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Registration and Molecular Characterization of *Cladosporium* Fungus Isolated from the Durum Wheat Cultivar Beni-Sweif- 5

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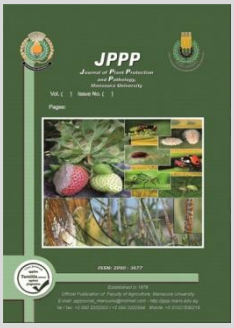

ABSTRACT

This study was carried out in the 2023/2024 wheat-growing season to identify the pathogen that infected the durum wheat cultivar Beni-Sweif-5 grown in the production sector, Sids agricultural research station. The stems and spike holders of the wheat cultivar showed dark browning ring symptoms. Initial infection symptoms appeared as brown discoloration then turned into dark brownish, then followed by drooping of the spikes to the ground. This cultivar was sown in 200 feddans (83.36 hectares) grown on Sids Agricultural Research Station farm (Agric. Res. Stn.), Beni-Sweif governorate. Disease incidence ranged from 10-15 % in all the growing areas. The isolated fungus characterized morphologically as *Cladosporium cladosporioides* in the Mycology Dept., Plant Pathology Research Institute (PPATHRI), ARC. The internal transcribed sequence (ITS) region of isolated fungus amplified by ITS1 and ITS4 primers pair, morphological and molecular studies confirmed that the causal fungus is *Cladosporium cladosporioides*. Phylogenetic relationships showed high homology 100 % with other *Cladosporium* isolates on the Gen Bank database. This isolate is released with accession number OM722086 in the NCBI/Gene Bank database. To our knowledge, this is the first record of the fungus *Cladosporium cladosporioides* on durum wheat in Egypt.

Keywords: Durum wheat, *Cladosporium cladosporioides*, ITS, Phylogenetic relationships.

INTRODUCTION

Durum wheat (*Triticum turgidum* L.) is consumed in specific geographical areas as a major food source with high protein content and a firm texture (De Santis *et al*., 2021; Martínez-Moreno *et al*., 2022; Grosse-Heilmann *et al*., 2024). Durum wheat annual production is about 35-40 million tons, representing approximately 7% of complete wheat worldwide productivity (Kadkol and Sissons 2016; Xynias *et al*., 2020; Broccanello *et al*., 2023). Nutritionally, it consists of 70% carbohydrates, 12%–16% protein, 1.9% fat, 1.6% minerals, and 1.6% fiber (Saini *et al*., 2022). Three to four hundred thousand feddans of durum wheat are grown annually concentrated in Egypt's middle and upper governorates (Economic Affairs Sector, 2023). Egypt's annual imports of durum wheat are about 2.72 billion USD (WITS, 2023). Many abiotic and biotic stresses threaten the productivity of durum wheat, reducing its production (Bouanaka *et al*., 2021; Laribi *et al*., 2021). Biotic diseases are restricted plant organs e.g. aerial tissues include rusts, powdery mildew, Fusarium head blight, and some minor diseases (Ogórek *et al*., 2012; Haile *et al*., 2019; Bhavani *et al*., 2021; Hadjout *et al*., 2022). Majority of mentioned pathogens belong to the phyla Ascomycota e.g. *Cladosporium* spp. (Doehlemann *et al*., 2017; Sultana *et al*., 2019). *Cladosporium cladosporioides* is a saprotrophic fungus which commonly develops on necrotic or decaying parts of plants as a secondary infection and found in several crops, including wheat (Bensch *et al*., 2010; Zhu *et al*., 2024). Some papers reported that *Alternaria*, *Cladosporium*, and *Rhizopus* are the common isolated fungi from cereal and wheat seeds (Pitt and Hocking, 2009; Riba *et al*., 2010; Djaaboub *et al*., 2020). Early fungal plant pathogen detection and identification are crucial to avoid disease outbreaks that might cause economic losses (Hariharan *et al*., 2021). The present study aims for isolation and identification the pathogen that result in dropping and death of durum wheat stems collected from, Beni-Sweif-5 grown at Sids Station, Beni-Sweif Governorate, Egypt.

