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Plant association with dark septate endophytes: When the going gets tough (and stressful), the tough fungi get going

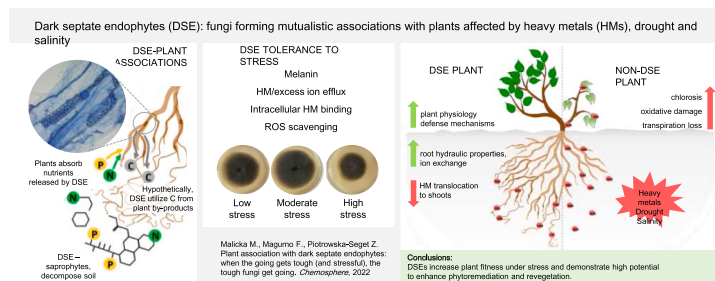
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HIGHLIGHTS

- DSEs are saprophytes improving plant nutrition by mineralization of soil substrate.
- DSEs robustly colonize the roots of plants in extreme habitats.
- DSEs show high tolerance to heavy metal (HM) pollution, drought, and salinity.
- DSEs favor HM sequestration in root cell walls preventing their transfer to shoots.
- DSEs affect the physiology and root architecture of drought-affected plants.

GRAPHICAL ABSTRACT



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ABSTRACT

Dark septate endophytes (DSEs) comprise a diverse and ubiquitous group of fungal generalists with broad habitat niches that robustly colonize the roots of plants in stressful environments. DSEs possess adaptation strategies that determine their high tolerance to heavy metal (HM) contamination, drought, and salinity. Most DSEs developed efficient melanin-dependent and melanin-independent mechanisms of HM detoxification and osmoprotection, including intracellular immobilization and extracellular efflux of HMs and excess ions, and the scavenging of reactive oxygen species. DSEs form mutualistic relationship with plants according to the hypothesis of “habitat-adapted associations”, supporting the survival of their hosts under stressful conditions. As saprophytes, DSEs mineralize a complex soil substrate improving plants’ nutrition and physiological parameters. They can protect the host plant from HMs by limiting HM accumulation in plant tissues and causing their sequestration in root cell walls as insoluble compounds, preventing further HM translocation to shoots. The presence of DSE in drought-affected plants can substantially ameliorate the physiology and architecture of root systems, improving their hydraulic properties. Plant growth-promoting features, supported by the versatility and easy culturing of DSEs, determine their high potential to enhance phytoremediation and revegetation projects for HM-contaminated, saline, and desertic lands reclamation.

1. Introduction

Dark septate endophytes (DSEs) comprise a diverse and ubiquitous

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Nomenclature

ABA	abscisic acid
AMF	arbuscular mycorrhizal fungi
CAT	catalase
DSE	dark septate endophyte
GPC	<i>Gaeumannomyces-Phialophora/Harpophora</i> species complex
GSH	glutathione
HM	heavy metal
IAA	indole acetic acid
MIC	minimal inhibitory concentration
MTP	metal tolerance protein
PAC	<i>Phialocephala fortinii</i> – <i>Acephala applanta</i> species complex
PAL	phenylalanine ammonia-lyase
PCS	phytochelatin synthase
POD	peroxidase
PME	pectin methylesterase
ROS	reactive oxygen species
SOD	superoxide dismutase
SOM	soil organic matter

group of ascomycetous root endophytes (Jumpponen and Trappe, 1998). They represent a group of fungal generalists with broad habitat niches that reside inter- and intracellularly in the roots of hundreds of mycorrhizal and non-mycorrhizal plant families, spanning from herbs to trees (Berthelot et al., 2016; Liu et al., 2017). DSEs can be found worldwide, and have been abundantly found in the roots of plants inhabiting different harsh and arid environments, such as mountain peaks, subantarctic regions, deserts (Fracchia et al., 2011; Gonzalez Mateu et al., 2020; Tejesvi et al., 2010; Väre et al., 1992) and areas affected by high salinity and heavy metal (HM) contaminations (Li et al., 2011; Qin et al., 2017).

DSEs develop inside the root cells forming typical structures such as melanized, distinctly septate hyphae, and microsclerotia. The latter present morphological differences from the true sclerotia produced by many fungi, but similarly they act as resting structures, dispersal propagules, and storage of substances like glycogen, proteins and polyphosphates that improve the fungal viability under harsh conditions (Peterson et al., 2008; Yu et al., 2001; Yung et al., 2021). Melanin, a complex polymeric compound accumulated in the cell wall of DSEs, has been widely recognized as the main responsible of the protective features of these fungi. In addition to antioxidative and thermo-protective characteristics, melanin protects hyphae from desiccation and mechanical disruption, improving cell wall rigidity, hydrophobicity, and decreasing its permeability (Li et al., 2019; Potisek et al., 2021; Zhan et al., 2011).

