DARK SEPTATE ENDOPHYTES: A REVIEW

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Abstract

Dark septate endophytes (DSEs) are facultative biotrophic, root-colonizing fungi that live within a plant for a part of their life cycle without causing any apparent, overt negative effects. In addition to plant roots, DSEs can also live on organic debris as well as in biological soil crusts. They are often referred to as "pseudomycorrhizae", due to their association with plant roots and their mutualistic effect on them. DSEs, however, do not form nutrient exchange structures like the arbuscular mycorrhizal fungi (AMF). DSEs have been found in more than 600 different plant species. Although their precise ecological functions are not yet well understood, there is increasing evidence that they enhance nutrient acquisition and plant growth, and improve the plant's ability to tolerate biotic and abiotic stresses.

Resumen

Los endofitos septados oscuros son hongos biotróficos facultativos que colonizan las raíces y viven dentro de la planta durante una parte de su ciclo vital sin causar ningún efecto negativo aparente. Además de las raíces de las plantas, los endofitos septados oscuros también pueden vivir en los restos orgánicos y en las costras biológicas del suelo. A menudo se les denomina "pseudomicorrizas", debido a su asociación con las raíces de las plantas y su efecto mutualista sobre ellas. Sin embargo, estos hongos no forman estructuras de intercambio de nutrientes como los hongos micorrícicos arbusculares. Se han encontrado endofitos septados oscuros en más de 600 especies de plantas diferentes. Aunque todavía no se conocen bien sus funciones ecológicas precisas, cada vez hay más evidencias de que aumentan la adquisición de nutrientes y mejoran la capacidad para tolerar el estrés biótico y abiótico, favoreciendo el crecimiento y desarrollo de las plantas hospedadoras.

Main characteristics of DSEs

Endophytic fungi reside mostly within plant tissues and grow within roots, stems, or leaves, without causing symptoms of disease (Rodriguez et al. 2009; Hardoim et al. 2015). Although endophytic fungi are known as important components of plant micro-ecosystems (Cordovez et al. 2019), their ecological function is not fully clear. One important group of fungal endophytes is formed by the dark septate endophytes (DSE), a group of anamorphic and root-inhabiting fungi, with dark septate or hyaline hyphae colonizing plant roots (Jumpponen and Trappe, 1998; Addy et al. 2005). The influence of DSEs on host plants relies on 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). For comprehensive reviews on DSE, readers are referred to Arora et al. (2019); Skiada et al. (2019); Barberis et al. (2021); Santos et al. (2021); Malicka et al. (2022).

Colonization

DSEs colonize the parenchymal root tissue inter and intracellularly, often forming microsclerotia. Upon first contact between the fungal hyphae and the plant cell, a hyphopodium is formed on the root epidermis. A cytoplasmic aggregation associated with the accumulation of endoplasmic reticulum and translocation of the nucleus below the hyphopodium contact site is activated. Cytoplasmic aggregation occurs at all sites of fungal contact with plant cells, and even when the fungus advances along the root cortex. The plant cell reacts by forming a papilla below the site of penetration; with the nucleus below the site of contact. The fungus penetrates the cell by means of single or multiple lobular hyphae. To penetrate the cell walls, the hyphae constrict or narrow in a process called stenosis (Jumpponen and Trappe 1998). Sometimes cell viability is not maintained, so not all colonized cells remain alive.

Colonization seems to be positively correlated with several parameters such as soil enzymatic activity; also, the presence of microsclerotia is correlated with soil ammonium content and a negative correlation with soil organic matter and pH (Xie et al. 2017). The level of root colonization depends on the growth stage of the plant. It may also vary within the same plant species, e.g. that of *Trifolium dasyphyllum* ranges from 0 to 13% (de Mesquita et al. 2018). Even in the same plant it is possible to observe complex consortia of DSEs.