MATERIALS AND METHODS

Observation of symptoms:

This study was conducted in 2023/2024 growing season to identify the pathogen infected the durum wheat cultivar Beni-Sweif-5 grown in the production sector farm, Sids agricultural research farm in the last of previous season. Stems and spike holders of the durum wheat cultivar Beni-Sweif-5 (200 feddans) grown in Sid's farm of the production sector, Beni-Sweif governorate showed dark browning rings around them.

The length of dark brown rings ranges from 4 - 6 mm (Fig. 1 A, B and C). Initial infection symptoms appeared as brown discoloration then turned to dark brownish and finally the stem bent causing the drooping of spikes to the ground. Disease incidence ranged from 10-15 % in all the growing areas. Infected samples were left to dry at room temperature, then maintained in the envelop bags until use.

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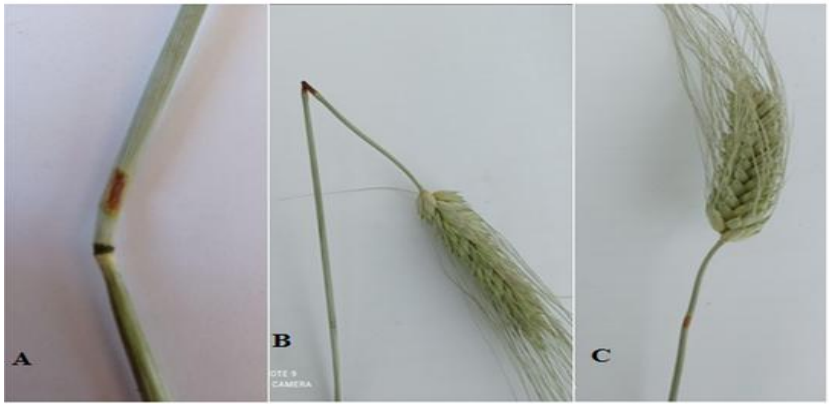

Fig. 1. (A, B, and C). Symptoms of infected spike holder of the durum wheat cultivar Beni-Sweif-5.

Fungus isolation and identification

For the etiology of this pathological phenomenon, twenty samples of the infected stems and spike holders were collected and preserved in paper bags. For isolation, small sections of diseased parts were sterilized in sodium hypochlorite solution (1%) for 2 minutes, dipped in sterile water three times, dehydrated then cultured on Potato Dextrose Agar (PDA) amended by 0.01% streptomycin. Plates were maintained in darkness for one week at 25℃. The isolated fungus was examined then identified microscopically at the Mycology Department, PPATHRI, ARC. Morphological identification of isolated fungus was done as reported by Barnett and Hunter (1987) and Tashiro *et al.* (2013).

Molecular identification

For further confirmation, DNA extracted from a purified fungal isolate using the SIGMA Company Miniprep (Quick-DNA™ Fungal/Bacterial kit). To perform polymerase chain reaction (PCR), DNA was measured then diluted to with nuclease-free water 50 ng/µL. The specific primers ITS1 and ITS4 are used to amplify the ITS regions of the nuclear rDNA (White *et al*., 1990). Setting up the PCR reaction (25 µL) requires DNA template, MyTaq Red Mix(8 µL), 1 µl of each primer (20 pmol), and nuclease-free water(15 µL). The PCR amplification conditions following Mohamed (2022). BLAST was used to align the sequenced DNA for species identification and to search for similarity with other fungal species registered in nucleotide database of GenBank. The Neighbor-Joining tool from BLAST Tree Viewer is used for molecular phylogenetic and evolutionary analysis.