The placement of DSEs within a defined taxonomic unit in the *Ascomycota* is impossible as they represent an artificial assemblage of fungi sharing similar morphological and functional traits (Piercey et al., 2004). Most DSEs are classified into the orders *Helotiales* and *Pleosporales*. Other species are found in other orders such as: *Chaetothyriales*, *Capnodiales*, *Calosporiales*, *Chaetosphaeriales*, *Eurotiales*, *Hypocreales*, *Magnaporthales*, *Microascales*, *Sordariales* and *Xylariales* (Addy et al., 2005; Jumpponen, 2001; Knapp et al., 2015). The globally distributed and the most studied DSE guilds belong to the *Phialocephala fortinii* – *Acephala applanta* complex (PAC), and the *Gaeumannomyces-Phialophora/Harpophora* species complex (GPC) (Sieber, 2002). PAC includes numerous cryptic fungal species, formerly considered to belong to a single species, *P. fortinii* (*Helotiales*). This group is prevalently detected in the roots of coniferous, ericaceous, and orchidaceous plants of the Northern hemisphere; however, the scant research on DSEs

occurrence in tropical and subtropical regions suggests that PAC species might be distributed worldwide (Addy et al., 2005; Grünig et al., 2011; Lukešová et al., 2015; Queloz et al., 2011). Highly polymorphic GPC includes DSEs belonging to the genera *Phialophora/Cadophora* (*Chaetothyriales/Helotiales*) and *Harpophora* (*Magnaporthales*) with anamorphs in the *Gaeumannomyces* and *Magnaporthe* genera, known to include also significant fungal pathogens (Gams, 2000; Yuan et al., 2010; Zhang et al., 2011). *Phialophora* comprises species widely colonizing trees and, to a lesser extent, herbaceous plants, whereas the genus *Harpophora* is typically associated with the roots of herbaceous plants, primarily *Poaceae* (Berthelot et al., 2017; Likar and Regvar, 2009; Sieber, 2002). These typical DSE guilds were described more than ten years ago after the first surveys on DSE distribution (Addy et al., 2005; Grünig et al., 2011; Sieber, 2002). The dynamic acceleration of DSEs investigations that occurred in the last few years has clearly shown that these guilds were only the tip of the iceberg of DSE diversity. Almost every new study that focuses on DSE diversity, particularly in harsh and stressful environments, reports the emergence of new members of the DSE group, pointing out a high diversity of the order *Pleosporales* (Fracchia et al., 2011; Qin et al., 2017; Shadmani et al., 2021).

Because of their high functional and species diversity, it is not easy to draw general conclusions and statements on DSEs and on the nature of plant-DSE associations (Mayerhofer et al., 2013; Newsham, 2011). Even though DSEs ability to colonize plant roots resembles the behavior of mycorrhizal fungi, other morphological, functional, metabolic, and genetic features exclude DSEs from this group of plant symbionts. Some authors claim that similarly to mycorrhizal fungi, DSEs may form a hyphal network that assists in plants' interactions with the rhizosphere and enables plants to access water and nutrients beyond the depletion zone (Liu and Wei, 2019). However, there is no clear evidence of direct nutrient exchange between a plant and DSE since any specialized interfaces for the mutualistic transfer of nutrients have never been detected (Ruotsalainen et al., 2021). Potentially, a direct nutrient exchange between DSEs and their host could occur in vascular plant tissue that, contrary to mycorrhizal fungi, can be penetrated by DSEs, but this hypothesis needs further experimental elucidation (Addy et al., 2005; Hou et al., 2020; Peterson et al., 2008; Su et al., 2021). Even if the direct nutrient transfer in the plant-DSE associations would have been confirmed, their relationship relies mainly on indirect nutrient exchange (Ruotsalainen et al., 2021).

Recent insights into DSE genetics revealed that DSEs hold a remarkably high proportion of saprophytic genes in their genomes (Knapp et al., 2018; Schlegel et al., 2016). DSEs produce a broad range of hydrolytic and oxidative enzymes, including amylases, cellulases, laccases, lipases, pectinases, polyphenol oxidases, proteolytic enzymes, and xylanases, and show incomplete ligninolytic activity (Caldwell et al., 2000; Suroño and Narisawa, 2017; Vohník et al., 2012). They support their host via the mineralization of a complex soil substrate, thereby releasing nutrients that plants can easily absorb (Ruotsalainen et al., 2021; Vohník et al., 2012). It is hypothesized that in return, DSEs require only a minor investment of plant resources because being saprophytes, they might obtain carbon (C) from plant metabolic waste by-products (Ruotsalainen et al., 2021) (Fig. 1). Despite the high number of studies on DSE symbiosis, the reasons of their intraradical and intracellular settling are still to be clarified (do they seek a safe, stable niche or shelter inside the roots from stress factors present in soil?).