It is not easy to establish general conclusions on the nature of the association of DSEs with plants. Like mycorrhizal fungi, DSEs have the ability to colonize plant roots, but other morphological, functional, metabolic, and genetic features exclude DSEs from being mycorrhizal symbionts. Although DSEs may form a hyphal network to establish a connection between plants and their rhizosphere to allow the plant to have access to water and nutrients, clear evidence of direct nutrient exchange between them has not been published as the existence of specialized interfaces for mutualistic transfer of nutrients have never been detected (Ruotsalainen et al. 2021). Nevertheless, a potential direct nutrient exchange could occur in vascular plant tissue that, contrary to mycorrhizal fungi, can be penetrated by DSEs, but further experimental work is required. Currently, plant-DSE associations rely on indirect nutrient exchange and are considered as mutualistic transitional phase in the evolution of mutualistic mycorrhiza from free-living saprophytes and pathogens (Ruotsalainen et al. 2021).

Microscopic structures

After colonization, DSEs develop inside the root cells forming typical structures such as melanized, distinctly septate hyphae (figure 1). Melanin is a complex polymeric compound that accumulates in the cell wall of DSEs, for which this type of fungi is named. Melanin is widely accepted as the main responsible of the antioxidative and thermo-protective characteristics of DSEs.
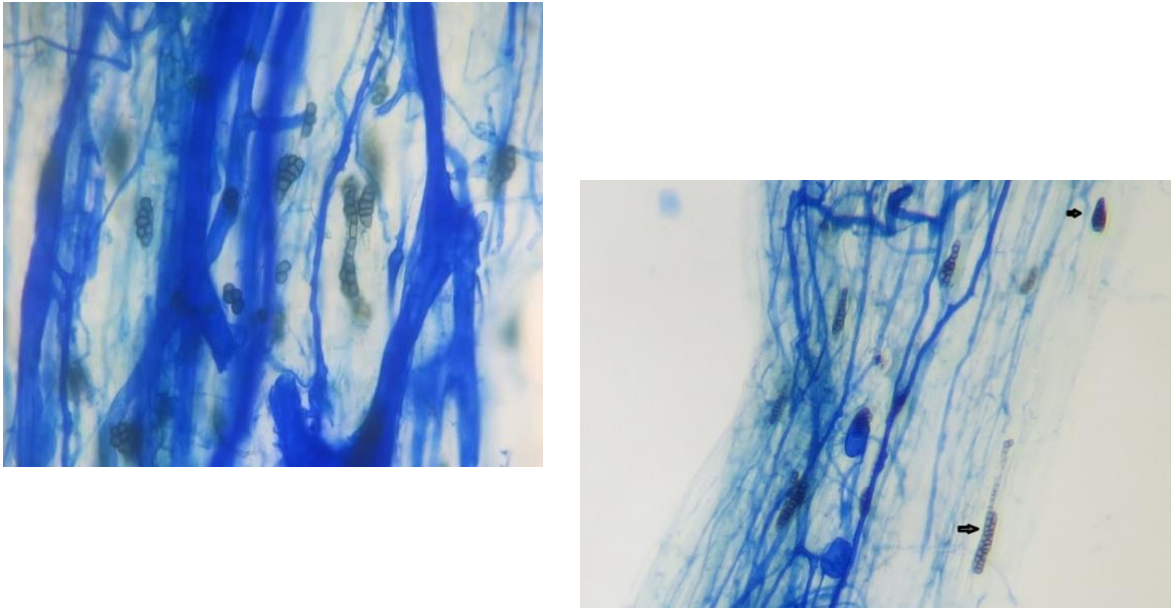
Figure 1. Melanized hyphal structures of a DSE colonizing *Crotalaria juncea* growing in acid soils of San Martín (Peru) (Photograhs by José C. Rojas García).

