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Protocol of cryopreservation of arbuscular mycorrhizal fungi.

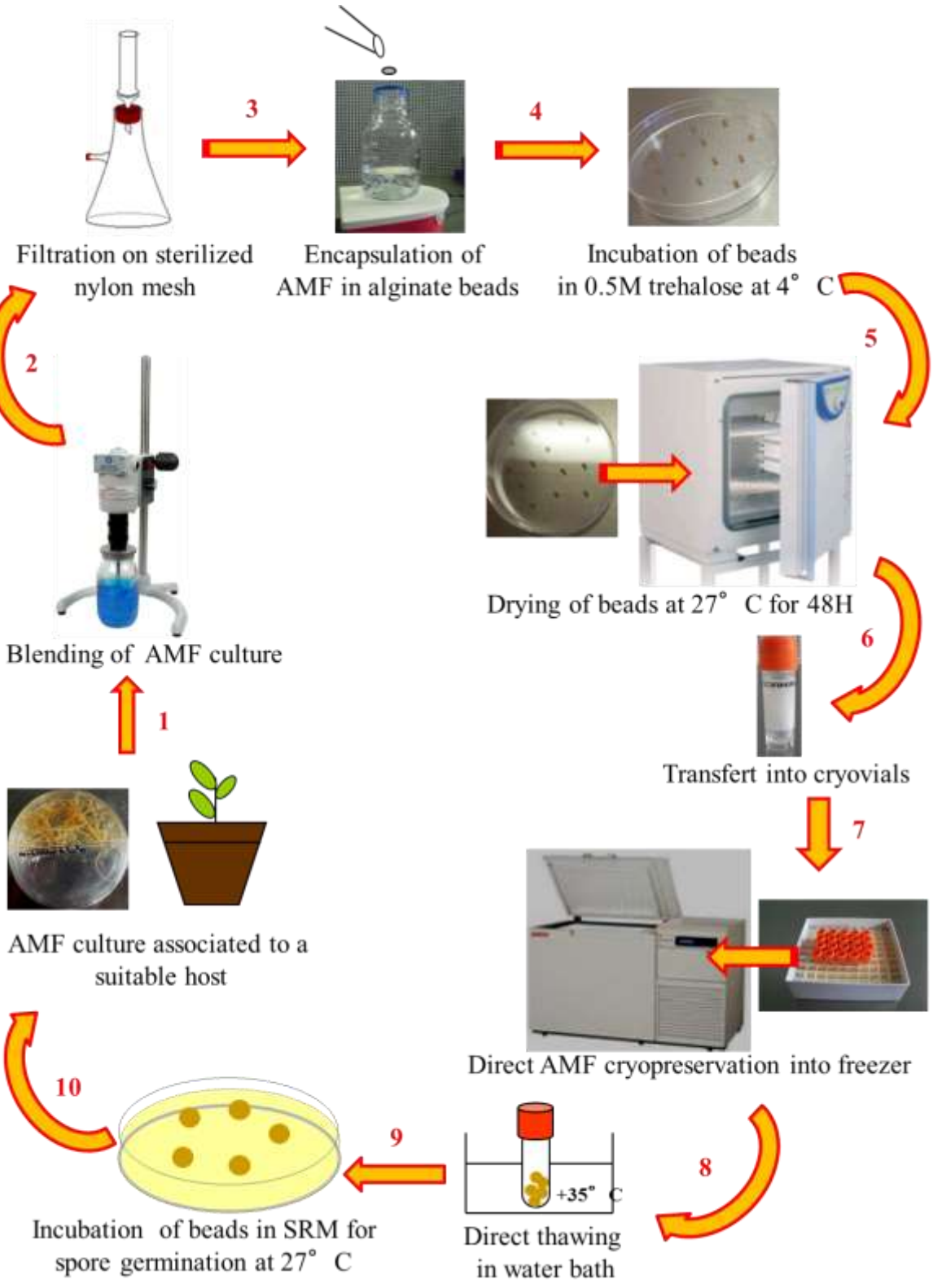
In
I. Lalaymia, S. Cranenbrouck and S. Declerck.
Maintenance and preservation of ectomycorrhizal and arbuscular mycorrhizal fungi
Mycorrhiza (2013) – DOI 10.1007/s00572-013-0541-8

Adapted from
I. Lalaymia, S. Cranenbrouck, X. Draye and S. Declerck
Preservation at ultra-low temperature of in vitro cultured arbuscular mycorrhizal fungi via entrapment-drying
Fungal Biology (2012) – 116:1032-1041.
I. Lalaymia, S. Declerck, F. Naveau and S. Cranenbrouck,
Cryopreservation of arbuscular mycorrhizal fungi from root-organ and plant
Mycorrhiza (2013) – doi:10.1007/s00572-013-0525-8

Protocol:

(1) Gelling medium, containing spores and roots of a 5-month old in vitro culture, is extracted from the Petri dishes, poured into 100 ml of sterilized (121 °C for 15 min) deionized water and subsequently blended twice for 30 s at 20,000 rpm in a sterilized (121 C for 15 min) mixer
(2) The mixture is filtered on a sterilized (121 °C for 15min) nylon filter (40 mm).
(3) The supernatant (spores and mycorrhizal/nonmycorrhizal root pieces) is encapsulated in 2 % (w/v) solution of sodium alginate (50±5 propagules per bead).
(4) The encapsulated propagules are incubated overnight in 0.5Mtrehalose and
(5) dried at 27 °C for 48 h (bead water content of approximately 8.1±4.6 %).
(6) Beads are transferred into 2-ml cryovials.
(7) The cryovials are cryopreserved in a freezer at −130 °C following a two-step decrease in temperature: a fast decrease (∼12 °C min−1) from room temperature (+20 °C) to −110 °C followed by a slow decrease in temperature (∼1 °C min−1) from −110 to −130 °C.
(8) For revival, the encapsulated AMF propagules are directly plunged in a water bath at +35 °C.
(9) The beads are dropped into sterilized (121 °C for 15 min) MSR medium, cooled in a water bath to 40 °C, and incubated at 27 °C for germination.
(10) After 4-weeks incubation, beads containing germinated propagules are associated with an excised root under in vitro culture conditions to reinitiate the fungal life cycle.

Lalaymia et al. (2013 – doi:10.1007/s00572-013-0525-8) adapted as follows this cryopreservation protocol to in vivo produced propagules: Pot cultures, at least 5 months old, are sampled. Spores are collected by wet sieving and decanting, while roots are collected with forceps and blended in a mixer in 100 ml deionized water for 30 s at 20,000 rpm, and filtered as above. The spores and the supernatant of the blended roots are mixed together and encapsulated in alginate beads, dried, cryopreserved, and thawed as described above. After thawing, the encapsulated propagules are placed directly in contact with roots of plants in pots containing a sterilized (2×15min at 121 °C, with 12-h interval) substrate. The plants are grown for at least 8 weeks in a growth chamber before assessing the fungal viability following cryopreservation.


Filtration on sterilized nylon mesh
3
Encapsulation of AMF in alginate beads
4
Incubation of beads in 0.5M trehalose at 4° C
5
Drying of beads at 27° C for 48H
6
Transfert into cryovials
7
Direct AMF cryopreservation into freezer
8
+35° C
Direct thawing in water bath
9
Incubation of beads in SRM for spore germination at 27° C
10
AMF culture associated to a suitable host
1
Blending of AMF culture
2