DSEs are considered as a 'by-product mutualist transitional phase' in the evolution of mutualistic mycorrhiza from free-living saprophytes and pathogens (Ruotsalainen et al., 2021). *Falciphora oryzae* (*Magnaporthales*), a beneficial endophyte of wild rice and a close relative of the pathogen *Magnaporthe oryzae*, is a clear example of the transition from pathogenic to endophytic behavior. Comparative analysis of *F. oryzae* and *M. oryzae* transcriptomes showed several changes in the expression pattern of the genes involved in the transduction of extracellular signals from the host and the modulation of the plant immune response (Xu et al., 2015). The influence of DSEs on a host plant relies on the fine

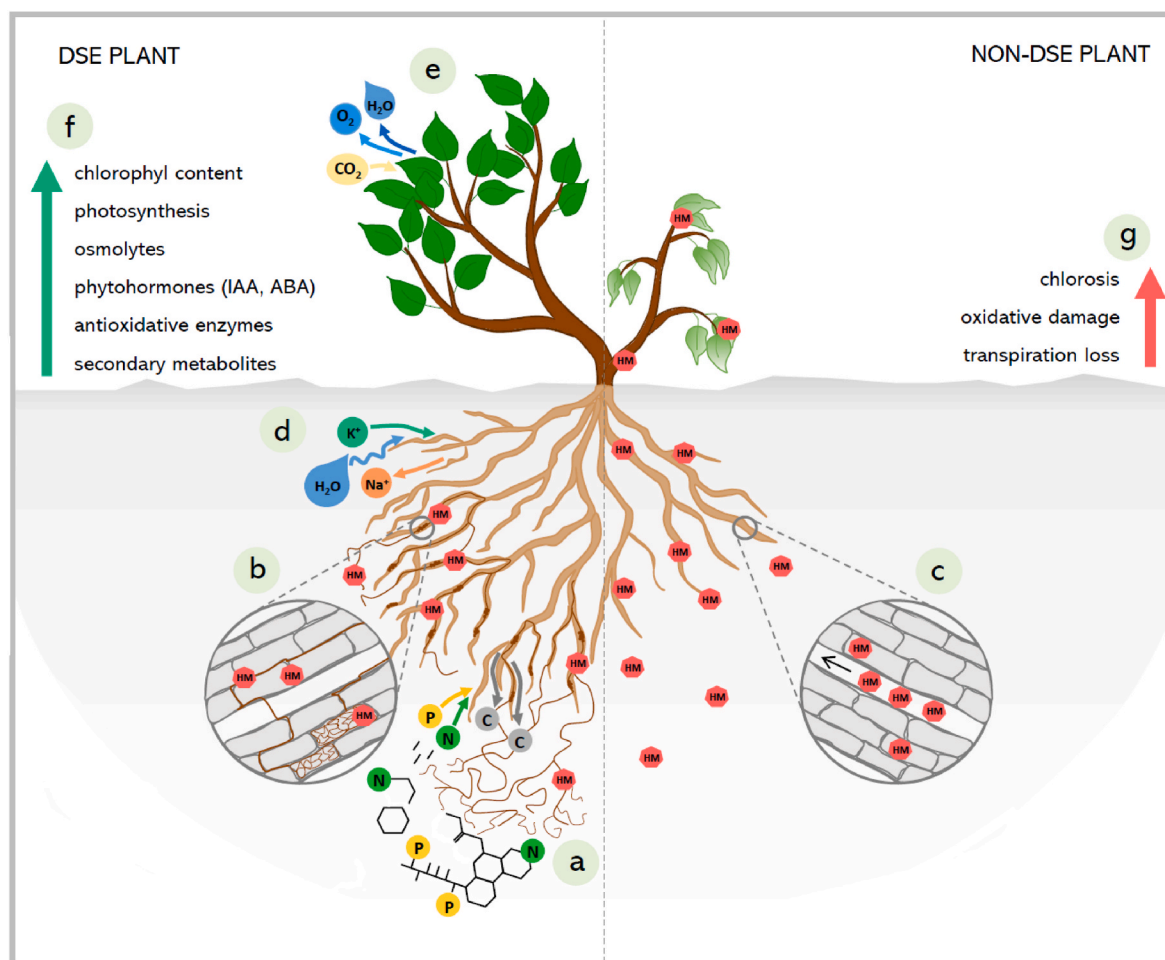


Fig. 1. The effects of DSE colonization on the fitness of HM- and drought-affected plants. DSE support plant growth improving their nutrition. In exchange for plant derived carbon (C), DSE efficiently decompose soil substrate increasing the pool of nutrients (P and N) available for plants (a). DSE protect plants from heavy metals (HMs) either by their direct immobilization by fungal hyphae or favoring their sequestration in plant cell walls, reducing HM soluble pool (b) and their further translocation to root vascular tissue and shoots (c). DSE increase plant tolerance to drought and salinity improving root hydraulic properties. DSE-colonized plants produce longer roots of smaller diameter and higher biomass, characterized by efficient ion exchange and water uptake (d). Moreover, the presence of DSE in drought-affected plants improves stomatal conductance, gas exchange and transpiration (e). Mutual associations between DSE and plants support plant physiology, increasing the chlorophyll content, photosynthesis rate and the production of phytohormones, like auxin (IAA) and abscisic acid (ABA), as well as plant defense mechanisms, like production of osmolytes, antioxidative enzymes, and secondary metabolites (f). Exposure of non-DSE-colonized plants to drought and HM results in their low biomass, chlorosis, increased oxidative damage and transpiration loss (g).

tuning and maintenance of a complex plant-fungal interaction, a delicate balance between the fungal virulence, root C allocation, plant defense mechanisms, and environmental factors (Mandyam and Jumpponen, 2015; Reiningger and Schlegel, 2016).