Additionally, melanin protects hyphae from desiccation and mechanical disruption, improving cell wall rigidity, hydrophobicity, and decreasing its permeability (Potisek et al. 2021). DSEs also form microsclerotia (figure 2). Although they present morphological differences from the true sclerotia produced by many fungi, similar to them they act as resting structures and serve for dispersal of propagules, and storage of substances like glycogen, proteins and polyphosphates involved in the improvement of the fungal viability under harsh conditions (Yung et al. 2021).
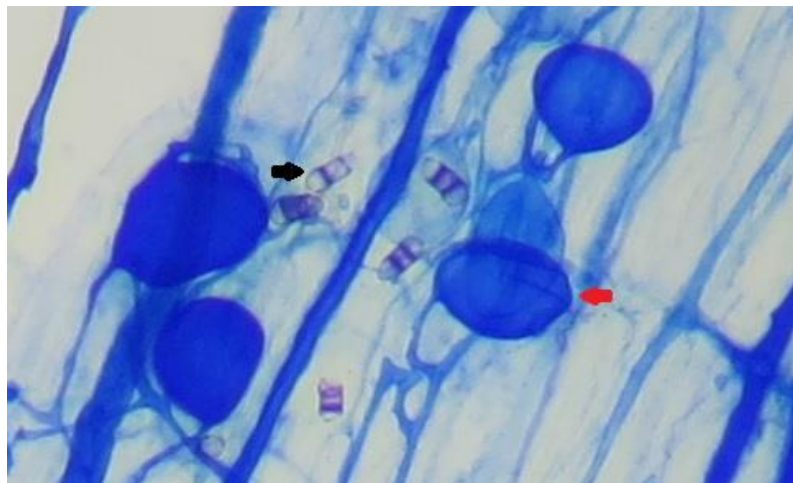

Figure 2. Coinfection of endophytic fungi in legumes: AMF vesicles (red arrow) and intracellular microsclerotia of DSE (black arrow) colonizing roots of *Centrosema macrocarpum* growing in alkaline soils of San Martin (Peru) (Photograh by José C. Rojas García).

Ecophysiology

It has been suggested that the first endophytic associations of plants with fungi occurred some 400 million years ago, and it is widely accepted that they probably helped plants to colonize the terrestrial space. It is therefore reasonable to assume that, after millions of years of co-evolution, DSEs influence adaptation of host plants to the changing situations that have occurred over time on planet Earth. Unlike arbuscular mycorrhizal fungi, which are obligate biotrophs, DSEs include a number of saprotrophic fungi from bulk soil and the rhizosphere and rhizoplane of many plants. Most of the DSEs perform a part of their life cycle as endophytes in the plant's interior without producing any damage to their hosts. Although most of them are not pathogenic, some species are.

DSEs have a short generation time and have small, reduced genomes compared to similar free-living species. Those characteristics allow fungi to evolve at much faster rates than plants, making them more adaptable to changing environmental conditions. In fact, there is evidence that DSEs can transfer to the host plant genes for resistance to infection by phytopathogens and those encoding plant growth promoting capabilities.

DSEs have been found in about 600 different hosts, are widely distributed, and are often the dominant fungal groups in plants growing in harsh habitats, such as Arctic, Antarctic, and alpine habitats, heavily polluted environments, arid ecosystems, and saline fields. It is in these harsh habitats where DSEs play fundamental roles in promoting plant growth and development as well as in mitigating biotic and abiotic stresses. They are also found in pastures, agricultural crops, aquatic systems, forests, swamps, dunes, and mountains (Barberis et al. 2020). Widely considered, the distribution, composition and colonization by DSEs are influenced by plant species, soil depth, and soil fertility level (Hou et al. 2019). Although it is recognized that DSEs influence plant succession in specific ecosystems, little is known about their abundance and variation in local environments, as well as their effect on plant density gradients (Barberis et al. 2020).

Fungal DSEs communities vary according to season, location, soil characteristics and host plant. They can survive as decomposers on leaves after the death of plant tissue, suggesting that mutualism may have derived from saprophytism (Suryanarayanan 2013). Interactions between DSEs and plants correlate according to the stage of the plant growth (e.g. mature leaves are colonised more than young leaves) and in response of the holobiont (plant and associated microorganisms) to environmental changes. Understanding the ecology of DSEs will provide insight into their ecological adaptability and their role in ecosystem restoration and reduction of desertification of arid ecosystems.

