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A New Disease of Gladiolus Caused by Binucleate *Rhizoctonia* sp.

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Abstract: Fungi with Rhizoctonia-like mycelia were isolated from root and stem of Gladiolus (*Gladiolus hybrida* L.) grown in commercial glasshouse in Mahallat, Iran, during the summer and fall of 2003. Isolated fungi were identified as either binucleate or multi nucleate *Rhizoctonia* sp. On the basis of hyphal characteristics and nuclear number, twenty three isolates of *Rhizoctonia* sp. were obtained from infected corms and stems. Of the 23 isolate, 9 had binucleate and 14 had multinucleate vegetative hyphal cells. Representative isolates of binucleate *Rhizoctonia* sp. were characterized for anastomosis, optimum temperature *in vitro* and virulence on Gladiolus. Isolates of binucleate *Rhizoctonia* failed to anastomose with tester isolates of Anastomosis Groups (AG)-A throug-S (not including AG-J and AG-M). The optimum temperature range for growth rate of binucleate *Rhizoctonia* sp. was 24-28°C. Growth rate of binucleate *Rhizoctonia* sp. was more rapid than *R. solani*. Five isolates from each group caused severe corm rot and mortality of plant during rooting. Isolates of binucleate *Rhizoctonia* caused corm and stem rot and mortality only on 35-day-old plants. This is the first detailed report of corm and stem rot disease of Gladiolus caused by binucleate *Rhizoctonia*. Further field studies are needed on the ecology and pathogenicity of *Rhizoctonia* sp. to formulate steps for controlling corm and stem rot of Gladiolus.

Key words: Anastomosis, hyphal fusion, ornamental plant, Gladiolus

INTRODUCTION

Pests and diseases are considered to be a limiting factor in agricultural production and are responsible for a considerable proportion of crop losses. Knowledge of the presence of particular diseases in a crop is important for an integrated approach to pest and disease control to enable sound management decisions. Gladiolus (*Gladiolus hybrida* L.) is one of the most famous and remarkable flowers among bulbous ornamental plants in the world and are among the best selling flowering potted plants in Iran. Many attempts are being made to improve the quality and increase the production of these attractive flowering plants. One of the constraints on this flower production is the occurrence of fungal disease.

During the summer and fall of 2003 and 2004 a root and stem rot disease was observed in commercially grown Gladiolus in Mahallat prefecture, Iran. Infected corms and stems turned dark brown or black (Fig. 1). The upper part of the leaves showed a yellows and necrosis symptoms, because roots and stems suffered from severe rot. Plants died a few days later due to disruption of translocation of water and nutrients. Mycelia were often found on infected corms and stems near the soil surface. Disease was frequently observed on young plants. Fungi with Rhizoctonia-like mycelia was consistently isolated from the diseased plants. Although *Rhizoctonia solani*, have been reported as a major plant-pathogenic fungus causing severe economic damage to many species of ornamental plants, occurrence of binucleates *Rhizoctonia* sp. on Gladiolus has not been reported to date[1].

The genus *Rhizoctonia* represents many fungi which are mutual symbionts as well as pathogens[2-4]. Pathogenic *Rhizoctonia* sp. attacks and cause severe diseases on many economically important plants[5]. Isolates of Rhizoctonia vary greatly in their colony morphology, growth characteristics and pathogenicity toward plants[5]. However, most *Rhizoctonia* sp. can be classified into uninucleate, binucleate, or multinucleate groups, based on the number of nuclei in their vegetative hyphal cells[3].

At present, *R. solani* is considered a species complex rather than a single species[2, 5]. Attempts have been made to divide the species complex into more homogeneous groups[2,4,5]. The most widely used method to separate *R. solani* is based on hyphal anastomosis. Hyphae of isolated will only anastomose with hyphae of isolates, if they are within the same Anastomosis Group (AG)[2,3,6]. Recently, *R. solani* was divided into 14 AGs designated as AG 1 through 13 and Bridging Isolates (BI) group[3,7]. Isolates belonging to AG BI have the ability to

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Fig. 1: Disease symptoms on corm and foliage of Gladiolus grown in greenhouses

anastomose with members of other AGs[6]. Binucleate *Rhizoctonia* sp. have been divided into 19 AGs designated as AG-A through-S[8]. Some AGs of binucleate *Rhizoctonia* sp. and *R. Solani* are further divided into subgroups based on cultural morphology, nutritional requirements, temperature effect on growth, host specificity, frequency of hyphal anastomosis and pathogenicity[3,8]. Present methods of distinguishing subgroups employ biochemical[9] and molecular techniques[10].

