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Plant biostimulants - Determination of Mycorrhizal fungi

Biostimulanzien für die pflanzliche Anwendung - Bestimmung von Mykorrhizapilzen

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65.080	Gnojila	Fertilizers

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TECHNICAL SPECIFICATION

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FINAL DRAFT
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Plant biostimulants - Determination of Mycorrhizal fungi

Biostimulanzien für die pflanzliche Anwendung - Bestimmung von Mykorrhizapilzen

This draft Technical Specification is submitted to CEN members for Vote. It has been drawn up by the Technical Committee CEN/TC 455.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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Contents

European foreword

This document (FprCEN/TS 17722:2021) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

This document is currently submitted to the Vote on TS.

This document has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association.

Introduction

This document was prepared by the experts of CEN/TC 455 'Plant Biostimulants'. The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European standards or European standardization deliverables to support the implementation of Regulation (EU) 2019/1009 of 5 June 2019 2019 laying down rules on the making available on the market of EU fertilising products ("FPR" or "Fertilising Products Regulation"). This request, presented as SR M/564, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe". The Working Group 5 "Labelling and denominations", was created to develop a work program as part of this request.

The technical committee CEN/TC 455 'Plant Biostimulants' was established to carry out the work program that will prepare a series of standards. The interest in biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

The Biostimulants used in agriculture can be applied in multiple ways: on soil, on plants, as seed treatment, etc. A microbial plant biostimulant consists of a microorganism or a consortium of microorganisms, as referred to in Component Material Category 7 of Annex II of the EU Fertilizing Products Regulation.

This document is applicable to all biostimulants in agriculture based on live microorganisms belonging to the Mycorrhiza.

Table 1 summarizes many of the agro-ecological principles and the role played by biostimulants.

Table 1 — Agro-ecological principles and the role played by biostimulants

Increase biodiversity	
By improving soil microorganism quality/quantity	
Reinforce biological regulation and interactions	
By reinforcing plant-microorganism interactions	
—	symbiotic exchanges i.e. *mycorrhiza*
—	symbiotic exchanges i.e. *rhizobiaciae/fava*
—	secretions mimicking plant hormones (i.e. *trichoderma*)
By regulating plant physiological processes	
—	for ex growth, metabolism, plant development
Improve biogeochemical cycles	
—	improve absorption of nutritional elements
—	improve bioavailability of nutritional elements in the soil
—	stimulate degradation of organic matter

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

1 Scope

This document was developed to provide a horizontal method for enumeration and genera/specie determination [1], [2], [3] of mycorrhizal fungi in plant biostimulants products in accordance to the Regulation of EU fertilizing products.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at https://www.electropedia.org/
- ISO Online browsing platform: available at https://www.iso.org/obp

3.1
mycorrhiza
symbiotic relationship between a filamentous fungus and a plant

Note 1 to entry: In a mycorrhizal association, the fungus colonizes the plants' root tissues either intracellularly (as with endomycorrhiza) or extracellularly (as with ectomycorrhiza). This beneficial interaction brings several advantages to the plants such as, for instance, enhancement of nutrients and water uptake.

[SOURCE: FprCEN/TS 17724, 3.2.2.7]

3.2
endomycorrhiza
symbiotic association characterized by a filamentous fungal partner that colonizes the plants' root tissues intracellularly

EXAMPLE Four main groups of endomycorrhizal associations exist like arbuscular, ericoid, orchidoid and sebacinoid mycorrhiza.

[SOURCE: FprCEN/TS 17724, 3.2.2.7]

3.3
arbuscular mycorrhizal fungi
AMF
AM fungi
biotrophic microscopic fungi belonging to the Glomeromycota phylum (synonymous Glomeromycota) that establish obligate symbiotic associations with more than 70% of plant species on Earth

Note 1 to entry: Arbuscular mycorrhizal fungi produces structures inside plant roots, such as vesicles and/or endospores, but also specialized nutrient exchange structures called arbuscules.

Note 2 to entry: The hyphae do not penetrate the plant cell protoplast, but instead, it invaginates the cortical cell membrane where it branches dichotomously to develop the arbuscule which is mean to be the place where the exchange of nutrients and water takes place between the plant and the fungus.

Note 3 to entry: Arbuscular mycorrhizal fungi extraradical mycelium forms an extensive network within the soil which increase plant nutrient availability and absorption.

3.4
ericoid mycorrhizal fungi
filamentous fungi belonging to the Ascomycota phylum that establish endomycorrhizal symbiotic associations specifically with Ericaceous plants (such as blueberry and cranberry)

Note 1 to entry: The intraradical growth phase is characterized by dense coil of hyphae in the outermost layer of root cells. Ericoid mycorrhizal fungi also have saprotrophic capabilities which can enable plant to access nutrients not yet available.

3.5
orchidoid mycorrhizal fungi
filamentous fungi belonging to the Basidiomycota phylum that establish endomycorrhizal symbiotic associations specifically with Orchids

Note 1 to entry: The hyphae of ochidoid mycorrhizal fungi penetrates the root cell and forms dense coil of hyphae where the nutrient exchange take place.