Different authors have reported that DSEs produce a broad range of hydrolytic and oxidative enzymes, which supports growth of their hosts via the mineralization of complex soil substrates, thereby releasing nutrients that plants can easily absorb. It is hypothesized that in return, DSEs obtain carbon compounds from plant metabolic waste by-products (Ruotsalainen et al. 2021).

Taxonomic classification of DSEs

DSEs constitute a paraphyletic group currently distributed in more than 40 classes, 100 orders, and some 1500 species, of which their taxonomic position is still unclear. Assignment of DSEs to a defined taxonomic unit within the Ascomycota is a difficult task as they constitute an artificial assemblage of fungi sharing similar morphological and functional traits (Piercey et al. 2004). Most DSEs are classified into the orders Helotiales, Hypocreales, and Pleosporaes. Other species are found in other orders such as Chaetothyriales, Capnodiales, Calospheriales, Chaetosphaeriales, Eurotiales, Hypocreales, Magnaporthales, Microascales, Sordariales and Xylariales. The most studied DSE belong to the PAC (*Phialocephala fortinii-Acephala applanata)* and GPC (*Gaeumannomyces-Phialophora/Harpophora*) species complexes. Fungal species within the PAC are considered to belong to the single species *P. fortinii* (Helotiales). PAC is mostly detected in the roots of coniferous,

ericaceous, and orchidaceous plants of the Northern hemisphere. Although research on DSEs is understudied in tropical and subtropical regions, previous obtained results suggest that PAC species might be distributed worldwide. Highly polymorphic DSEs ascribed to GPC include members of the genera *Phialophora/Cadophora* (Chaetothyriales/Helotiales) and *Harpophora* (Magnaporthales) with anamorphs in the *Gaeumannomyces* and *Magnaporthe* genera. Species of *Phialophora* colonize trees and, to a lesser extent, herbaceous plants, and those of the genus *Harpophora* associate with roots of herbs, primarily Poaceae. It is also to note that genera *Gaeumannomyces* and *Magnaporthe* also contain significant fungal pathogens. Since the first surveys on DSEs distribution (Sieber 2002; Addy et al. 2005; Grünig et al. 2011), current research shows the existence of a high diversity of DSEs, particularly in harsh and stressful environments, with special relevance of members of the order Pleosporales. Only a minority os plant species show little host plant specificity (Barberis et al. 2021).