The objective of the present study was to characterize *Rhizoctonia* sp. obtained form infected roots and corms of Gladiolus. Isolates were studied for nuclear appearance, cultural appearance, type of teleomorph, effect of temperature on mycelial growth and pathogenicity on Gladiolus.

MATERIALS AND METHODS

Isolation and culture maintenance. Gladiolus samples were obtained from commercial glasshouse-grown Gladiolus in Mahallat prefecture, Iran. Symptomatic corms and stems were washed thoroughly in running tap water for 30 min to remove adhered soil particles, air dried, then cut into 5 mm pieces. Roots and stems were surface disinfected with 0.5% sodium hypochlorite solution for 2 min and rinsed three times with sterile distilled water. Pieces of root and stem were dried separately on sterilized filter paper (Whatman No. 1), placed on petri dishes containing water agar acidified (pH 4.5) with 10% lactic acid (AWA) and incubated at 25°C in the dark. After 2 to 3 days, cultures were examined microscopically for hyphal characteristic typical of *Rhizoctonia* sp.[7]. Rhizoctonia like fungi were hyphal tipped and sub-cultured onto AWA, to avoid culture contamination. Isolates were transferred to test tube slants of 2% Potato Dextrose Agar (PDA) and maintained at 25°C. Pure cultures were stored in PDA slant tubes or on sterile barley grain at 4°C.

Nuclear conditions: Single 7 mm diameter disks of 2 to 3-day-old cultures of Rhizoctonia sp. growing on PDA were transferred to clean glass slides which had been dipped in 99.5% ethanol and flamed before use. Inoculated glass slides were incubated in moist chamber at 25°C in the dark. A drop of safranin O and 3% KOH was placed directly in the mycelium of 1 to 2-day-old cultures[11]. Twenty five cells of each isolate were examined for nuclei at ×400 magnification using bright field microscopy.

AG determination: AG identities were determined by using the glass-slide technique[11]. Single 7 mm-diameter disks were cut from the perimeter of a 2 to 3-day-old colony on PDA and placed on a clean glass slide. Tester isolates of binucleate Rhizoctonia AG-A through AG-S (not including AG-J due to lack of the tester isolates in our collection) were placed 3 to 4 cm away from each tested isolate. Slides were put in a moist chamber and incubated at 25°C for 24 to 48 h in the dark. Excess moisture was wiped from the bottom of the slide. When the hyphae from the two disks were overlapping, they were stained with safranin O and 3% KOH and examined microscopically to determine anastomosis reaction[6,11].

Cultural appearance: Single 7 mm-diameter mycelial disks from the margins of actively expanding young cultures on PDA placed in the center of 9 cm petri dishes containing 2% PDA of Czapek solution agar were incubated at 25°C in the dark. Cultures were evaluated after 25 days of incubation.

Hyphal growth rate: Radial growth of *Rhizoctonia* sp. was determined at 5, 15, 20, 25, 30 and 40°C. A single 7 mm-diameter mycelial disk from the margin of a 2 to 3-day-old colony was transferred to PDA in a petri dish. Measurements were taken 12 h after incubating petri dishes to allow the diffusion of temperature to agar. Colony radius was measured at 24 h intervals until the colony reached the edge of the petri dishes. Treatments were replicated three times and the experiment was repeated twice.

Induction of teleomorphs: According to method used by Oniki *et al.*[12], fresh cultures of each isolate were grown separately on a modified Potato Yeast Extract Agar (PYEA). After autoclave sterilization, the medium was

acidified to pH 4.5 with 10% lactic acid. Cultures were incubated at 27°C and when the hyphae reached the margins of the dish, they were covered to the rim with air dried soil aggregates. The cultures were incubated at room temperature with the petri dish lids removed. Humidity was maintained by watering the soil 1-3 times daily, while excess moisture was drained. Production of hymenia on the surface of soil was expected within 12-14 days afterwards.