2. DSEs improve plant nutrition under stress conditions

The mutualistic interactions between DSEs and plants have been primarily revealed under stress factors and in nutrient-poor soils, invoking the hypothesis of “habitat-adapted symbiosis”. According to this hypothesis, plants develop associations with DSEs to improve their survival in harsh conditions caused by drought, high salinity, and HM contamination (Rodríguez et al., 2008) (Fig. 1).

Newsham’s meta-analysis (2011) suggested that the most critical plant growth-promoting feature of DSEs is the enhancement of organic nitrogen (N) mineralization in soil. DSEs synthesize a large amount of arginine that seems to be their main N storage and source for the plant (Tienaho et al., 2019). The ability of DSEs to increase the bioavailability of organic N for the host plant might be of high ecological importance since the role of arbuscular mycorrhizal fungi (AMF) in providing N under high C content is negligible (Ingraffia et al., 2020). Indeed, DSE

colonization likely dominates over mycorrhizal fungal colonization in those harsh environments where soil organic matter (SOM) contents are high (Han et al., 2021; Hou et al., 2019; Huusko et al., 2017; Kauppinen et al., 2014; Tejesvi et al., 2010). AMF specialized in acquiring inorganic phosphorus (P) and N, have been reported abundantly present at early plant successional stages and in poor sandy soils. DSE colonization occurs mostly in mid/late successional stages and forests where their saprophytic activity might be more beneficial for plants than the symbiosis with AMF (Huusko et al., 2017; Kauppinen et al., 2014; Tejesvi et al., 2010). Nevertheless, DSEs might also support the activity of mycorrhizal fungi through the solubilization of inorganic P, thus increasing the pool of bioavailable P (Priyadharsini and Muthukumar, 2017; Spagnoletti et al., 2017). Breaking down insoluble inorganic compounds, DSEs can increase the availability of micronutrients and trace elements and contribute to an improved plant nutrition (Berthelot et al., 2019; He et al., 2019; Liu et al., 2021; Yung et al., 2021). The above findings are supported by the latest research showing an increase in the total N, P, and C content in stress-affected plants colonized by DSEs (Ban et al., 2021; Farias et al., 2020; Hou et al., 2020; Qiang et al., 2019). Considering that DSEs seem to be less vulnerable to stress factors than mycorrhizal fungi, they might be selected by plants as alternative

symbiotic partners to support host nutrition in adverse environmental conditions (Deram et al., 2011; Hou et al., 2020; Ruotsalainen et al., 2007).

3. Heavy metal-tolerant DSEs protect plants from heavy metal toxicity

DSEs are abundantly present in HM-polluted environments and some of them demonstrate high resistance to HM (Ban et al., 2012; Shadmani et al., 2021). A few studies showed that the root colonization by DSEs is favored in the presence of low concentrations of HM in soil (Hou et al., 2020; Li et al., 2011; Regvar et al., 2010; Ruotsalainen et al., 2007; Su et al., 2021). For example, different DSE species: *Alternaria alternata* and *Alternaria* sp. (Pleosporales), *Microdochium bolleyi* (Xylariales), *Bipolaris zeicola* (Pleosporales), and *Pleosporales* sp., isolated from Cd-enriched landfills in Iran, have shown a higher resistance *in vitro* to Cd (minimal inhibitory concentration – MIC = 1254.5 mg Cd kg⁻¹) than non-DSE endophytic fungi obtained from the same sites (MIC = 800 mg Cd kg⁻¹) (Shadmani et al., 2021). The intensity of the colonization of tomato roots by *Phialophora mustea*, a DSE isolated from a HM polluted site in France, was 5% and 10% higher in cultures contaminated with 10 mg Cd kg⁻¹ and 600 mg Zn kg⁻¹, respectively, in comparison to uncontaminated cultures (Berthelot et al., 2016). Similarly, the colonization of maize roots with *Gaeumannomyces cylindrosporus* (Magnaporthales) was 10%–15% higher in cultures contaminated with 50 mg Cd kg⁻¹ and 500 mg Cd kg⁻¹, respectively, compared to cultures non-contaminated with HM (Ban et al., 2017).

DSEs possess adaptation strategies that determine their high tolerance to HM. They developed efficient ways of immobilizing HM in their cell walls, intracellular complexes, and compartments, as well as efficient systems of extracellular efflux of metal ions and scavenging of reactive oxygen species (ROS) (Ban et al., 2012; Hou et al., 2020; Li et al., 2019; Potisek et al., 2021; Su et al., 2021; Yu et al., 2021; Zhan et al., 2017; Zhao et al., 2015). It seems that the activation of superoxide dismutase (SOD) is the first line of DSE defense in neutralizing peroxide radicals (Ban et al., 2012; He et al., 2021; Li et al., 2019). Increased SOD activity is often accompanied by the activation of catalase (CAT), peroxidase (POD) and higher synthesis of glutathione (GSH) (Yu et al., 2021). Elevated mitochondrial ATP production and the overexpression of genes involved in redox homeostasis, DNA repair and maintenance of cell wall integrity was also reported (Su et al., 2021; Zhao et al., 2015). These transcriptional changes are often paired to remarkable alterations of DSE morphology. DSE mycelia grown on a medium with HM were much darker, thicker, swollen, and more prone to twisting and looping. The septal spacing of the mycelia was shorter and the number of chlamydo-spores was significantly increased compared to DSE non-affected by HMs (Ban et al., 2012; Hou et al., 2020).

