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RESEARCH ARTICLE

First Report of Powdery Mildew Caused By Erysiphe Cruciferarum on Camelina Sativa in Bulgaria

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ABSTRACT

Camelina (Camelina sativa L. Crantz) belongs to the Cruciferous family and is a relatively new crop for Bulgaria. At the end of June of the same year, symptoms of powdery mildew (Erysiphe cruciferarum Opiz ex L. Junell) were detected, with an attack rate exceeding 40%. A white mycelium was observed on the upper side of the leaves and the stems, later the leaves turned yellow. The structure of the white colonies with hyphal growth is observed under a microscope. Conidiophores are cylindrical, 19.9 to 41.7 × 8 to 17.1 µm and composed of 3 to 4 cells. The chasmothecia of the pathogen was also discovered. Fruiting bodies are 110 µm (80-130 µm) in diameter, with colourless, club-shaped asci with colourless, ovoid ascospores. Pathogenicity experiments performed according to Koch's rules were conducted in a greenhouse at 22 °C, 14 h photoperiod and 70 % humidity. As a result, the typical symptoms of powdery mildew appeared 8 days post-infection. It was re-isolated and identified by morphological characteristics. Partial sequence analysis of the ITS5 - 5.8 - ITS4 region of the nuclear ribosomal DNA with universal primers identified the pathogen as E. cruciferarum. The phylogenetic tree was built using the neighbour-joining by BLAST software. According to the literature, the article is the first report of powdery mildew caused by E. cruciferarum on camelina in Bulgaria.

INTRODUCTION

Camelina (Camelina sativa (L.) Crantz) is an annual plant of the Brassicacea family that contains oil (30-40%) with unique properties. Camelina oil is suitable for use in the food industry and biodiesel production. Camellia is resistant to some diseases such as brown spot (Alternaria brassicae (Berk.) Sacc.), and phomosis (Phoma lingam (Tode: Fr.) Desmaz) (Séguin-Swartz et al. 2009). On the other hand the plant is sensitive to some diseases such as white rust (Albugo candida (Pers. ex Chev.) Kuntze) (Séguin-Swartz et al. 2009), stem rot (Sclerotinia sclerotiorum (Lib.) de Bary) and powdery mildew (Erysiphe spp.) (Föller et al. 1998). Powdery mildew of crucifers is caused by the fungus Erysiphe cruciferarum (Opiz exL. Junell). It infects all aerial parts, significantly reducing yields by reducing plant growth and seed development (Ali et al., 2017; Runno-Paurson et al. 2021). Outbreaks of powdery mildew have been reported in cruciferous species in various parts of the world, followed by significant yield losses in India (Meena et al. 2018), in Australia (Uloth et al. 2017), in Poland (Sadowski et al. 2001), in Turkey (Mert-Türk et al. 2008), in Estonia (Runno-Paurson, 2021) and in Greece (Vellios et al. 2017). In our country, camellia is a new crop and powdery mildew attack has

not been reported so far. The symptoms of fungal disease were found at the end of June 2023, with a high degree of attack. The purpose of the study is to identify the fungal pathogen that causes the disease using morphological observations, molecular identification and pathogenicity tests.

MATERIALS AND METHODS

Field experiments

Field trials were conducted on the certified organic field at the Agroecological Center of the Agricultural University -Plovdiv. The soil type is Mollic fulvisols – FAO, with a low humus content of 3.7% and neutral pH. A randomized complete block design was used for setting a small plot experiment of a local Bulgarian landrace (K3) camelina variety that was grown in 2023. The sowing was executed with plot seeder Wintersteiger AG with 800 germinating seeds/m^2. In this study, we present the results from the spring cultivation in small plots of 10m2 (1.4 m x 7.7m). Fertilization with approved for organic farming solid fertilizer -30kg/ha of active substance nitrogen was made before soil cultivation. No significant environmental or biotic stress factors were observed during the vegetation of the camelina in the following year.

Phenotypic characteristics, such as spore-producing structures formed as a result of asexual or sexual reproduction it is important for the identification of fungal species within the mycological community (Hyde et al. 2010). The use of morphology in fungal species identification is very important for understanding the developmental cycle and ecological requirements of the pathogen (Ciardo et al. 2007).

Pathogenicity test

Pathogenicity experiments performed according to Koch's postulates were carried out in a greenhouse at 22 °C, 14 h photoperiod and 70% humidity. Inoculation of the experimental plants (at 14 days) was carried out by gently pressing the upper surface of naturally infected leaves with the upper surface of the leaves of healthy experimental plants for about 5 seconds according to Choi et al. 2009.