Pathogenicity test: In pathogenicity test, the virulence of isolates of binucleate *Rhizoctonia* sp. on Gladiolus Cv. silk was determined. Five representative isolates form each group were tested. Inoculum of *Rhizoctonia* sp. was prepared by growing each isolate in a 500 mL Erlenmeyer flask containing 100 g of barley grain and 100 mL of distilled water. Flasks were sterilized at 121°C for 20 min and inoculated with three 7 mm diameter mycelial disks of the isolates cut from the edges of 3-day-old *Rhizoctonia* sp. growing on PDA. Flasks were incubated at 25°C for 10 days in the dark and shaken regularly to aid uniform colonization. Infested barley grain was air dried for 1 week and stored at 4°C until needed[13]. Soil containing peat moss, perlite, vermiculite, mountain soil, (55:23:15% wt.) was partially sterilized on two consecutive days at 60°C for 30 min, then infested with 2% (wt./wt.) ground barley grain colonized with *Rhizoctonia* sp. Healthy, uniform corms were carefully grown into 1000 g of *Rhizoctonia*-infested soil in 15 cm-diameter plastic pots. Soils amended with sterile barley grain served as controls. Pots were covered with black vinyl sheet, incubated at room temperature for 24 h to stimulate the growth of *Rhizoctonia* sp. then transferred to the greenhouse (at 25 to 30°C).

Disease severity was determined 2-6 weeks after inoculation. A randomized block design with isolate as single treatment was replicated five times. Data were subjected to one-way ANOVA. Treatment means were separated with fishers protected LSD at $p<0.05$. Data containing 0 values were transformed to square root before analysis. The experiment was repeated twice. Data from repeated experiments were combined because there was no interaction between experiment and treatment effects.

RESULTS

Isolation and AG determinations: Of 23 Rhizoctonia isolates recovered from diseased corms and stems of Gladiolus, 9 had binucleate and 14 had multinucleate vegetative hyphal cells (Fig. 3). The nine binucleate Rhizoctonia isolated failed to anastomose with tester isolates of AG-A through-S (not include were AG-J and AG-M).

Fig. 2: Symptoms resulted from binucleate *Rhizoctonia* isolates in pathogenicity test

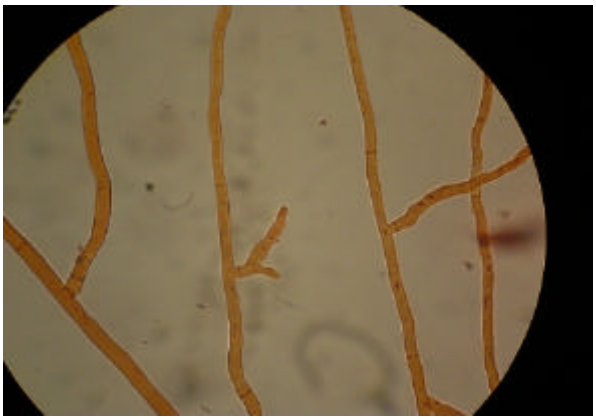

Fig. 3: Nuclei (N) in vegetative hyphae of binucleate *Rhizoctonia* sp.

Nuclear number: Determination of the number of nuclei in vegetative hyphal cells is an important process in the identification of *Rhizoctonia* sp. The number of nuclei per cell varied from 4 to 15 for isolates of multinucleate Rhizoctonia. All of isolates of binucleate Rhizoctonia contained only two nuclei per hyphal cell (Fig. 3).

Colony morphology: The mycelium of binucleate isolates growing on PDA was white to light tan when young, but become brown to dark brown with age. The diameter of hyphal cells were range from 2.5-4.3 μm, which were more thinner than those were belong to *R. solani*. Concentric rings of light and dark mycelium were apparent. Also in the case of binucleate Rhizoctonia, sclerotia were not produced in PDA medium. Whereas, isolates of *R. solani* formed sclerotia 0.5 to 3 mm in diameter and changed from brown to dark brown color with age. Concentric rings of dark and light mycelium were visible in isolates of binucleate Rhizoctonia grown on PDA.

