Phylogenetic diversity and toxigenic potential of *Fusarium tricinctum* Species Complex strains associated to Fusarium head blight on durum wheat

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Several *Fusarium* species are agents of Fusarium head blight (FHB), a serious worldwide cereal disease causing qualitative and quantitative yield losses. A lot of information is available for the main fungal species, such as *F. graminearum* and *F. culmorum*, involved in FHB. However, the "less aggressive" species, like *F. acuminatum* and *F. tricinctum*, belonging to the *Fusarium tricinctum* Species Complex (FTSC), have poorly been studied until now. A population of FTSC strains isolated from Italian durum wheat grains was molecularly characterized by partial sequencing of the translation elongation factor 1-alpha region (*TEF1*α) and RNA polymerase second largest subunit region (*RPB2*). Furthermore, greenhouse and field experiments were carried out in order to define their pathogenetic role. The strains capacity to biosynthesize, *in vitro* and *in vivo*, secondary metabolites (enniatins A, A1, B, B1 and beauvericin) was also investigated by HPLC-MS/MS. The molecular characterization allowed the identification of two phylogenetically distinct populations of *F. tricinctum* without evident biological differences. Specific symptoms caused by *F. tricinctum sensu stricto* strains were observed. Inoculated durum wheat heads showed "peacock eye"-shaped necrosis and necrotic spots at the glume level. Secondary metabolites production was independent from the phylogenetic group. *In vitro*, enniatins were produced in significant amounts but no beauvericin was detected. *In vivo*, the enniatin levels were higher than the beauvericin ones. These results allowed to clarify the role of *F. tricinctum sensu stricto* in the disease while further studies are necessary to better understand the involvement of each member of FTSC and their relationship within the FHB complex.

A fast and specific assay for the surveillance of the invasive forest pathogen *Heterobasidion irregulare* based on Loop-mediated isothermal AMPlification (LAMP)

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The North American species *H. irregulare* Garbel. & Otrosina was accidentally introduced in central Italy during the World War II. The pathogen has become invasive and is currently distributed in pine and oak stands over about 105 km of coast around Rome, often in association with significant mortality of Italian stone pine (*Pinus pinea* L.) trees. Based on its observed and potential impact, *H. irregulare* is recommended for regulation in Europe by the European and Mediterranean Plant Protection Organisation (EPPO). In the frame of the EU Project EMPHASIS (grant agreement 634179), a fast and specific molecular diagnostic tool based on Loop-mediated isothermal AMPlification (LAMP) coupled with two different DNA extraction methods was developed and optimized for the detection of *H. irregulare*. The LAMP assay was successfully validated both in controlled conditions and in the field on different samples, including fruiting bodies, infected plants, wood colonized by the pathogen, and airspora collected by using woody spore traps. The detection of the pathogen in airspora was optimized both in terms of methods and in terms of length of incubation period of traps. The limit of detection of this assay is approximately 20 picograms of target DNA with a detection time of about 40 minutes. This molecular diagnostic tool may be used for the surveillance in the ports of entry of wood imported from North America and for the monitoring of pine forests, especially those located in a buffer zone surrounding the infested area.

Biogenic extracellular synthesis of gold and silver nanoparticles by *Trichoderma harzianum* and *Trichoderma longibrachiatum* and their effectiveness against seed-borne fungal pathogens

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Nanoparticles present a multifunctional platform for a diverse range of applications in the modern agricultural concept of precision farming due to their small size, high surface to volume ratio and unique optical properties. Antimicrobial nano-materials, pathogen detection by the use of nano-sensors, spray-induced gene silencing (SIGS) are only some of the potentialities of the nanotechnology in crop protection. The biosynthesis of gold and silver nanoparticles (AuNPs and AgNPs) by *Trichoderma harzianum* and *T. longibrachiatum* was evaluated. Fungi were isolated from deep-sterilized tissues of *Cupressus sempervirens* and *Gladiolus* cv. 'Red Balance', respectively, and were molecularly identified using ITS and *tef-1* sequences analyzed by *Trich*OKEY and *Tricho*BLAST. Cell-free extracts of the fungi were challenged with 1 mM silver nitrate and 0.5 mM tetracholoroauric acid solutions. The formed nanoparticles were characterized by means of spectroscopic and microscopic analyses including UV-VIS spectroscopy, TEM and XRD, EDAX and FT-IR analyses. Antifungal activity using a microdilution assay in 96-well microtiter plates against *Colletotrichum lupini*, *Fusarium oxysporum* f. sp. *basilici* and *Botrytis cinerea* was assessed. The results were transformed to percentage of controls and the IC_{50} and IC_{90} values were graphically obtained from the dose-response curves. The AgNPs of *T. harzianum* and *T. longibrachiatum* strongly reduced the growth rate of all tested pathogens (85-100%) and only the AuNPs of *T. longibrachiatum* gave rise to a reduction of growth between 18 and 56% depending on the pathogen tested. The promising results obtained open up opportunities for further research on effective and ecofriendly solutions for the control of seed-borne diseases.