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Disease Note: First Report of *Cladosporium ramotenellum* Schub., Zalar, Crous & Braun, 2007 (Fungi: Dothideomycetes) as a Potential Contaminant of Date Palm Tissue Culture

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Abstract: *Cladosporium ramotenellum* Schub., Zalar, Crous & Braun, 2007 an ascomycetes fungus, was isolated from cultured date palm tissues at callus induction stage in Date Palm Research Centre, University of Basrah. Morphometric and molecular diagnoses using internal transcribed spacer (ITS) primers were applied. Notably, *C. ramotenellum* was not previously recorded as a contaminant of cultured date palm tissues in Iraq.

Keywords: *Cladosporium ramotenellum*, Date palm, Fungal contamination, Molecular identification, Morphology.

Introduction

Date palm (*Phoenix dactylifera* L.) is one of the most important evergreen fruit trees in a number of countries in the world. Its importance lies in the value of its fruits, which contain nutrients with high nutritional value, such as sugars, mineral salts, some vitamins and a percentage of proteins; its trees are also used in construction, craft and fuel industries (Abass, 2017).

Tissue culture is one of the modern technologies used in the propagation of many plants from different plant families, including date palm. Date palm is propagated *in vitro* from the developing apex and axillary buds through organogenesis or somatic embryogenesis through the callus stage, from which vegetative embryos are formed (Johnson *et al.*, 2015). However, tissue culture faces great challenges; the most important of which is the problem of microorganism contamination, which causes callus tissue deterioration (Hameed & Abass, 2006).

Fungi are one of the main aggregates that contaminate date palm tissue in very high proportions during all stages of tissue culture course (20-30% as a contamination percentage) (Abass, 2013). In this study, *Cladosporium ramotenellum* Schub., Zalar, Crous & Braun, 2007 was isolated (Fig. 1) and diagnosed as one of the fungal contamination in date palm tissue cultures in Basrah Governorate, Iraq. The fungal isolate was grown on potato dextrose agar medium under standardized conditions to identify the pathogen (Fig. 2 A-C). The results showed that its phenotype matches the description in

a number of relevant literature (Braun *et al.* 2006; Ding *et al*., 2019).

Fig. (1): Fungal contamination of date palm tissue cultures. *C. ramotenellum* fungus colony on date palm callus.

Fig. (2): Morphological traits of *C. ramotenellum* on PDA medium.

(A) Colony morphology on PDA after 15 day

(B) Conidiophores (C): Scale bar = 10 µm.

Arrows refer to conidiophores.

The colony showed diffuse hyphae with radial growth in the form of concentric rings, a black centre with grey ring and black edges. The conidia were pear-shaped to oval with pointed end, had 0-3 septa, light yellow in colour and 9.6-24 µm × 2.4-4.8 µm in size.

The fungal hyphae were branched and wide, had closely spaced divisions in the form of knots or bulges and transparent to light brown in colour. The conidiophores were short, wider than the conidia and dark brown in colour.

The comparison of phenotypic traits amongst reference literature has not given reliable discrimination, because its phylogenetic description matches a number of *Cladosporium* species, particularly *C. ramotenellum* (Abass, 2013, Razaq & Abass, 2021).

Therefore, the internal transcribed spacer region of the isolate was amplified using primers ITS1 and ITS4 and sequenced. The 518-bp amplicon had 98% similarity to *C. ramotenellum* (has been deposited in GenBank as an Accession OL589159.1). Thus, the isolated fungus was identified as *C. ramotenellum* by phylogenetic analysis (Data not shown).

Several studies confirmed that the fungal contamination is common in the cultivation of date palm tissue and inhibits the growth of embryonic callus, which causes plant death (Abass, 2017; Abdel-Karim, 2017). This study is the first to record the fungus *C. ramotenellum* as one of the contaminant of cultured date palm tissues in Iraq. More studies are required to apply a new control measures to restrict the fungal contamination damage on date palm tissue cultures.

Contributions of Authors

A.N. Ahmed: Samples collection; and preparing the experiments of molecular diagnosis.

M.H. Abass: Contribute in research plan; writing the manuscript and preforming bioinformatics.

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Conflicts of interest

The authors declare that they have no conflict of interests.

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التسجيل الأول للفطر *Cladosporium ramotenellum* كمسبب لتلوث مزارع أنسجة نخيل التمر

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المستخلص: عزل النوع الفطري *Cladosporium ramotenellum* من مزارع أنسجة نخيل التمر في مختبرات زراعة الأنسجة في مركز أبحاث النخيل/ جامعة البصرة وتم تشخيصه مظهرياً، فضلاً عن التشخيص الجزيئي باستعمال الواسم الجزيئي Internal transcribed spacer (ITS). ومن الجدير بالذكر أن هذا التسجيل يعد الأول من نوعه كمسبب لتلوث مزارع أنسجة نخيل التمر في العراق.

الكلمات المفتاحية: *Cladosporium ramotenellum*، نخيل التمر، التلوث الفطري، تصنيف جزيئي، مظهري.