High DSEs tolerance to HMs was reported to be associated with the substantial synthesis of melanin, rich in hydroxyl groups with high HM-binding capacity (Ban et al., 2012; Potisek et al., 2021; Yamaji et al., 2016). The level of melanin accumulation in DSE hyphae is species- or even strain-specific and linked to fungal response driven by extracellular conditions (Potisek et al., 2021). Examining two different strains (Cad#148 and Cad#149) of *Cadophora* sp. (*Helotiales*), Potisek et al. (2021) reported that the melanin content in both strains was negatively correlated with Cd concentration. However, Cad#148 accumulated seventeen times more melanin than Cad#149 and showed higher tolerance to Cd in the medium. In contrasting reports, the hyphal melanin content of *G. cylindrosporus*, *Acrocalymma vagum* (Pleosporales), and *Scytalidium lignicola* (Leotiomyces inc. sed.), showed a positive correlation with Pb and Cd concentration *in vitro*, and with intracellular Cd accumulation (Ban et al., 2012; Hou et al., 2020). The latest research on the effect of melanin synthesis inhibition on DSE growth demonstrated that among different DSE albino strains of *Cadophora* sp., *Lepidotontidium* sp. (*Helotiales*), and *P. mustea*, only one *Cadophora* sp. strain was more sensitive to Cd contamination than the wild,

melanin-producing strains (Berthelot et al., 2020; Potisek et al., 2021). However, reduced Cd accumulation has been measured in the mycelium of all tested albino DSE strains. Those results suggest that melanin is crucial for immobilizing HM in the fungal cell wall.

Besides, the repertoire of HM detoxifying mechanisms of DSEs is much broader. Indeed, DSEs can detoxify HMs in a melanin-independent manner by their efflux or deposition in vacuoles (Liu et al., 2021; Su et al., 2021). The richest fractions of Cd in *Cadophora* sp. mycelium were those bound to O-ligands and S-ligands (Potisek et al., 2021). O-ligands are primarily associated with melanin, chitin, and chitosan present in the fungal cell wall, and with organic acids whose increased metabolism in DSEs has been reported (Potisek et al., 2021; Zhao et al., 2015). Cd bound to S-ligands indicated its detoxification by thiol groups present in GSH, γ -glutamyl cysteine, sulfur-rich proteins, and metallothioneins (Potisek et al., 2021). To avoid the intracellular accumulation of HM, DSEs can regulate the expression of metal ions transporters and compartmentalize ions through vesicle membrane fusion (Su et al., 2021; Zhao et al., 2015). Upon exposure to Pb and Zn, *Exophiala pisciphila* (*Chaetothyriales*) showed a down-regulation of its membrane transporters for the uptake of Zn²⁺, Fe²⁺ and Ca²⁺, and up-regulation of transporters involved in the efflux of HM ions from the cytosol, outside a cell or inside vacuoles (Cao et al., 2019; Zhao et al., 2015). Exposure of *E. pisciphila* and *Falciphora oryzae* to Cd have revealed the sequestration of Cd²⁺ and Zn²⁺ in vacuoles and chlamydo-spores (Liu et al., 2021; Su et al., 2021), as a consequence of the over-expression of SNARE protein Syntaxin 1 involved in chlamydo-spore formation and vacuole enlargement (Su et al., 2021).

DSEs of diverse taxonomic affiliation (*Chaetothyriales*, *Helotiales*, *Pleosporales*, *Magnaporthales*) robustly colonize the roots of different plants, representing crops (*Oryza granulata*, *Zea mays*, *Hordeum vulgare*), herbaceous plants (*Lolium perenne*, *Nocca caerulea*, *Phragmites australis*) and trees (*Clethra barbinervis*, *Salix* sp., *Populus tremula*, *Betula pendula*), improving their performance in soils contaminated with HMs (An et al., 2015; Berthelot et al., 2017, 2018; Deram et al., 2008; He et al., 2017; Hou et al., 2020; Jin et al., 2018; Likar and Regvar, 2009; Su et al., 2021; Wang et al., 2016; Yung et al., 2021; Zhan et al., 2017; Zhu et al., 2018). The presence of DSEs in 90–100% of the roots collected from HM contaminated soils has been well documented (Shadmani et al., 2021; Zhang et al., 2013). *Phialocephala fortinii* colonization was indicated as the main factor determining the HM tolerance of *C. barbinervis*, a tree naturally inhabiting a mine site in Japan contaminated with Pb, Zn, and Cu. Inoculation of *C. barbinervis* with this fungus decreased the concentration of Pb in the roots from 1400 to 200 $\mu\text{g g}^{-1}$ dry weight, drastically increased (ten-fold) the height and biomass of seedlings in comparison to the uninoculated plants and doubled their biomass in comparison to plants inoculated with non-DSE fungi (*Rhizoderma veluwensis* or *Rhizoscyphus* sp.) isolated from the same mining site (Yamaji et al., 2016) (Fig. 1).