DSEs and stress conditions

Following the hypothesis of "habitat adapted symbiosis", the interactions between DSEs and plants reach special importance in plants growing in nutrient poor soils or under stress conditions caused by drought, high salinity, and heavy metals contamination (Rodriguez et al. 2009) (figure 3).
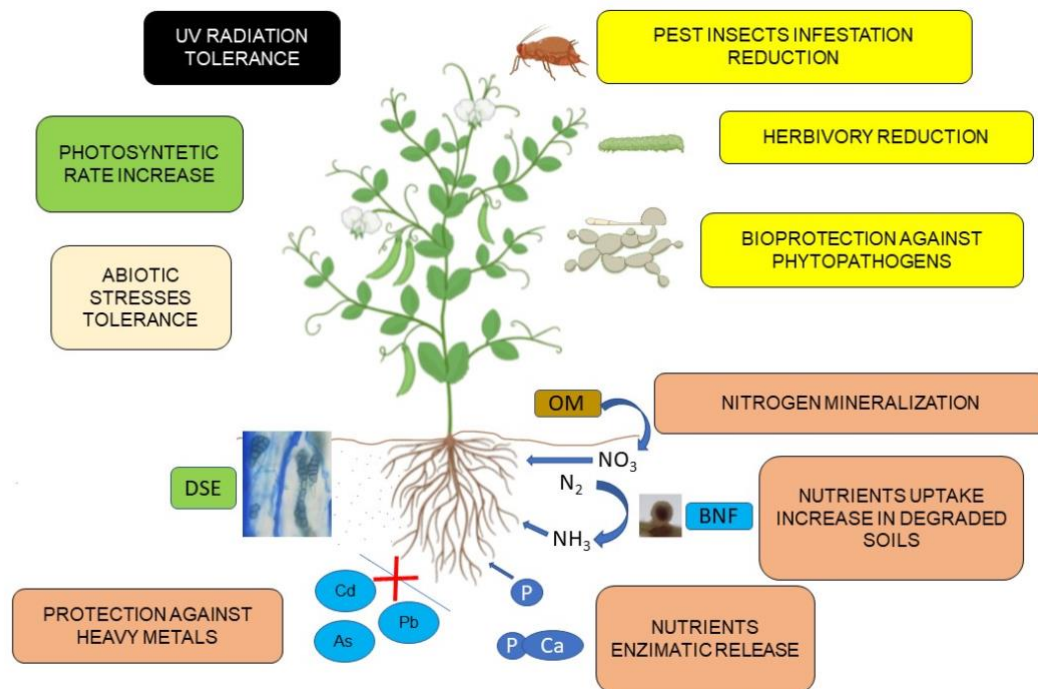

Figure 3. Main benefits derived from DSE-plants associations.

The meta-analysis carried out by Newsham (2011) revealed that a feature of DSEs related to plant growth is the enhancement of organic nitrogen (N) mineralization in the surrounding soil. Results by Tienaho et al. (2019) showed that DSEs synthesize and store large amount of arginine that will be used as an N source for the plant. 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 in providing N under high C content is negligible (Ingraffia et al. 2020). DSEs may also participate in solubilization of inorganic phosphate, which would support the P-solubilization activity of mycorrhizal fungi, thus increasing the pool of bioavailable P to the plant (Priyadharsini and Muthukumar 2017; Spagnoletti et al. 2017). DSEs can also increase the availability of micronutrients and trace elements from organic compound s in the soil and contribute to improved plant nutrition. In fact, increased contents in the total N, P, and C of stress-affected plants colonized by DSEs have

been reported. Thus, it is more likely that DSEs could be selected by plants as alternative symbiotic partners to support host nutrition in adverse environmental conditions (Deram et al. 2011; Hou et al. 2020; Ban et al. 2021; Akhtar et al. 2022).

DSEs and heavy metals toxicity

The presence of DSEs is very common in soils contaminated with heavy metals; to which DSEs usually show high resistance (Ban et al. 2012; Shadmani et al. 2021). Some authors have reported that the presence of low concentrations of metals in the soil favor root colonization by DSEs (Li et al. 2011; Su et al. 2021). The species *Alternaria alternata, Microdochium bolleyi* and *Bipolaris zeicola* have been isolated from Cd-containing landfills that show higher resistance to Cd than non-DSE fungi obtained from the same sites in Iran (Shadmani et al. 2021). Also, *Phialophora mustea,* a DSE isolated from a heavy metal-contaminated soil in France, colonized tomato roots with 5-10% higher intensity in cultures contaminated with Cd compared to uncontaminated cultures (Berthelot et al. 2016). The colonization of maize roots with *Gaeumannomyces cylindrosporus* was 10-15% higher in cultures contaminated with Pb in comparison with uncontaminated cultures (Ban et al. 2017). The tolerance of DSEs to heavy metals seems to be related not only with the development of immobilization mechanisms in their cell walls, intracellular complexes, and compartments, but also with efficient systems of extracellular efflux of metal ions and scavenging of reactive oxygen species (Zhan et al. 2017; Hou et al. 2020; Potisek et al. 2021; Su et al. 2021; Yu et al. 2021). The activation of superoxide dismutase (He et al. 2021) often accompanied by increases in catalase and peroxidase activities, and higher glutathione synthesis (Yu et al. 2021) are responsible for the neutralization of peroxide radicals. Overexpression of genes involved in redox homeostasis, DNA repair and maintenance of cell wall integrity, and high mitochondrial ATP production were also published (Zhao et al. 2015; Su et al. 2021).

