

PCR Combined with GFP-Tagged *Verticillium dahliae* Confirmed the Seeds' Transmission of Sunflower Verticillium Wilt

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Abstract

Verticillium Wilt of sunflower (*Helianthus annuus* L.) is a widespread and destructive disease caused by the soil-borne fungal pathogen *Verticillium dahliae* (*V. dahliae*). The quick spreading of Sunflower Verticillium Wilt in the sunflower planting region of China caused us to consider the possibility of seeds' transmission of the pathogen. Therefore, knowledge about the contamination of the seeds by *V. dahliae* is critical for understanding the infection cycle and also to develop ways to control the spreading of this disease. In this study, sunflower seedlings were inoculated with conidial suspensions of a GFP-tagged isolate. Colonization was studied with a confocal microscope. After 12 to 96 hours of post-inoculation (hpi), conidia germinated and formed hyphal colonies on the root tips and in the root elongation zones. Hyphae colonized cortical tissues and vascular elements 2 weeks after inoculation (2wpi). 10 wpi later, the xylem of the upper stem, sunflower disc, and sunflower seed parts, including the pericarp and seed coat, had been colonized by the pathogen. Moreover, pathogen DNA could be detected by RT-PCR in the pericarp and seed coat. Additional experiments that detected the transmission rate of seeds from different sunflower cultivars were conducted with PCR. Our results indicated that the transmission rate of sunflower seeds ranged from 10 to 25% among all tested cultivars. In conclusion, seed transmission is the main way for the long distance transmission of sunflower *V. dahliae*, and seed pretreatment should be done to control the infection of sunflower seedlings in the future.

Keywords: sunflower (*Helianthus annuus* L.); *Verticillium dahliae*; seed transmission

向日葵黄萎病的种子带菌研究

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摘要

向日葵黄萎病是一种由大丽轮枝菌引成的极具破坏性的真菌性病害。该病原体通过种子带菌进行长距离传播，并能够引起向日葵黄萎病的快速蔓延。因此，了解向日葵种子是否携带黄萎菌是确定向日葵黄萎病侵染周期、制定控制这种病害方法的关键。在本研究中，应用 GFP 标记大丽轮枝菌的分生孢子悬浮液接种向日葵幼苗。利用共焦显微镜观察该病原菌的定殖过程。接种 12~96 小时后，分生孢子开始萌发形成菌丝并附着在根尖和根伸长区。接种 2 周后，菌丝定殖在根的皮肤组织以及维管束里。接种 10 周后，地上部分的向日葵茎秆、向日葵花盘以及向日葵种子的各部分结构（包括果皮、种皮），均有病原菌的定殖。此外，通过 RT-PCR 在种子的果皮和种皮结构中检测到该病原体的 DNA。同时，我们利用 PCR 技术检测不同品种的向日葵种子的带菌率，结果表明，所有供试向日葵种子的带菌率为 10~25%。总之，种子带播是向日葵黄萎病进行长距离传播的主要途径，进行种子预处理是减少向日葵幼苗被侵染的关键。

关键词：向日葵；大丽轮枝菌；种子带菌