylogenetic tree shows that the all examined isolates of AG-*Erica* were grouped in the same clade (Figure 4).

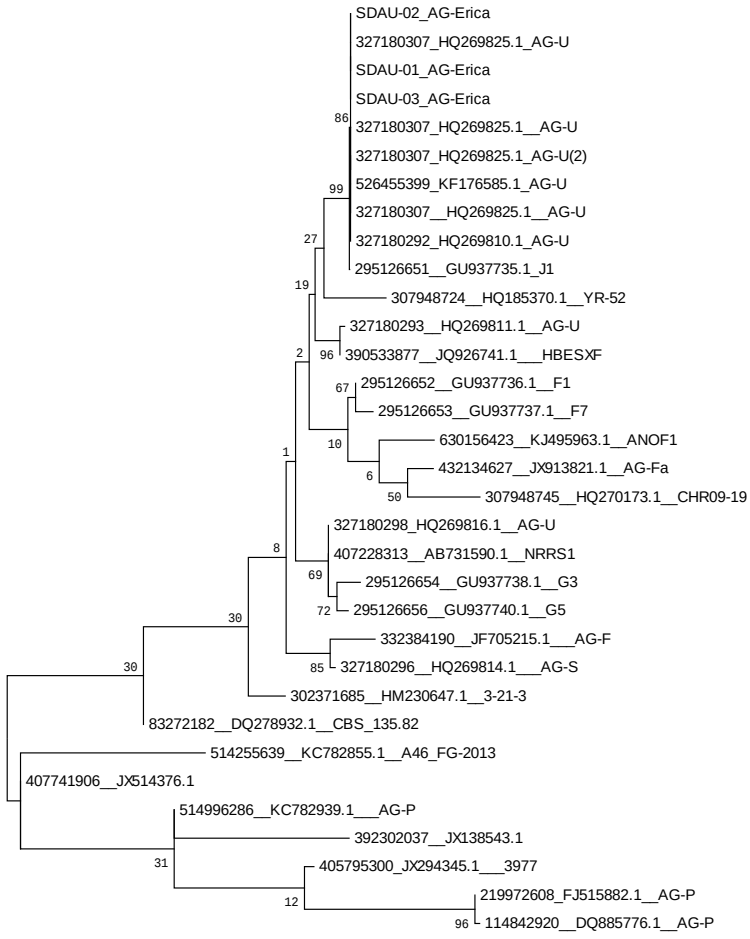

Figure 4. Phylogenetic tree based on rDNA ITS sequences of AG-*Erica* strains and other *Rhizoctonia* species registered in GenBank.

Summary

According to reports at home and abroad, there are very few diseases that occurred in the *Erica* sp. except root rots. In this study, the pathogen of *Erica* sp. was proved to be binucleate *Rhizoctonia* species through Koch's postulation and molecular biology techniques. This is the first report that *Rhizoctonia* cause leaves and stems disease on *Erica* sp. The new results can play an important role of studying the disease and protection of *Erica* sp. The occurrence of the disease and the relationship between the environmental conditions, and how to prevent and control disease need to be further studied.

Acknowledgement

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References

[1] P.L. Yan, D.H. Shu, *Erica*—European popular potted flower, China Flowers & Horticulture. 2 (2014) 37.

[2] C. Eken, E. Demirci, Identification and pathogenicity of *Rhizoctonia* solani and binucleate *Rhizoctonia* anastomosis groups isolated from forage legumes in Erzurum, Turkey, Phytoparasitica. 31 (2003)76-80.

[3] E. M. Babiker, S.H. Hulbert, K.L. Schroeder, T.C. Paulitz, Evaluation of Brassica species for resistance to *Rhizoctonia* solani and binucleate *Rhizoctonia* (*Ceratobasidum* spp.) under controlled environment conditions, Eur. J. Plant Pathol. 136 (2013)763-772.

[4] R.V. Abgona, K. Kageyama, M. Hyakurnach, Biocontrol of *Rhizoctonia* damping-off of cucumber by non-pathogenic binucleate *Rhizoctonia*, Eur. J. Plant Pathology. 102(1996)227-235.

[5] P.S. Bains, H.S. Bennypau, L D.R. Lynch, L.M. Kawchuk, C.A. Schaupmeyer, *Rhizoctonia* disease of potatoes (*Rhizoctonia solani*): fungicidal efficacy and cultivar susceptibility, Amer. J. Potato Res. 79 (2002)99-106.

[6] I. Mnif, A.G. Campistany, J.C. León, I. Hammami, M.A. Triki, A. Manresa, D.Ghribi, Purification and identification of *Bacillus subtilis* SPB1 lipopeptide biosurfactant exhibiting antifungal activity against *Rhizoctonia bataticola* and *Rhizoctonia solani*, Environ. Sci. Pollut. Res. 23 (2016)6690-6699.

[7] M. Hyakumachi, A. Priyatmojo, M. Kubota, H. Fukui, New anastomosis groups, AG-T and AG-U, of binucleate *Rhizoctonia* spp. causing root and stem rot of cut-flower and miniature roses, Phytopathology. 95 (2005)784-792.

[8] A. Priyatmojo, Y. Yotani, K. Hattori, K. Kageyama, M. Hyakumachi, Characterization of *Rhizoctonia* spp. causing root and stem rot of miniature rose, Plant Dis. 85(2001)1200-1205.

[9] M.A. Cubeta, E. Echandi, T. Abenerthy, R. Vilgalys, Characterization of anastomosis groups of binucleate *Rhizoctonia* species using restriction analysis of ribosomal RNA gene, Phytopathology. 81(1991)1395-1400.

[10]Z.L. Liu, J.B. Sinclair, Differentiation of intraspecific groups within anastomosis group 1 of *Rhizoctonia solani* using ribosomal DNA internal transcribed spacer and isozyme comparisons, Can. J. Plant Pathol. 15(1993)272-280.

[11]S. Kuninaga, T. Natsuki, T. Takeuchi, R. Yokosawa, Sequence variation of the rDNA ITS regions within between anastomosis groups in *Rhizoctonia solani*, Curr. Genet. 32(1997)237-243.