The mycelia of DSEs grown with heavy metals are much darker, thicker, swollen, and more prone to twisting and looping. The septal spacing of the mycelia is shorter and the number of chlamydospores was significantly increased compared to DSEs non-affected by the metals (Ban et al. 2012; Hou et al. 2020). Tolerance of DSEs to heavy metal has been associated to the synthesis of melanin, a compound rich in hydroxyl groups with high binding capacity of heavy metals (Ban et al. 2012; Potisek et al. 2021;). Accumulation of melanin in hyphae of DSEs seems to be species, or even strain-specific, and is linked to fungal response driven by extracellular conditions (Potisek et al. 2021). Although Potisek et al. (2021) reported a negative correlation between the melanin content and Cd concentration in *Cadophora* sp., other authors found that melanin content in hyphae of *G. cylindrosporus, Acrocalymma vagum*, and *Scytalidium lignicola* was positively correlated with Pb and Cd (Ban et al. 2012; Hou et al. 2020). Moreover, among different albino strains of *Cadophora* sp., *Leptodontidium* sp. and *P. mustea*, only one strain of albino *Cadophora* sp. 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 DSEs strains. Those results suggest that melanin is crucial for immobilizing heavy metals in the fungal cell wall.

DSEs can also detoxify heavy metals in a melanin-independent manner either by their efflux or deposition in vacuoles (Zhao et al. 2015; Liu et al. 2021; Su et al. 2021). The richest fractions of Cd in the mycelium of *Cadophora* sp. were those bound to O-ligands and S-ligands, which 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 (Zhao et al. 2015: Potisek et al. 2021). After exposure to Cd and Zn, *Exophiala pisciphila* and *Falciphora oryzae* accumulated Cd in their vacuoles and chlamydospores (Liu et al. 2021; Su et al. 2021).

DSEs of the orders Chaetothyriales, Helotiales, Pleosporales, and Magnaporthales colonize the roots of several crops and trees improving their performance in soils contaminated with heavy metals (for more information see Malicka et al. 2022). The presence of DSEs in 90-100% of the roots collected

from heavy metals contaminated soils is well documented (Shadmani et al. 2021). Root colonization by *P. fortinii* was the main factor determining the heavy metal tolerance of *Clethra. barbinervis,* a Japanese tree growing wild in a mine site contaminated with Pb, Zn, and Cu. Colonization by *P. fortinii* decreased the concentration of Pb in the roots and produced an enhanced biomass in comparison to plants inoculated with the non-DSE *Rhizoderma veluwensis* or *Rhizoscyphus* sp. isolated from the same mining site (Yamaji et al. 2016). The presence of DSEs in plant seems to trigger changes in the subcellular heavy metals distribution and in the regulation of plant genes responsible for the translocation and detoxification of the metals (Shen et al. 2020; Su et al. 2021). The cell wall remodeling in response to roots colonization by *E. pisciphila* was the effect of the increased activity of the enzymes pectin methylesterase involved in demethylation ov cell wall pectins, extending their capacity of cation binding, and the phenylalanine ammonia-lyase and the cell wall peroxidase which together catalyze the polymerization of lignin monomers (Shen et al. 2020).