3. Molecular identification

DNA was isolated with a HiPurA Fungal DNA purification kit (Himedia, Mumbai, India) according to the manufacturer's instructions. Control of the purity and concentrations of genomic DNA was performed by agarose gel electrophoresis and by the benchtop fluorometer (Qubit 2.0, Thermo Fisher Scientific) with a dsDNA average value of 3.32 ng/µl. Fungal pathogen identification sequences of large subunit (LSU) ribosomal RNA (rRNA) were determined with the universal primers with 1 µL each of 10 µm internal transcribed spacer ITS-5 (5' – TCCGTAGGTGAACCTGCG - 3') and NL-4 (5'-GGTCCGTGTTTCAAGACGG - 3') (White et al. 1990; Kurtzman and Robnett, 1998). The PCR reaction mixture in the total volume of 20 µL contained 0.5 µL Taq polymerase (5 U/µL, Canvax, Spain). The temperature programme of thermal cycling was initial denaturation at 96 for 2 min, followed by 30 cycles consisting of denaturation at 96 °C for 1 min, annealing at 55 °C for 45 s, and extension at 72 °C for 2 min, followed by a final extension at 72 °C for 5 min according to Petkova et al. 2022. The PCR products were cut out from the 1.2% gel and purified by using the Clean-Easy™ Agarose Purification Kit (Canvax Biotech, Spain). Purified samples were sent for DNA sequencing to Microsynth (Microsynth AG, Balgach, Switzerland). A BLAST search of nucleotide sequences was performed using the platform of the National Centre for Biotechnology Information with accession number in GenBank PP702051.1 (https://www.ncbi.nlm.nih.gov/nuccore/PP702051, accessed on 19 April 2024). The phylogenetic tree was built according to the neighbour-joining method by BLAST pairwise alignment between a query and the database sequences searched (Mkumbe et al. 2018; Singh Saharan et al. 2023).

RESULTS AND DISCUSSION

Symptoms

The observed phenotypic manifestations are typical for classical powdery mildew damage. On the upper side of the leaves and the stems can be observed small spots of a whitish coating, which grow and cover the entire leaf petura and the stem. Later, the leaves turn yellow, necrotize and dry (Fig. 1 A and B).

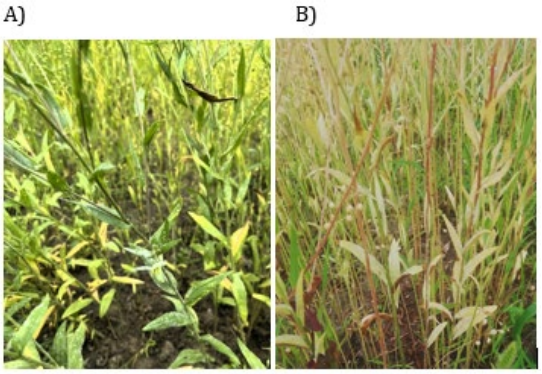

Figure 1. Symptoms of the disease caused by Erysiphe cruciferarum PP702051.1 on camelina K3 local Bulgarian variety. A) Severe powdery mildew attack on camelina variety K3; B) Symptoms of yellowing on camelina leaves, stems, and siliques in the final stage of vegetation.

Powdery mildew symptoms on camelina grown on the experimental field

The mycelia structure is observed under a microscope. Conidiophores are cylindrical, 19.9 to 41.7 × 8 to 17.1 µm and composed of 3 to 4 cells. At the tip of the conidiophores, a single colourless conidia was formed, oblong to cylindrical or oval, 22.6 - 47.9 µm long and 8.9 - 19.5 µm wide (average 35.3 - 14.2 µm). These characteristics are consistent with other reports of E. cruciferarum Opiz ex L. Junell (Choi et al. 2009; Vellios et al. 2017; Kim et al. 2013; Fu and Yan, 2022).

The chasmothecia of the pathogen with typical spherical were also found. The immature ones were yellowish, and the mature ones were brown (Figs. 2 A). Their size was at an average of 110 µm (80 - 130 µm) in diameter, with six colourless, club-shaped asci measuring 50.1 - 72.9 x 27.2 - 42.9 µm (average 55.2 x 33.9 µm) (Fig. 2 B and C). Colorless, ovoid ascospores are located in the asci. These features are consistent with previous data on E. cruciferarum (Vellios et al. 2017).

