SLOVENSKI STANDARD
oSIST prEN 17722:2023

01-maj-2023

Rastlinski biostimulanti - Ugotavljanje prisotnosti in števila mikoriznih gliv

Plant biostimulants - Determination of mycorrhizal fungi

Pflanzen-Biostimulanzien - Bestimmung von Mykorrhizapilzen

Biostimulants des végétaux - Détermination des champignons mycorhiziens

Ta slovenski standard je istoveten z: prEN 17722

ICS:

65.080	Gnojila	Fertilizers

oSIST prEN 17722:2023 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

DRAFT
prEN 17722

April 2023

ICS 65.080

Will supersede CEN/TS 17722:2022

English Version

Plant biostimulants - Determination of mycorrhizal fungi

Biostimulants des végétaux - Détermination des champignons mycorhiziens

Pflanzen-Biostimulanzien - Bestimmung von Mykorrhizapilzen

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

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

This draft European Standard was established by CEN in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and United Kingdom.

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.

Warning : This document is not a European Standard. It is distributed for review and comments. It is subject to change without notice and shall not be referred to as a European Standard.

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

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

Ref. No. prEN 17722:2023 E

Contents

Page

European foreword

This document (prEN 17722:2023) 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 CEN enquiry.

This document will supersede CEN/TS 17722:2022.

This document has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s) / Regulation(s).

For relationship with EU Directive(s) / Regulation(s), see informative Annex ZA, which is an integral part of this document.

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 laying down rules on the making available on the market of EU fertilising products (“FPR” or “Fertilising Products Regulation”). This standardization request, presented as SR M/564 and M/564 Amd1, also contributes to the Communication on “Innovating for Sustainable Growth: A Bio economy for Europe”. Working Group 5 “Labelling and denominations”, was created to develop a work program as part of this standardization request.

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 plant 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 plant 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 plant biostimulants, and will promote and support commercialisation of the European plant biostimulant industry.

The plant 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 Fertilising Products Regulation.

This document is applicable to all plant 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 plant biostimulants.

Table 1 — Agro-ecological principles and the role played by plant 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
— e.g. 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 standard was developed to provide a horizontal method for enumeration and genera/species determination [1], [2], [3] of mycorrhizal fungi in microbial plant biostimulant in accordance with the Regulation (EU) 2019/1009 of the European Parliament and of the Council.

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 terminology databases for use in standardization at the following addresses:

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

3.1
arbuscular mycorrhizal fungus
AMF
AM fungus
biotrophic microscopic fungus belonging to the Glomeromycota phylum (synonymous Glomeromycota) that establishes obligate symbiotic associations with more than 70 % of plant species on Earth

Note 1 to entry: Arbuscular mycorrhizal fungi produce 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, they invaginate the cortical cell membrane where they branch dichotomously to develop the arbuscule which is meant 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 fungus extraradical mycelium forms an extensive network within the soil which increases plant nutrient availability and absorption.

3.2
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 belong mainly to the Ascomycota and Basidiomycota phylum with around 5 % to 10 % of coniferous and deciduous trees.

Note 2 to entry: In some cases, the hyphae can 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 increases plant nutrient availability and absorption. Since these fungi have septate hyphae, hyphal fragments along with spores are considered long-term effective propagation structures.

[SOURCE: EN 17724:—[1], 3.2.2.6.2]

[1] Under preparation

3.3
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: EN 17724:—[1], 3.2.2.6.1]

3.4
ericoid mycorrhizal fungus
filamentous fungus belonging to the Ascomycota phylum that establishes 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 the plant to access nutrients not yet available.

3.5
in vivo
production performed in open area (greenhouse, tunnel, open field)

3.6
in vitro
production performed in monoxenic conditions

3.7
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: EN 17724:—[1], 3.2.2.6]

3.8
orchidoid mycorrhizal fungus
filamentous fungus belonging to the Basidiomycota phylum that establishes endomycorrhizal symbiotic associations specifically with Orchids

Note 1 to entry: The hyphae of orchidoid mycorrhizal fungus penetrate the root cell and form dense coil of hyphae 100 exchange takes place.

3.9
propagule
component of the fungus able to initiate a symbiosis with root

3.10
sebacinoid mycorrhizal fungus
endophytic filamentous fungus belonging to the Basidiomycota phylum, more specifically the order Sebacinales, which establishes mutualistic symbiotic relationship with a wide variety of plant hosts

EXAMPLE The model species *Piriformospora* spp.

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

3.11
serendipita mycorrhizal fungus
serendipitaceae (formerly Sebacinales Group B) belonging to a taxonomically, ecologically and physiologically diverse group of fungi in the Basidiomycota (kingdom Fungus)

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 are associated to all families of herbaceous angiosperms (flowering plants) from temperate, subtropical and tropical regions.

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

3.12
spore
very small and very tough cell able of germination under favourable conditions, caused by the fungus which ensure its dissemination

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

3.13
Unit Potential Mycorrhizal
UPM
unit 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 UPM 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 Table 2 to obtain the quantification in UPM.

The methods are:

Method N° 1: Spore isolation and counting MTT

Method N° 2 A + B: Staining procedures and counting vesicles and spores in roots + extraradical

N°2 A Procedure for enumeration of spores

N°2 B Procedure for clearing and staining root samples and enumeration of vesicles in the stained root samples

Method N° 2 B: Staining procedures and counting vesicle in roots

Method N°3: Endomycorrhiza Bioassay, the MPN test (Most Probable Number of mycorrhizal propagule)

Method N°4: Ectomycorrhiza and Ericoid count on plates

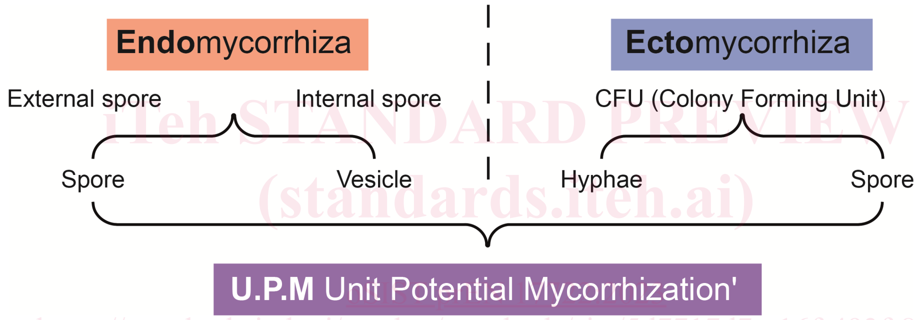

Figure 1 — Different types of mycorrhizas and propagules

Table 2 — Methods to use for enumeration of UPM with plant cultures and without plant cultures

Origin of product	SPORES Extractable	Other propagules, roots extractable	Endo mycorrhiza	Ectomycorrhiza	Ericoid	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