DSEs and plant drought tolerance

Halophytes thriving in coastal habitats, deserts and invasive species with a wide range of ecological tolerance, are the main ecosystems where halo- and xero-tolerant DSEs can be found. Data from field surveys in desert areas of the USA, Mexico, and Argentina show that DSEs represented by PAC and Pleosporales are the main endophytic fungal communities in the roots of desert flora where they were found in nearly 100% of the plants tested (Porras-Alfaro et al. 2008; Khidir et al. 2010; Fracchia et al. 2011). Pleosporales and other species of DSEs represented 60% of the endophytic fungal isolates from the Great Hungarian Plane (Knapp et al. 2012, 2015) and more than 50% of the fungi obtained from the roots of xerophytic shrubs in Northwest China (Xie et al. 2017; Hou et al. 2019; Zuo et al. 2020; He et al. 2021). Members of the Pleosporales fungi are frequent root colonizers of halophytes of the families Poaceae, Amaranthaceae, Convolvulaceae, Cyperaceae, and Boraginaceae growing in saline coastal habitats (Qin et al. 2017).

Optimal conditions for DSEs growth in culture media are those corresponding to moderate osmotic pressure. DSEs isolated from the roots of halophytes showed sensitivity to 4% KCl and NaCl in the growth medium but maintain hyphal viability even up to a 12% salt concentration, which suggests that DSEs can be considered halotolerant, but not of halophilic nature fungi (Qin et al. 2017). DSEs salt tolerance could be related to their ability to accumulate osmolytes like trehalose and mannitol, which maintain cell turgor and stabilize proteins (Usuki and Narisawa 2007; Dastogeer and Wylie 2017).

Under moderate drought and salinity, inoculation of plants with DSEs of the orders *Chaetothyriales, Calosphaeriales, Capnodiales,* and *Pleosporales* increased the total and shoot biomass and resulted in a clear amelioration of the physiology and architecture of the root system (Qin et al. 2017; Zhang et al. 2017; Pan et al. 2018; He et al. 2019; Li et al. 2019; Qiang et al. 2019; Gonzalez Mateu et al. 2020).

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 (Li et al. 2019; Hou et al. 2021). The root biomass of plants affected by drought and salinity can be increased up to 95% in DSE-colonized plants compared to non-colonized plants, proving their better performance in the uptake and allocation of nutrients and water (Li et al. 2019; He et al. 2019, 2021; Qiang et al. 2019; Zuo et al. 2020). Experimental evidence suggests that inoculation of xerophytic plants with DSEs isolated from the desert plants *Alternaria chlamydosporigena, Paraphoma chrysanthemicola,* and *Bipolaris sorokiniana* improved the adaptability of *Artemisia ordosica* to salt stress by stimulating the synthesis of indol acetic acid and by enhancing the K^+ accumulation and Na^+ exclusion from the roots, increasing their biomass and length up to 40% (Li et al. 2018; Hou et al. 2021). In a pot experiment with soil exposed to drought stress, the presence of DSEs increased the abundance of

saprotrophic and symbiotrophic fungi and prevented the development of fungal pathogens, an effect more likely due to the interaction with other members of the plant microbiome (He et al. 2021).

DSEs and plant tolerance to stress

In addition to the mechanisms developed to cope against heavy metals and drought stresses, DSEs can also support plants growing in adverse conditions by regulating their photosynthesis and respiration (figure 3).

Reduced stomatal conductance, decreased concentrations of photosynthetic pigments, and low capacity of photosystems are usually the result of plant growth limitations under drought and heavy metals stress (Zhang et al. 2020; Sitko et al. 2022). A number of plants, including *Z. mays*, *Triticum aestivum*, *Sorghum bicolor*, *Hordeum vulgare*, *Phragmites australis*, and *Glycorrhiza uralensis* had increased chlorophyll content, efficiency of PSII photochemistry, transpiration rate, and stomatal conductance, all this leading to a better gas exchange and higher level of (Zhang et al. 2017; He et al. 2019; Qiang et al. 2019; Shadmani et al. 2021). Similarly, DSEs-inoculated trees of the genera *Salix caprea, Betula pendula, Populus tremula, P. tomentosa,* and *Vochysia divergens* growing under heavy metals and salt stress had higher chlorophyll concentrations, transpiration and photosynthetic rates than the uninoculated trees (Ban et al. 2017; Pan et al. 2018; Farias et al. 2020).