3.6
sebacinoid mycorrhizal fungi
endophytic filamentous fungi belonging to the Basidiomycota phylum, more specifically the order Sebacinales, which establishes mutualistic symbiotic relationship with a wide variety of plant host

EXAMPLE The model species Piriformospora spp.

Note 1 to entry: Sebacinoid mycorrhizal fungi colonizes plant roots with intracellular mycelium where the nutrient exchanges take place.

3.7
serendipita mycorrhizal fungi
serendipitaceae (formerly Sebacinales Group B) belong to a taxonomically, ecologically and physiologically diverse group of fungi in the Basidiomycota (kingdom Fungi)

Note 1 to entry: While historically recognized as orchid mycorrhizae, recent based phylogenetic studies have demonstrated both their pandemic distribution and the broad spectrum of mycorrhizal types they form.

Note 2 to entry: Serendipita mycorrhizal fungi is associated to all families of herbaceous angiosperms (flowering plants) from temperate, subtropical and tropical regions.

Note 3 to entry: Serendipitaceae mycorrhizal fungi should be considered as a previously hidden, but amenable and effective microbial tool for enhancing plant productivity and stress tolerance.

3.8
ectomycorrhiza
hyphal sheath, or mantle, covering the root tip and an extracellular Hartig net of hyphae surrounding the plant cells within the root cortex

Note 1 to entry: Beneficial symbiotic associations established by filamentous fungi belonging mainly to the Ascomycota and Basidiomycota phylum with around 5 - 10 % of coniferous and deciduous trees.

Note 2 to entry: In some cases the hyphae may also penetrate the plant cells, in which case the mycorrhiza is called an ectendomycorrhiza. Outside the root, ectomycorrhizal extraradical mycelium forms an extensive network within the soil which increase plant nutrient availability and absorption. Since these fungi have septate hyphae, hyphal fragments along with spores are considered long-term effective propagation structures.

[SOURCE: FprCEN/TS 17724, 3.2.2.7]

3.9
spores
very small and very tough cells able of germination under favourable conditions, caused by the fungi which ensure their dissemination

Note 1 to entry: There are sexual, asexual or vegetative spores [1].

3.10
propagules
component of the fungus able to initiate a symbiosis with root

3.11
in vivo
production performed in open area (greenhouse, tunnel, open field)

3.12
in vitro
production performed in monoxenic conditions

3.13
Unit Potential Mycorrhizal
UPM
unity of counting for mycorrhiza

where

U is unit, spore or propagule of any type able to initiate mycorrhiza formation in a host plant's root;

P is potential, since the development of the symbiosis depend on different factors (soil, plant, agriculture practises, competition with other soil borne microorganisms, etc);

M is mycorrhizal, since the inoculum is able to synthesize new mycorrhizae in association with plant roots depending on factors previously cited.

EXAMPLE U.P.M per gram (% spores, % propagules) (*in vivo*, *in vitro*).

4 Methods for the quantification of Mycorrhiza

4.1 General

According to the type of mycorrhiza analysed (see Figure 1), the method to be used is listed in the Table 2 to obtain the quantification in U.P.M.

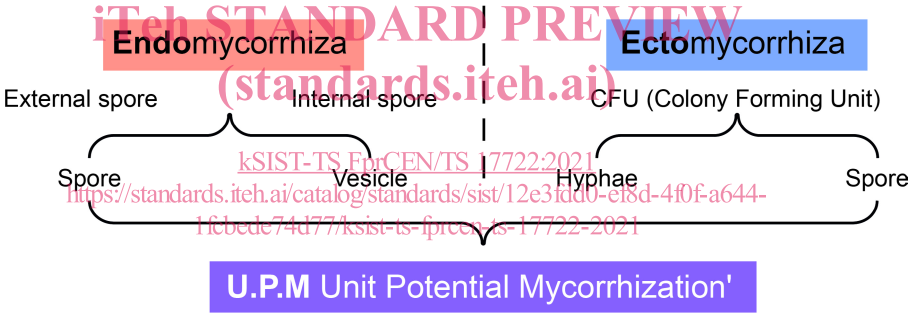

Figure 1 — Different type of mycorrhizas and propagules

Table 2 — Methods to use for enumeration of U.P.M. with plant cultures and without plant cultures

Origin of product	SPORES Extractable	Other propagules, roots extractable	Endo mycorrhiza	Ectomycorrhiza	Ericoïd	Orchidoid	Sebacinoid	Serendipita
IN VITRO 1	Yes	NO	Method N°1	Method N°4	Method N°4			
IN VITRO 2	Yes	Yes	Method N°1 to count the spores and Method N°2 to count propagules					
IN VIVO 1	NO	NO	Method N°3					
IN VIVO 2	Yes	NO	Method N°1	Method N°4	Method N°3			
IN VIVO 3	Yes	Yes	Method N°1 to count the spores and Method N°2 to count propagules					
IN VIVO 4	NO	Yes	Method N°2			Method N°3	Method N°3	Method N°3