Hyphal growth rates: Hyphal growth rates of isolates of binucleate Rhizoctonia and *R. solani* were similar except at 30°C. Isolates of binucleate Rhizoctonia and AG-4 grow at 10°C had the ability to grow at 30°C. Isolates of binucleate Rhizoctonia did not grow at 5 and 40°C; however these isolates also had an optimal temperature at 25°C and could grow as well up to 30°C, as isolates of binucleate *Rhizoctonia* sp.

Induction of teleomorphs: The method used by Oniki *et al.*[12] is appropriate for many isolates of binucleate as well as multinucleate *Rhizoctonia* sp.[3]. However, some isolates including our isolates, cannot be induced to produce the teleomorph stage under these conditions.

Pathogenicity study: In pathogenicity test, all isolates of binucleate Rhizoctonia, caused severe rot on corms during rooting showing severe damage (disease severity index=4). Differences in disease severity ratings among isolates were not significant (data not shown). At 34 to 37 days after inoculation, disease symptoms began to appear first on upper part of leaves and then on lower part of stems (Fig. 2). Corms also were infected as disease development progressed. Mortality occurred as roots were not formed and plants declined. Rhizoctonia sp. were re-isolated from diseased tissue plants completing koch's postulates. ANOVA indicated that isolates were highly significant factors (p=0.001) in disease severity index of Gladiolus Cv. Silk. No disease was observed on control treatments (Fig. 2).

DISCUSSION

Binucleate Rhizoctonia and multinucleate *R. solani*, were isolated from diseased corms and stems of Gladiolus. Isolates of binucleate Rhizoctonia failed to anastomose with the tester isolates of AG-A through AG-S (not include were AG-J and AG-M). Although tester isolates of AG-J and AG-M were not used in anastomosis determination. Isolates of AG-J cause diseases on bean, pea radish, onion, lettuce, tomato, soybean, cowpea and peanut[1,15]. Isolates of AG-M have not been reported to be plant pathogens[3]. Martin[14] observed inconsistent assessment of AG for binucleate Rhizoctonia recovered from strawberry. Some strawberry isolates did not anastomose with the tester isolate, although the RFLP banding patterns suggested that they were in the same AG with the tester isolate[14].

Regarding the induction of teleomorph, although the method used by Oniki *et al.*[12], is appropriate for many isolates of binucleate as well as multinucleate *Rhizoctonia* sp.[3]. However, the isolates tested here, could not be induced to produce the teleomorph stage under these conditions. This is agree with the previous report regarding *R. tuliparum* on flower-bulbs in Netherland indicating that the teleomorph has not been perceived yet[15]. Therefore, more information is needed for inferring that isolates of binucleate Rhizoctonia obtained from Gladiolus represent a new group. Additionally, molecular analysis would be helpful to characterize and identify isolates of binucleate Rhizoctonia[10].

Pathogenicity test revealed that isolates of binucleate Rhizoctonia and *R. solani* are causal pathogens of root and stem rot of Gladiolus. The evidence that all tested isolates could cause severe damage on Gladiolus during rooting suggested that much attention should be paid during this stage of production. For Gladiolus propagation, most commercial growers use the method employed in this study.

As reported by other investigators, pathogenicity of *Rhizoctonia* sp. is host dependent. Trujillo *et al.*[16] reported that isolates of AG 2-2 were more virulent that AG4 on carnation. On potato, isolates of AG 3 were more virulent that AG 5 and AG 5[17]. Isolates of *R. solani* AG 4 were generally more virulent on beet seedlings compared to isolates of AG 2. Host age influences virulence of *R. solani* of sugar beet[18]. Present observations that isolates of binucleate Rhizoctonia had an optimum growth rate at 24-28°C are similar to the results of studied on isolates obtained from soybean, carnation and potato[16,17].

To our knowledge, this is the first detailed report of corm and stem rot disease of Gladiolus caused by binucleate Rhizoctonia. Further field studies are needed on the ecology and pathogenicity of *Rhizoctonia* sp. to formulate steps for controlling corm and stem rot of Gladiolus.

