

# First report of *Fusarium subglutinans* (Wollenw. & Reinking) (1983) as a causative agent of leaf spot disease on broad bean *Vicia faba* L. in Iraq

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## Abstract

Broad bean (*Vicia faba* L.) is one of the most common vegetable crops in Iraq; cultivated in many areas. Broad bean production has been affected significantly by several diseases caused by either bacteria or fungi; among these pathogens *Fusarium* have a direct impact on faba beans production. The current study was conducted at the laboratories of the College of Agriculture - University of Basrah to identify the fungal pathogens of broad bean leaf spot disease. Isolation of the fungal pathogen was performed on PDA and PCA media; the morphological and microscopic identification revealed the identity of *Fusarium subglutinans*, followed a molecular diagnosis by ITS universal primers (ITS1 and ITS4) with a similarity percentage of 99%; the gene sequence was deposited at NCBI as LC769974.

Experimental tests confirmed the pathogen's capability to infect the vegetative system of the broad bean plants. This is the first recorded of *F. subglutinans* being identified as the cause of leaf spotting on broad bean plants, making it a notable discovery in both Iraq and globally.

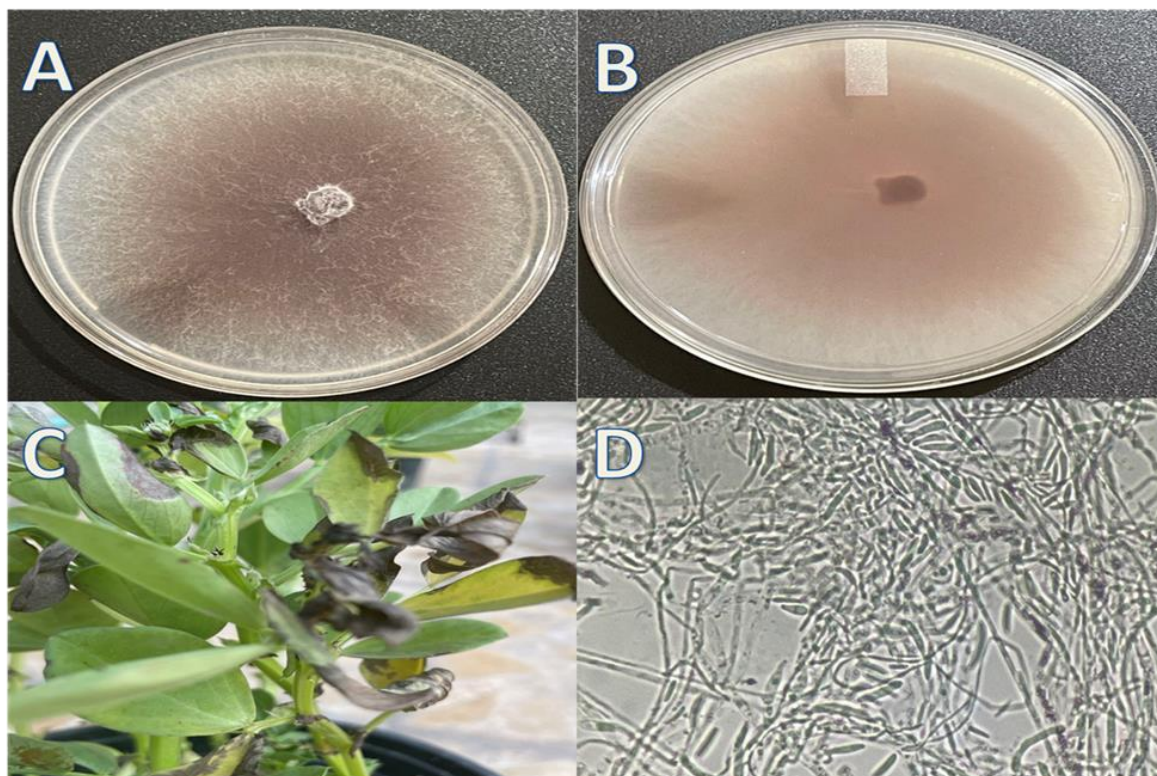
**Keywords:** broad bean, *Fusarium subglutinans*, ITS, molecular diagnosis, phenotypic.

The broad bean plant (*Vicia faba* L.) is a significant leguminous crop with a protein content of 20-30%, making it valuable for both human and animal consumption (Qahtan et al., 2021). It is native to the Mediterranean region and Central Asia, and it demonstrates adaptability to various climates worldwide (Crépon et al., 2010; Arya et al., 2021). Broad bean is widely consumed in China, Africa, the Middle East, and Asia (Martineau et al., 2022).

