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Binucleate *Rhizoctonia* AG-A pathogen of red clover in Serbia

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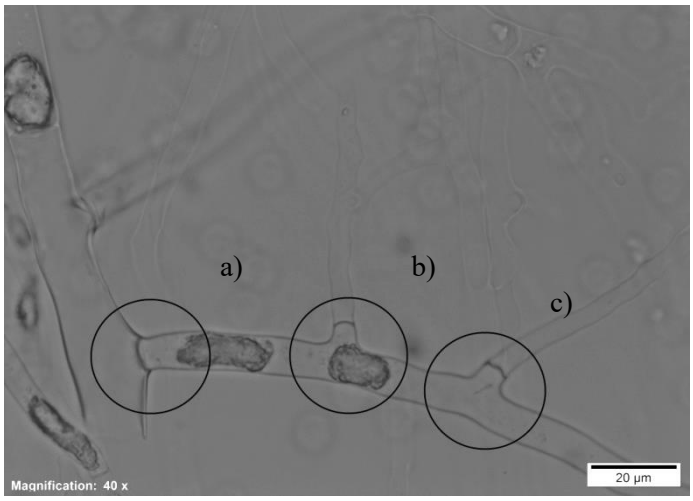
Red clover (*Trifolium pratense* L.) in Serbia has a long history of cultivating due to its favorable growing characteristics and its high nutritional value, but it is susceptible to the variety of fungal pathogens that can limit its yield and shorten the plants lifespan. *Rhizoctonia* spp. represent a very important genus of phytopathogenic fungi that inhabit the soil and can infect a vast verity of cultivated plants (Vojvodić, 2021). Sampling of red clover plant F2 with symptoms of stunting and necrosis of leaves and stems was done on 05.11.2020. on red clover monoculture plot in Globoder, Serbia (43°34'56.9"N 21°12'08.2"E). Plant F2 root surface was dark colored with sporadical cracks in the root epidermis. The root cross sections showed dark brown necrotic tissue of central cylinder, which was used for pathogen isolation. Obtained isolate had an average growth of 75,67 mm after seven days at 25° C and was designated F2B. After acquiring a hyphal tip fungal colony macromorphological and micromorphological features were examined on PDA medium. The culture had a woolly structure, mycelium color was paled beige with shades of light brown, sclerotia structures were not present. Microscope examination showed septate hyphae (average width 9,3 μm) with characteristic 90° branching, constriction at the branching point and a septa in the immediate vicinity (Picture 1.). Obtained morphological features were in accordance with the features of the *Rhizoctonia* genus. Pathogenicity of the isolate was confirmed on red clover plants and detached plant parts by three pathogenicity tests. The first test consisted of detached red clover leaves inoculation and measuring of lesion diameter (average 6.23 mm), the second was inoculation of red clover stem fragments (40 mm) with measuring of necrotic stem part length (average 27,65 mm) after seven days of incubation at 25° C. The third pathogenisity test consisted of inoculation of 150 days old red clover plants in semi controlled environment (Yli-Mattila et al., 2008). Inoculation spot was on the main clover roots, about 20 mm from the plant crown and scoring was done 60 days after. Leaves and stems symptoms were visible on seven plants, but the root symptoms were present in all 12 treatment plants, while control plants remained symptomless. Average width of the root necrotic tissue was 2,36 mm and length was 24,28 mm. Species level identification was done by isolating genomic DNA followed by PCR amplification of the internal transcribed spacer (ITS) using ITS1/ITS4 primer pair. BLAST analysis of the nucleotide sequence revealed that isolate F2B was identical with several reference sequences of binucleate *Rhizoctonia* AG-A deposited in NCBI GenBank. Based on morphological, pathogenic and molecular features of the isolate F2B, the pathogen of red clover that caused rot root was identified as binucleate *Rhizoctonia* AG-A.



Picture 1. Isolate F2B septate hyphae with characteristic 90° branching a), b) and c)

References

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