Recent analyses revealed that DSEs can protect a host by lowering HM accumulation in plant tissues and/or by sequestering HM in root cell walls as insoluble form, preventing further translocation to shoots (He et al., 2017; Shen et al., 2020) (Fig. 1). *F. oryzae*, introduced to the rhizosphere of *O. granulata*, acted as a biofilter by immobilizing Cd ions in the hyphae blocking their further translocation to the plant. Rice plants colonized by *F. oryzae* doubled their biomass and accumulated less Cd (also in the seeds) than non-DSE plants. In the presence of Cd, the hyphae of *F. oryzae*, characterized by the high capacity of Cd absorption, were able to penetrate the rice vascular tissue hindering Cd translocation to shoots, while, in the absence of pollutants, the fungal colonization was restricted only to the root cortex (Su et al., 2021). The roots of *Z. mays* inoculated with *E. pisciphila* accumulated approximately 1100 mg Cd kg⁻¹ whereas the control plant roots contained almost 2100 mg Cd kg⁻¹ when grown in a substrate spiked with 100 mg Cd kg⁻¹. DSEs presence in roots triggered changes in plant subcellular HM distribution and in the regulation of plant genes responsible for the translocation and detoxification of HMs (Shen et al., 2020; Su et al., 2021;

Wang et al., 2016). The colonization of *Z. mays* with *E. pisciphila* promoted Cd deposition in the plant cell wall (Wang et al., 2016). Consequently, the pool of non-toxic, insoluble Cd (such as: undissolved Cd, pectate, hemicellulose and protein-bound Cd, oxalate Cd forms, carboxylic and phenolic-bound Cd) increased, whereas the pool of soluble Cd and its translocation to the shoots decreased. This shift in Cd distribution in plants resulted from DSE-mediated transcriptional and enzymatic changes in the cell wall synthesis and activity of transporters (Shen et al., 2020; Wang et al., 2016). At the molecular level, the sequestration of Cd was related to the downregulation of plant ZIP transporters responsible for the cellular inflow of Cd²⁺, the upregulation of phytochelatin synthase (PCS) responsible for the capture of free Cd²⁺ in the cytosol, and the upregulation of metal tolerance protein (MTP) that takes part in the sequestration of excessive Cd²⁺ in vacuoles and other cellular compartments (Wang et al., 2016). The cell wall remodeling in response to roots colonization by *E. pisciphila* was the effect of the increased activity of three enzymes: the pectin methyltransferase (PME) that demethylates cell wall pectins, extending their capacity of cation binding, and the phenylalanine ammonia-lyase (PAL) together with the cell wall peroxidase that catalyze the polymerization of lignin monomers (Shen et al., 2020).

4. DSEs as key determinants of plant drought tolerance

Drought, often associated with high soil salinity, is probably the most severe factor limiting the crop yields and reclamation of disturbed areas. Therefore, the implementation of economically and ecologically efficient strategies to improve plant tolerance to drought is highly desirable. Halophytes, like the plants of coastal habitats, deserts, and invasive species with a wide range of ecological tolerance, are the reservoir of halo- and xero-tolerant DSEs with high potential to help plants to thrive under osmotic stress and enhance the revegetation of saline soils degraded by improper management practices (Farias et al., 2020; Gonzalez Mateu et al., 2020; He et al., 2021; Pan et al., 2018; Qin et al., 2017).

Numerous field surveys have demonstrated that DSEs represented by PAC and *Pleosporales* are the core of endophytic fungal communities in the roots of desert flora. Their consistent presence was reported in nearly 100% of root systems in desert areas of the USA, Mexico, and Argentina (Fracchia et al., 2011; Herrera et al., 2010; Khidir et al., 2010; Porras-Alfaro et al., 2008). *Pleosporales* accompanied by other DSE species were present in more than 50% of the roots of xerophytic shrubs in Northwest China (He et al., 2021; Hou et al., 2019; Li et al., 2015, 2018; Xie et al., 2017; Zuo et al., 2020) and on the Great Hungarian Plane where they represented 60% of the endophytic fungal isolates (Knapp et al., 2012, 2015). Pleosporalean fungi were reported as the most frequent root colonizers of halophytes (*Poaceae*, *Amaranthaceae*, *Convolvulaceae*, *Cyperaceae* and *Boraginaceae*), which inhabit saline coastal habitats (Qin et al., 2017). Several studies have revealed that moderate osmotic pressure (−0.08 MPa to −2.24 MPa) created the optimal conditions for DSE growth in culture medium (Li et al., 2019; Santos et al., 2017). DSEs isolated from the roots of halophytes showed sensitivity to 4% KCl and NaCl concentration in the growth medium; however, they maintained hyphal viability even up to a 12% salt concentration, which indicated their halotolerant but not halophilic nature (Qin et al., 2017). DSE salt tolerance might be related to their ability to accumulate osmolytes like trehalose and mannitol which maintain cell turgor and stabilize proteins (Dastogeer and Wylie, 2017; Usuki and Narisawa, 2007).