Pathogenicity assay

The pathogenicity test was conducted for Koch's postulates confirmation. In the November 2023 season, the Beni-Swief-5 cultivar seeds were sown in 10 plastic pots (50x50 cm) under a conditioned glasshouse at Gemmeiza Agricultural Research Station, ARC, Egypt. Ten wheat plants/pots were sprayed with the fungal isolate suspension at heading growth stage (GS 5) following Zadoks *et al*. (1974), and maintained in a conditioned glasshouse with 80% relative humidity at 25°C. The inoculum was obtained from fungal culture (15 days old) growing on PDA by flooding each plate with sterile distilled water and scraping gently the fungal colony to dislodge conidia with a flame-sterilized scalpel. The conidial suspension was filtered through a layer of cheesecloth, and the concentration spores were detected using the hemocytometer. The inoculum concentration was adjusted to 1.0×10^6 (conidia/mL) according to Zhang *et al*. (2022). Plants sprayed only with sterilized distilled water were kept as control. Initial brown discoloration around the stems of wheat plants developed after 8-10 days of inoculation similar to the symptoms observed in with naturally infected wheat. The causal fungus was reisolated from the infected stems then identified microscopically.

RESULTS AND DISCUSSION

Results

Culture Examination

The microscopic examination of the isolated culture showed that colonies were effuse, dark brown with a black reverse, and in 7 days the diameter reached 5.0 cm. After 4-5 days, the white mycelium turned into olive-grey to faint green, velvet, and tufted mycelium. Colony edges may be white to olive-grey and somewhat feathery (Fig. 2 A and B). This isolate was identified as typical of the genus *Cladosporium cladosporioides*.

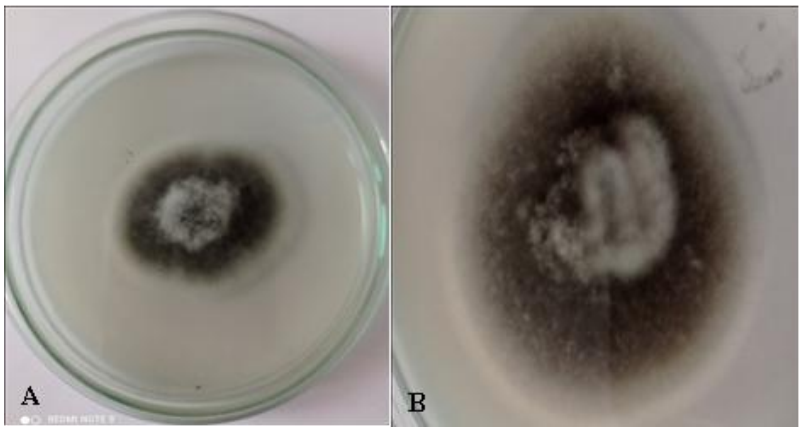

Fig. 2. (A and B). Colonies of *Cladosporium cladosporioides* are olive-green to olive-brown.

Ramoconidia have an oblong to cylindrical shape, with a mean length and width of (14.73 µm × 3.48 µm). Conidia are blastocatenated, branching chains that can readily disarticulated, single-celled, and subglobose to ellipsoid, with visible scars and denticle shaped projections were characteristic features of the genus Cladosporium. The conidia's average length and width diameters are 5.95 µm × 3.7 µm (Fig. 3 A, B and C).

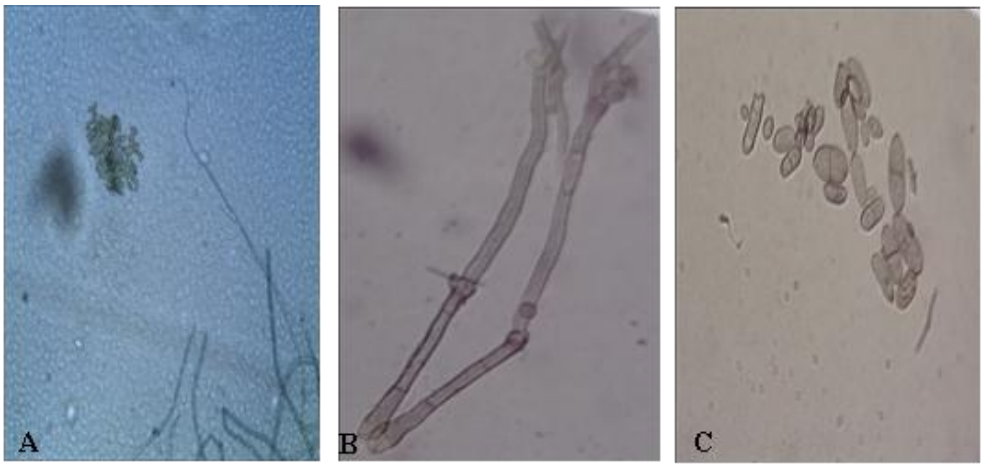

Fig. 3. (A, B and C). The identified mycelium and conidia of *Cladosporium cladosporioides* as reported at the Mycology Department, PPATHRI, ARC.