Samples of faba bean plants showing spots on both stems and leaves were collected during the fourth quarter of 2022 and the first quarter of 2033 in Basrah Governorate. A comprehensive investigation was carried out to examine the spots, resulting in the identification of *F. subglutinans* fungus as the causal agent. The fungus was isolated under specific conditions of low temperatures (15-20° C) and high humidity. The diagnosis was confirmed through cultivation of the fungus in PDA and PCA medium, as illustrated in Figure (1 A and B). This diagnosis



aligns with previous studies conducted by Borrás et al. (2001) and Harish et al. (2023). To validate the pathogenicity of the fungus on broad bean plants, Koch's postulates were followed. Bean seeds were cultivated in 12 cm pots containing a mixture of sterile peat moss and soil in a 2:1 ratio. Following a period of one and a half months, the plants were inoculated with a suspension of *F. subglutinans* fungus ( $1 \times 10^6$  spores/ml). After two weeks of inoculation, characteristic spotting symptoms manifested, as depicted in Figure (1C).



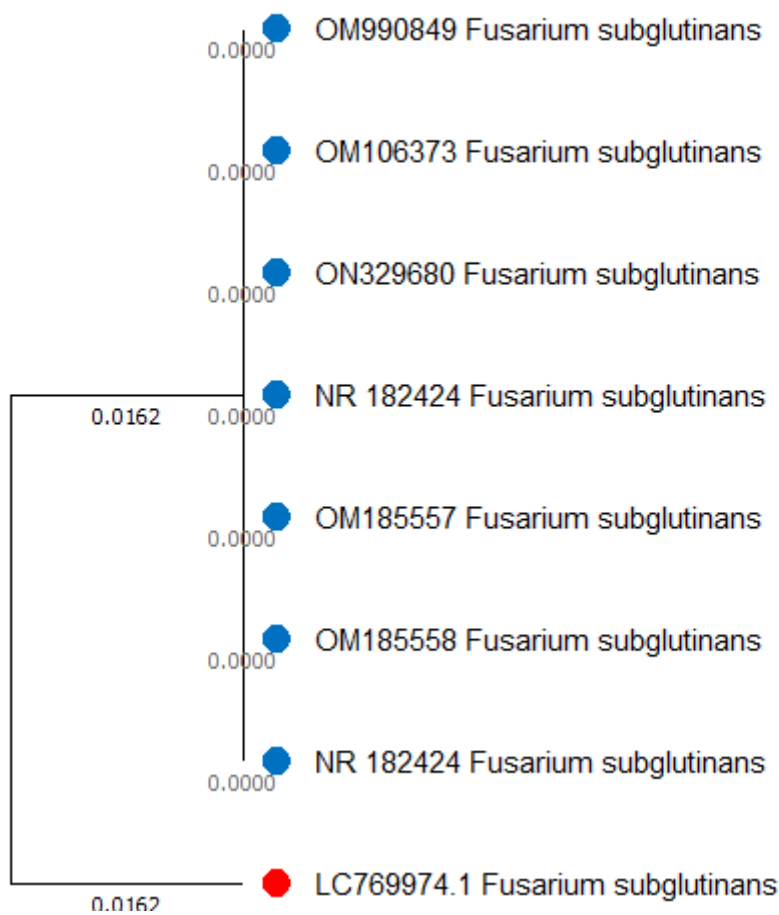
**Figure (1) *F. subglutinans* colony features on PDA.**

(A) Top colony appearance (B) Reverse colony appearance. (C) Pathological symptoms on faba bean (D) conidia (40x magnification).

As depicted in Figure (1A-B), the pure colony cultivated on PDA medium initially displayed a color spectrum ranging from white to creamy. After 7 days of incubation, the colony color changed into a light violet shade with a cottony texture. The reverse side of the colony appeared white to creamy. Complete plate coverage by the colony was achieved after 6-7 days of incubation at a temperature of 25° C.

It was also found that the fungus produces three types of conidia. The large spores are cylindrical and slightly curved, their length ranges from 9.9-23.31 x 3.34-4.99  $\mu\text{m}$ , and the transverse divisions are 3-4 divisions, while the small spores are slightly elongated and curved, and they are either unicellular or dioecious, with a length ranging from 4.99-9.99 x 2.34 -3.34  $\mu\text{m}$  (Figure 1D).

The ITS region was amplified using ITS1 and ITS4 primers over the duplication region 524- bp (Ahmed and Abass, 2022; Yaser and Abass, 2022). The results showed that the fungus is *F. subglutinans* with a match rate of 99%. It was deposited in the Genome Bank under the sequence number Accession No. (LC769974). Sequence characteristics of ITS genes were applied and the phylogenetic tree was drawn according to MEGA7 software with the available and similar sequence in National Center for Biotechnology Information (NCBI) using Neighbor-Joining method (Figure 2). The phenotypic and molecular diagnosis agreed with many studies revealed the identity of *F. subglutinans* (Shin et al., 2014; Han et al., 2015).



**Figure 2: The phylogenetic tree generated by the Neighbor-Joining method for the genetic affinity of *F. subglutinans* in comparison with the standard sequences of nitrogenous bases in the NCBI/GenBank**

Several studies have confirmed that *F. subglutinans* is a common cause of spotting on plants (Ichikawa and Aoki, 2000; Harish et al., 2023). This study is the first to record *F. subglutinans* fungus as a cause of leaf spots on broad bean plants in Iraq and the world. More studies are needed to apply new control measures to reduce the damage of blotch on faba bean.

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