Reports on the effect of plant inoculation with different DSE species suggest that the beneficial outcome of plant-DSE associations under drought stress requires certain compatibility between both organisms (Li et al., 2018; Perez-Naranjo, 2009). Nevertheless, the improvement of the performance of the host (usually xerophytes) and non-typical-host plants – mostly crops (rice, sorghum, maize, tomato) and medicinal plants (licorice, *Artemisia ordosica*), under water deficit was reported as

the effect of inoculation with a wide range of DSEs from *Chaetothiales*, *Calosphaerales*, *Capnodiales*, and *Pleosporales*. The highest DSE potential to improve plant fitness was reported in mesohaline conditions (~1 g NaCl kg⁻¹ soil) (Gonzalez Mateu et al., 2020; He et al., 2021; Hou et al., 2021). A few studies reported that DSE inoculation significantly increased the total biomass (17–39%) and shoot biomass (10–43%) of plants under moderate drought and salinity (Gonzalez Mateu et al., 2020; He et al., 2019; Li et al., 2018, 2019; Pan et al., 2018; Qiang et al., 2019; Qin et al., 2017; Santos et al., 2017; Zhang et al., 2017), but most importantly, DSEs substantially ameliorated the physiology and architecture of the root system (Fig. 1). Roots are the most sensitive organs to salt stress and respond rapidly with changes in length, volume and architecture to increase plant adaptability to osmotic stress and water deficiency (Hou et al., 2021; Li et al., 2019). The root biomass of plants affected by drought and salinity can be increased even up to 95% in DSE-colonized plants, compared to non-colonized plants (He et al., 2019, 2021; Qiang et al., 2019). For example, the DSE host pioneer desert plant *Hedysarum scoparium* and non-host plants (*Glycyrrhiza uralensis*, *Z. mays*) were cultured under mild water deficiency and inoculated with *Acrocalymma vagum*, *Paraphoma* sp. (*Pleosporales*), and *Cladosporium oxysporum* (*Cladosporiales*). DSE-colonized plants developed longer roots (up to 60%) of smaller diameter and higher biomass (up to 40%) than non-colonized plants proving their better performance in the uptake and allocation of nutrients and water (He et al., 2019; Li et al., 2019; Zuo et al., 2020). Moreover, the inoculation of drought-affected leguminous tree *Ormosia hosiei* with *A. vagum* resulted in the increased root biomass and volume by 20–40% and 50–60%, respectively. The presence of the endophyte raised the number and length of fine roots, which might be associated with higher indole acetic acid (IAA) synthesis, as well as prevented the root cells from ultra-structural damage caused by dehydration. Additionally, the endophyte promoted the accumulation of abscisic acid (ABA) that participates in leaf stomatal regulation reducing water loss by transpiration loss. By expanding the absorption range of the roots and maintaining their integrity, *A. vagum* significantly diminished water loss and enhanced water uptake in *O. hosiei* (Liu and Wei, 2019). Introduction of *Alternaria alternata* to the rhizosphere of *Triticum aestivum* under mild water deficit significantly improved the root development and accumulation of proline and soluble sugars that provided better osmotic adjustment and maintenance of plant cell turgor (Qiang et al., 2019). Other experiments have shown that inoculation of xerophytes (*A. ordosica*, *Ammopiptanthus mongolicus*) with DSEs isolated from desert (*Alternaria chlamyosporigena*, *Paraphoma chrysanthemicola* and *Bipolaris sorokiniana*) improved the adaptability of *A. ordosica* to salt stress by stimulating IAA synthesis and enhancing the K⁺ accumulation and Na⁺ exclusion from the roots, increasing their biomass and length up to 40% (Hou et al., 2021; Li et al., 2018).

Recent research on the effect of DSE inoculation on the rhizosphere microbial composition suggested that DSEs might indirectly support the growth of drought-affected plants via interactions with other members of the plant microbiome. The results of a pot experiment with DSEs and *G. uralensis* have indicated that the presence of DSEs increased the abundance of saprotrophic and symbiotrophic fungi and stunted the development of fungal pathogens. This DSE-mediated shift in the fungal community was observed only in soil exposed to drought stress (He et al., 2021).