Molecular identification

The primer set ITS1/ITS4 used for ITS region amplification and sequencing according to White *et al.* (1990) for confirmation of the microscopic identification. The fungal isolate was identified as *Cladosporium* sp. isolate LS_T4L3C39 with an ITS 1, 5.8S ribosomal RNA gene, complete sequence; and 28S ribosomal RNA gene, partial sequence par-nucleotide and showed homology100 percent with other Gene Bank *Cladosporium* depositing strains (Fig.4). The sequence has accession number OM722086 as deposited in NCBI data base.

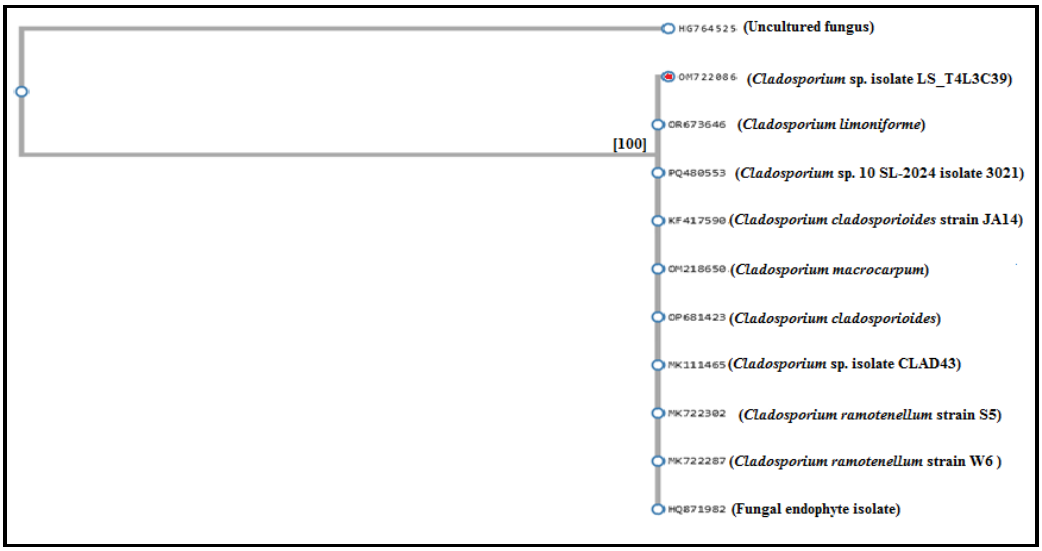

Fig.4. Phylogenetic tree using BLAST pairwise alignment between the isolated fungus and other fungi in the sequence database.

Discussion

Durum wheat is a worldwide cultivated crop, representing 7% of total produced wheat (Broccanello *et al*., 2023). In Egypt, it exposes to infect with some diseases including rusts especially brown rust and powdery mildew (Mohamed and Elkot, 2020). Observations of brown color rings around stems of the Beni-Sweif-5 durum wheat variety were detected in March 2023 and finally stems with the spikes were dropped down. Fernandez and Jefferson (2004) stated that isolation and sampling of fungi from spring and durum wheat were commonly at specific stages (milk to early-dough). The causal fungus was isolated on PDA media at 20-25 ^{0}C which attained a growth with, a 5.0 cm diameter in one week, effuse, dark brown with black reverse. After 4-5 days, the initially white mycelium turned into an olive-grey to dull green, tufted and velvety mycelium. Bensch *et al*. (2010) and Krzyściak *et al*. (2011) stated that *Cladosporium* sp. growth on PDA medium was moderate fast at 25 °C and colonies are velvet, meal, grey-green to olive-green and reverse black. Also, Domsch *et al*., (1980) stated that colonies of *Cladosporium cladosporioides* diameter about 3–4 cm on MEA in ten days at 20°C. Conidia were subspheric to lemoni-form, mainly smooth-walled, barely finely verrucous, olive-brown, single celled, 3-7 (-11) ×2-4 (-5) µm. Causative fungus was isolated and determined morphologically as *Cladosporium*

cladosporioides in the Mycology Dept., PPATHRI (Barnett and Hunter, 1987 and Tashiro, *et al*., 2013).

