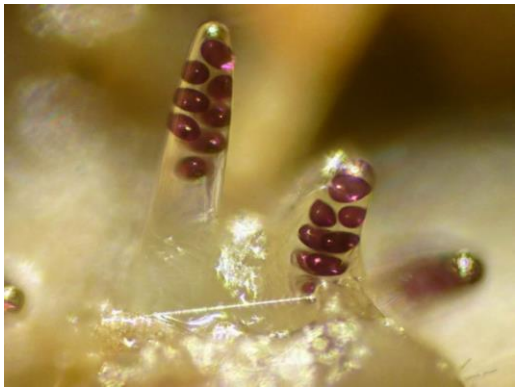
ASCOTUBE



In vitro and *in situ* unraveling truffle sexual reproduction using *Ascobolus immersus* as a test tube model

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Context — Truffles are fungi belonging to Pezizomycete class and forming ectomycorrhizal symbiosis with tree and shrubs. The Pezizomycetes comprise 200 genus and ~1,700 described species. They constitute an early diverging lineage of Ascomycota composed of saprophytic, mycorrhizal and pathogenic species found in soils, wood decays, leaves, roots and dung. Some species are well known due to their edible fructifications, such as truffles and morels. *Ascobolus immersus* is also famous since the late 1930s as a model organism for genetic studies. Despite their interest, to date, only the genomes of *T. melanosporum* and *Pyronema confluens* have been published. The *T. melanosporum* genome sequencing projects permitted highly microsatellite and mating type idiomorphs (MAT1-1 and MAT1-2) to be characterized (international patent n°WO2012/032098). An international collaboration between IAM unit and CNR-Perugia highlighted that *T. melanosporum* is a heterothallic species: truffles are strictly outcrossing species requiring two partners of different mating types to complete their life cycle, specifically when forming ascocarp. A non-random distribution pattern of *T. melanosporum* genotypes was observed, resulting in field patches exclusively colonized by genotypes of the same mating types. To date the mechanisms behind this selection or recognition of strains to colonize plant root system are unknown as well as the origin of paternal tissue (i.e. those that fertilize the maternal tissue harboured by root system). One question that should be addressed is if such mechanisms are driven by mating type genes or by another linked locus. Unfortunately, it is currently impossible to decipher the mechanisms behind truffle strains recognition *in vitro* due to the absence of genetic tools for truffles. More generally, genetic tools are lacking for many ecologically (e.g. *Cenococcum geophylum*, *Sphaerosporella brunnea*) and economically (e.g. Morels, *Terfezia boudieri*) important Ascomycota for which genomic resources are or will be soon available. The saprophytic pezizomycete, *A. immersus*, is a model organism for genetic studies. While found on dung in nature, axenic media have been developed to allow production of vegetative mycelium and completion of sexual cycle in lab conditions. To propagate itself, this heterothallic fungus relies on sexual reproduction only. Using reverse genetic strategy, several *A. immersus* genes have already been deleted, although the experimental procedure is not yet optimal.

Objectives — The aim of AscoTube is to unravel *in vitro* and *in situ* the black truffle sexual reproduction cycle. Since *A. immersus* belongs to Pezizomycete, as truffles, and its genome has been recently our hypothesis is that *A. immersus* could be used as a heterologous system to address fundamental questions for other Pezizomycetes and more generally Ascomycota species for which genetic tools are lacking. AscoTube is an innovative project that will produce several outputs in term of 1) new tools for fundamental basic research since *A. immersus* will become a handy test tube that any member scientific community can use to answer specific questions; 2) important knowledge about the sexual reproduction and strain recognition steps in truffles; 3) assessment of outcrossing occurrence between closely related truffle species such as *T.*

melanosporum and *T. indicum* that could lead to new policy recommendation and 4) the identification and localisation of maternal and paternal tissues in situ for optimizing truffle orchards management. The main results obtained so are:

- One publication in an international journal highlighting male and female genotype *in situ*
- One divulgation paper, eight seminar and one poster
- The recruitment of one postdoc: Simone Belmondo

The project started by the *in situ* analysis that was recently published in Environmental Microbiology. This analysis questioned the importance of ascospores in truffle sexual reproduction. New experiments will be developed to investigate this important point. Simone Belmondo was hired 1st April 2017 for 18 months, he moved to Saclay 1st November 2017 to start the work with *A. immersus*. It is now producing *A. immersus* CRISPR-CAS9 strains that will facilitate genetic studies.

Valorization —

Preprint

De la Varga H, Le Tacon F, Lagoguet M, Todesco F, Varga T, Miquel I, Barry-Etienne D, Robin C, Halkett F, Martin F, Murat C (2016) Five years investigation of female and male genotypes in Perigord black truffle (*Tuber melanosporum* Vittad.) revealed contrasted reproduction strategies. bioRxiv. DOI:http://dx.doi.org/10.1101/073650

International review

De la Varga H, Le Tacon F, Lagoguet M, Todesco F, Varga T, Miquel I, Barry-Etienne D, Robin C, Halkett F, Martin F, Murat C (2017) Five years investigation of female and male genotypes in Perigord black truffle (*Tuber melanosporum* Vittad.) revealed contrasted reproduction strategies. Environmental Microbiology, 19 (7): 2604-2615

Diffusion

Murat C, Selosse MA, Taschen E, Schneider-Maunoury L, Richard F, Martin F, Le Tacon F (2017). Quelques considérations sur le réensemencement par ascospores des truffières à Tuber melanosporum. Le Trufficulteur 98, 12-13.

Seminars

Murat, C. (2017) Le cycle de reproduction sexuée de la truffe noire, Syndicat des trufficulteurs du Vaucluse, 14 octobre 2017 .

Murat, C. (2017) L'evoluzione della simbiosi microbica studiata mediante il sequenziamento dei genomi fungini. Université d'Urbino (Italy), 17 février 2017.

Belmondo, S. (2017) Characterization of fungal genes involved in the establishment and the functioning of the arbuscular mycorrhizal symbiosis. INRA Centre Grand Est Nancy (France), 19 mai 2017.

Murat, C. (2016). La trufficulture avance, grâce à la recherche. Grands salon de l'hotel de ville de Nancy, 11 novembre 2016.

Murat, C. (2016). Le programme d'expérimentation national CulturTruf et les dernières avancée de la recherche. Syndicat des trufficulteur de la Drôme des collines. Génissieux, 29 octobre 2016.

Murat, C. (2016). Le programme d'expérimentation national CulturTruf et les dernières avancée de la recherche. Syndicat des trufficulteur de l'Ardèche. Serrière, 28 octobre 2016.

Murat, C. (2016). Le programme d'expérimentation national CulturTruf et les dernières avancée de la recherche. Syndicat des trufficulteur de l'Ain. Belley, 21 octobre 2016.

Murat, C. (2016). Dynamique de la reproduction sexuée de *Tuber melanosporum*. Journée de restitution des résultats de la recherche. Dijon, 22 avril 2016.

Posters

Belmondo, S., Grognet P., Paolocci F., Malagnac F., Martin F., Murat C. (2017) AscoTube: in vitro and in situ unraveling truffle sexual reproduction using Ascobolus immersus as a test tube model. Doc & Post-Doc Day. INRA Centre Grand Est Nancy (France), 14 juin 2017.