5. DSEs improve plant tolerance to stress, supporting their metabolism and physiology

Besides the mechanisms described for constraining HM and drought stresses, DSEs can also support plants growing in adverse conditions by regulating their photosynthesis and respiration (Fig. 1). Severe plant growth limitations under drought and HM stress are the effect of reduced stomatal conductance, decreased concentrations of photosynthetic pigments, and low capacity of photosystems (Sitko et al., 2022;

Zhang et al., 2020). The use of several DSE species increased chlorophyll content, the efficiency of PSII photochemistry, transpiration rate, and stomatal conductance indicating better gas exchange and higher level of photosynthesis in *Zea mays*, *Triticum aestivum*, *Sorghum bicolor*, *Hordeum vulgare*, *Phragmites australis*, and *Glycorrhiza uralensis* (He et al., 2017, 2019; Qiang et al., 2019; Shadmani et al., 2021; Zhang et al., 2017). Better stomatal conductance might be related to the increased K^+ uptake by DSE-colonized roots (Berthelot et al., 2017; Li et al., 2018). Higher chlorophyll concentrations, transpiration rates, and photosynthetic performance have also been reported in DSE inoculated trees (*Salix caprea*, *Betula pendula*, *Populus tremula*, *Populus tomentosa*, *Vochysia divergens*) growing under HM and salt stress (Ban et al., 2017; Berthelot et al., 2017; Farias et al., 2020; Likar and Regvar, 2009; Pan et al., 2018).

DSE colonization significantly affects plant physiology by modulating the production of phytohormones, secondary metabolites, and antioxidative enzymes, which help a host plant to mitigate adverse stress by preventing the tissues from structural damage, maintaining their normal development and physiological functions (He et al., 2017; Hou et al., 2021; Liu and Wei, 2019) (Fig. 1). Some DSEs can synthesize IAA, exerting a stimulatory effect on plant growth (Berthelot et al., 2016; Priyadharsini and Muthukumar, 2017; Qiang et al., 2019). Furthermore, DSE colonization can influence the hormone signal transduction and hormone-regulated gene expression in plants (Andrade-Linares et al., 2011). The presence of DSEs likely induces phenylpropanoid pathway that is responsible for the production of secondary metabolites (Lahlali et al., 2014; Shen et al., 2020; Zhang et al., 2017). Lahlali et al. (2014) demonstrated that *Cladophialophora chaetospora* (*Chaetothyriales*)-induced systemic resistance in *Brassica napus* was associated with higher activity of PAL, the first committed enzyme in the phenylpropanoid pathway. Inoculation of *S. bicolor* and *Z. mays* with *Exophiala pisciphila* supported the production of anthocyanins, polyphenols and flavonoids, and enhanced enzymatic activities of cinnamyl alcohol dehydrogenase, PAL and guaiacol peroxidase in plants affected by water deficit (Shen et al., 2020; Zhang et al., 2017). High anthocyanin content was also reported in an HM hyperaccumulator *Noccaea caerulea* after inoculation with *Leptodontidium* sp. and *Phialophora mustea* (Yung et al., 2021).

The presence of DSEs can significantly reduce the oxidative cellular damage in stress-affected plants by reinforcing their antioxidative machinery (Berthelot et al., 2017, 2018; He et al., 2019; Qiang et al., 2019; Zhang et al., 2017; Zhu et al., 2018) (Fig. 1). The associations of stress-affected plants with various DSEs belonging to *Chaetothyriales* and *Pleosporales* resulted in the plant overexpression of the key antioxidative enzymes – SOD, CAT and POD, by 14%–62% compared to a non-inoculated control (He et al., 2019; Hou et al., 2021; Li et al., 2019; Pan et al., 2018; Wang et al., 2016; Zhan et al., 2017; Zhang et al., 2013; Zhu et al., 2018). The protective effect of DSE inoculation might be additionally enhanced by the increased content of GSH in plants that acts as an antioxidant and HM-ligand peptide (Ban et al., 2012; Hou et al., 2020; Wang et al., 2016). In addition to the beneficial influence of DSEs on plant antioxidant activity, melanin produced by DSEs inside plant roots might directly complex and neutralize oxygen radicals formed by plants exposed to stress (Redman et al., 2002).

6. Conclusions

For several years, DSEs have been described as an elusive group of endophytes with unclear classification and forming ambiguous associations with plants. Recent investigations on DSEs shed new light on their occurrence, significance, and benefits to host plants. Several features define the high DSE tolerance to abiotic stresses, and intrinsically explain their occurrence in the roots of plants inhabiting harsh environments at various latitudes. On the other hand, according to the hypothesis of ‘habitat-adapted symbiosis’, plants are keen to interact mutualistically with DSEs to improve their survival under exposure to stress. DSEs are saprophytes with little needs of plant-derived C, which,

compared to mycorrhiza, makes the symbiosis less burdensome for a host plant growing in adverse environmental conditions. They can grant multiple benefits to their plant symbiotic partners, ranging from a higher bioavailability of nutrients to protection against several stressors. Even though their relationships are not as intimate as those of other endophytes, DSEs have been shown to modulate plant metabolism and physiology to a great extent.

DSEs are versatile generalists of low host specificity, but their positive effect on plants still requires certain host compatibility and might be limited under severe stress and low SOM content. The potential application of DSEs in phytoremediation and revegetation of degraded lands is welcomed by many in the scientific community. Nevertheless, the performance of more tailored experiments, for specific interactions and utilization potentials is necessary to better harness the DSEs utility and further assess their environmental impact.

CRediT authorship contribution statement

Monika Malicka: Conceptualization, Writing – original draft, Visualization, Funding acquisition. **Franco Magurno:** Writing – review & editing. **Zofia Piotrowska-Seget:** Writing – review & editing.

Declaration of competing interest

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