PCR was used for amplification of the ITS region, and then it was sequenced by the primer pair ITS1/ITS4. The fungal isolate was identified as *Cladosporium* sp. isolate LS_T4L3C39 with an ITS1, 5.8S rRNA gene, complete sequence, and 28S rRNA gene, partial sequence par-nucleotide. The phenotypic tree showed homology 100 percent with other Gene Bank *Cladosporium* depositing strains. The identified sequence was deposited in NCBI with accession number OM722086.

Artificially spraying with *Cladosporium* isolate develops the identical symptoms that showed on the durum wheat stems as in the natural infection. Vujanovic *et al*. (2012) reported that 17 species were detected on durum wheat as pathogens, among them *Cladosporium*, and crop rotation severely affected the domination of two pathogenic fungi. Briceño and Latorre (2007) reported that *Cladosporium*, a saprotrophic fungus, commonly develops as a second infection on necrotic or decaying plant parts and has been found in several crops, including wheat. Also, Farr *et al. (*1989) stated that *Cladosporium* sp. often causes a greenish-black discolouration on the ear heads. *C. cladosporioides* is an emerging threat that affects both plants and humans (Solairaj *et al*., 2022). In Egypt, *Cladosporium cladosporioides* was reported as the first record on mango leaves Abd Elghany and Kamhawy (2023). We believe that it is the first record of *Cladosporium cladosporioides* on Durum wheat.

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التسجيل و التوصيف الجزيئ لفطر كلادوسبوريوم المعزول من صنف القمح الديورام بنى سويف ٥

مصطفى محمود الشامى ، منى السيد محمد و أسامة عبد البديع

قسم بحوث أمراض القمح – معهد بحوث أمراض النباتات – مركز البحوث الزراعية – مصر

الملخص

أجريت هذه الدراسة في موسم زراعة القمح ٢٠٢٤/٢٠٢٣ لتحديد العامل الممرض الذي أصاب صنف القمح الصلب بنى سويف-٥ المنزرع في قطاع الإنتاج بمحطة البحوث الزراعية سدس. تمثلت أعراض الاصابة بوجود حلقات بنية تحولت الى اللون البنى الداكن حول سيقان وحوامل السنابل المصابة ثم حدث تحلل لمنطقة الاصابة مما أدى الى سقوط السنابل على الأرض. زرع هذا الصنف في ٢٠٠ فدان (٨٣,٣٦ هكتارا) في مزرعة محطة البحوث الزراعية بسدس في محافظة بني سويف. تراوحت نسبة الإصابة بالمرض بين ١٠-١٥٪ في جميع مناطق النمو. تم تعريف الفطر المعزول مورفولوجيا بأنه كلادوسبوريم كلادوسبوريدس (*Cladosporium cladosporioides*) في قسم علم الفطريات بمعهد أبحاث أمراض النبات(PPATHRI) التابع لمركز البحوث الزراعية. كما أكدت منطقة التسلسل الحمض النووى الريبوزى الداخلي (ITS) للفطر المعزول الذي تم تضخيمه بواسطة زوج البادئات ITS1 و ITS4 أن الفطر المسبب هو Cladosporium cladosporioides. أظهرت العلاقات التطورية تشابها كبيرا بنسبة ١٠٠٪ مع عزلات Cladosporium الأخرى في قاعدة بيانات Gen Bank. تم تسجيل هذه العزلة برقم التعريف OM722086 في قاعدة بيانات NCBI / Gene Bank. وعلى حد علمنا ، هذا هو أول تسجيل للفطر Cladosporium cladosporioides على القمح الديورام في مصر.

الكلمات الدالة: كلادوسبوريم كلادوسبوريدس , العلاقات التطورية , قمح الديورام