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REFERENCES

1. Benson, D.M. and D.K. Cartwright, 1996. Ornamental Diseases Incited by *Rhizoctonia* sp. In: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Sneh, B., S. Jabaji-Hare, S. Neate and G. Dijst, Eds. Kluwer Academic Publishers, The Netherlands, pp: 303-314.

2. Anderson, N.A., 1982. The genetics and pathology of *Rhizoctonia solani*. Ann. Rev. Phytopathol., 20: 329-344.
3. Sneh, B., L. Burpee and A. Ogoshi, 1991. Identification of *Rhizoctonia* Species. APS Press, The American Phytopathological Society. St. Paul, Minnesota, USA.
4. Andersen, T.F. and H.N. Rasmussen, 1996. The Mycorrizal Species of *Rhizoctonia*. In: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Sneh, B., S. Jabaji-Hare, S. Neate and G. Dijst, Eds. Kluwer Academic Publishers, The Netherlands, pp: 379-390.
5. Adams, G.C., 1988. *Thanatephorus cucumeris* (*Rhizoctonia solani*) a Species Complex of Wide Host Range. In: Advances in Plant Pathology. Vol 6, Genetics of Plant Pathogenic Fungi. Sidhu, G.S., Ed. Academic Press, New York, pp: 535-552.
6. Carling, D.E., 1996. Grouping in *Rhizoctonia solani* by Hyphal Anastomosis Reaction. In: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Sneh, B., S. Jabaji-Hare, S. Neate and G. Dijst, Eds. Kluwer Academic Publishers, The Netherlands, pp: 37-47.
7. Carling, D.E., R.E. Baird, S. Kuninaga and R.D. Gitaitis, 1999. Characterization of anastomosis group (AG) 13 of *Rhizoctonia solani*. (Abstr.) Phytopatholog, 89: 511.
8. Ogoshi, A., 1987. Ecology and pathogenicity of anastomosis and interspecific groups of *Rhizoctonia solani* Kuhn. Ann. Rev. Phytopathol., 25: 125-143.
9. Stevens-Johnk, J. and R.K. Jones, 1994. Comparison of whole-cell fatty acid compositions in interspecific groups of *Rhizoctonia solani* AG-1. Phytopathology, 84: 271-275.
10. Salazar, O., M.C., Julian and V. Rubio, 2000. Primers based on species rDNA-ITS sequences for PCR detection of *Rhizoctonia solani*, *R. solani* AG2 subgroups and ecological types and binucleate *Rhizoctonia*. Mycol. Res., 104: 281-285.
11. Kronland, W.C. and M.E. Stanghellini, 1988. Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. Phytopathology, 78: 820-822.
12. Oniki, M., A. Ogoshi, T. Araki, R. Sakai and S. Tanaka, 1985. The perfect state of *Rhizoctonia oryzae* and *R. zeae* and the anastomosis groups of *Waitea circinata*. Trans. Mycol. Soc. Japan 26: 189-198.
13. Liu, Z. and J.B. Sinclair, 1991. Isolates of *Rhizoctonia solani* anastomosis group 2-2 pathogenic to soybean. Plant Dis., 75: 682-687.
14. Martin, F. N. 2000. *Rhizoctonia* sp. recovered from strawberry roots in Central Coastal California. Phytopathology, 90: 345-353.
15. Sneh, B., S. Jabaji-Hare, S. Neate and G. Dijst, 1996. *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Kluwer Academic Publishers.
16. Trujillo, E.E., R. Shimabuku, C.A. Cavin and M. Aragaki, 1988. *Rhizoctonia solani* anastomosis grouping in carnation fields and their pathogenicity to carnation. Plant Dis., 72: 863-865.
17. Carling, D.E. and R.H. Leiner, 1990. Virulence of isolates of *Rhizoctonia solani* AG3 collected from potato plant organs and soil. Plant Dis., 74: 901-903.
18. Hert, L.J., 1996. Sugar Beet Diseases Incited by *Rhizoctonia* sp. In: *Rhizoctonia* Species: Taxonomy, Molecular Biology, Ecology, Pathology and Disease Control. Sneh, B., S. Jabaji-Hare, S. Neate and G. Dijst, Eds. Kluwer Academic Publishers, The Netherlands, pp: 341-350.