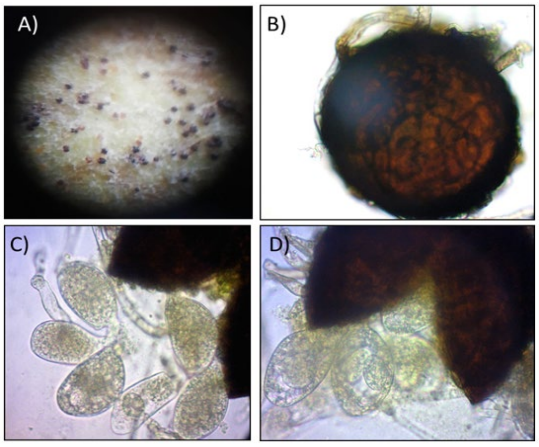

Figure 2. A) Microscopic observation of mature and immature chasmothecia (with magnification x 2); B) Microscopic observation of chasmothecia (with magnification x 40). C) and D) Asci emerging from a ruptured chasmothecia (with magnification x 40).

2. Pathogenicity test

External manifestations are typical of classical powdery mildew. In the current experiment and for the control variants, naturally healthy leaves were used. On the 8th day, the typical symptoms of powdery mildew appeared. In the control the plants are healthy. It was re-isolated and identified by morphological characteristics that match the original isolate. These symptoms were similar to previous reports on Erysiphe cruciferarum (Choi et al. 2009; Kim et al. 2013; Fu and Yan, 2022).

3. Molecular identification

Phenotypic characterization, such as spore-producing structures formed as a result of asexual or sexual reproduction, as the sole means of identifying fungal species, and even today it is still adopted as a means of species identification within the mycological community (Hyde, 2010). The use of morphology in fungal species identification is very important to understanding the evolution of morphological characters (Ciardo et al. 2007).

Fungal isolate used in plant pathogenicity tests was identified using universal primers for amplification of the ITS5 - 5.8 - ITS4 region of the nuclear ribosomal DNA according to the method of White et al. 1990. The pathogen was identified as Erysiphe cruciferarum according to the BLAST search with accession number in GenBank PP702051.1. Furthermore, the ITS sequence clustered with Erysiphe cruciferarum on different Brassicaceae host plants in the phylogenetic tree in Figure 3. The dendrogram in Figure 3 showed 98.92% identity with Erysiphe cruciferarum isolate Fi5 found on Lepidium apetalum in China in 2017 (MK341128.1) and Erysiphe cruciferarum isolate ZM2022 on Pugionium cornutum (L.) in China (Bao et al. 2023) and 98.32% identity with Erysiphe cruciferarum (AB104516.1) from Iran reported by Khodaparast et al. 2023. Erysiphe cruciferarum on Camelina sativa has been reported in Domokos province in central Greece (Vellios et al. 2017) and in Montana, USA (Fu and Yan, 2022).

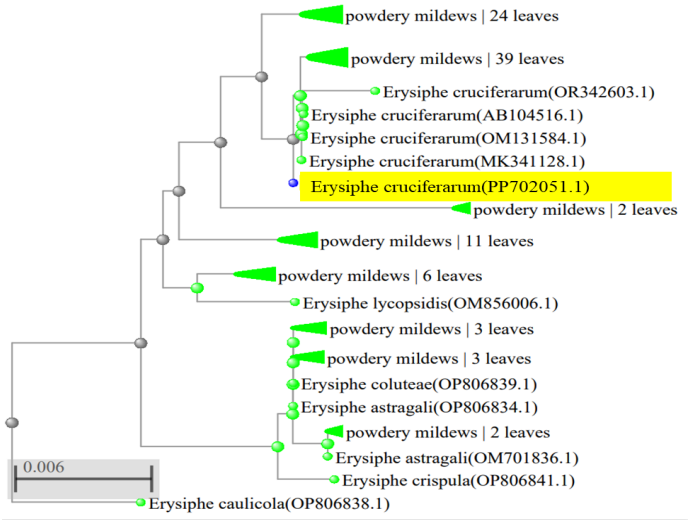

Figure 3. The neighbor-joining phylogenetic tree built by BLAST showing genetic relationships and reference sequences from GenBank. Whith green color accessions of Erysiphe spp. and with yellow color is powdery mildews on Camelina staiva from Bulgaria (PP702051.1).

CONCLUSIONS

As far as is known, this is the first report of powdery mildew caused by Erysiphe cruciferarum on Camelina sativa in Bulgaria.

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