Mitigation of adverse stress produced by DSEs has been considered to be due to changes in the plant physiology by modulating the production of phytohormones, secondary metabolites, and antioxidative enzymes, thus preventing the plant tissues from structural damage, maintaining their normal development and physiological functions (Liu and Wei 2019; Hou et al. 2021). In addition to the synthesis of indol acetic acid which exerts a stimulatory effect on plant growth (Berthelot et al. 2016; Priyadharsini and Muthukumar 2017; Qiang et al. 2019), DSE can affect the hormone signal transduction and hormone-regulated gene expression in plants (Andrade-Linares et al. 2011).

The presence of DSEs likely induces metabolic pathway responsible for the production of secondary metabolites. Lahlali et al. (2014) reported the induction of systemic resistance in *Brassica napus* after inoculation with *Cladophialophora chaetospira*. Also, the inoculation of *S. bicolor* and *Z. mays* with *E. pisciphila* enhanced the production of anthocyanins, polyphenols and flavonoids, as well as the enzymatic activities of cinnamyl alcohol dehydrogenase, phenyl ammonia lyase, and guaiacol peroxidase in plants affected by water deficit (Zhang et al. 2017; Shen et al. 2020). Yung et al. (2021) also found higher anthocyanin content in *Noccaea caerulescens* after inoculation with *Leptodontidium* sp. and *P. mustea*. The antioxidant pathways involved in reduction of oxidative cellular damage is also enhanced in stress-affected plants (Zhang et al. 2017; Zhu et al. 2018: Berthelot et al. 2018; He et al. 2019; Qiang et al. 2019). Overexpression of the antioxidative enzymes superoxide dismutase, catalase and peroxidase has been reported in stress plants treated with DSEs of the orders Chaetothyriales and Pleosporales (Pan et al. 2018; He et al. 2019; Li et al. 2019; Zhu et al. 2018; Hou et al. 2021). Increased content of the antioxidant glutathione is also assigned to inoculation with DSEs (Ban et al. 2012; Hou et al. 2020). Additionally, melanin produced by DSEs inside plant roots could be responsible for the binding and neutralization of the oxygen radicals formed by plants exposed to stress (Redman et al. 2002).

DSEs and resistance to biotic stress

There is evidence on the role of DSEs in plant-microorganisms interactions. Due to their close relationship with the host, great physiological versatility, and low specificity, DSEs are suitable biocontrol agents for plant diseases (Santos et al. 2021). Among the mechanisms of action, antibiosis and the development of resistance to phytopathogens have been most reported.

The DSE *Veronaeopsis simplex* Y34 produced a 71% reduction in *Fusarium* wilt disease in *B. campestris* and still had good growth (Khastini et al. 2012), and Zhang et al. (2022) reported that inoculation of rice with *P. fortinii* strain J2PC4 increased its resistance to Southern rice black-streaked dwarf disease virus. The increase in the activity of antioxidant enzymes such as superoxide dismutase and peroxidase activity, as well as the deregulation of genes related to defense in rice, such as PR1a and AOS, constitute the mechanisms of action that increase resistance to this disease.

Co-inoculation of *Picea abies* seedlings with the DSE *P. sphaeroides*, the root pathogen *Heterobasidion parviporum* or coinfected with both fungi revealed that *H. parviporum* infection triggered reprogramming of host metabolism, and that the endophyte *P. sphaeroides* counteracted the negative effects of *H. parviporum* on the growth of the spruce seedlings (Wen et al. 2022). Also, inoculation of *Eustoma grandiflorum*, an economically important ornamental flower crop worldwide, inoculated with *Hyaloscypha variabilis* J1PC1 suppressed the root rot disease caused by *Pythium irregulare* producing a positive effect on shoot growth (Marian et al. 2022). There is also evidence about the reduction of herbivory by insect pests in crops due to the interactions of the plants with DSEs (Zhang et al. 2022).

The effect of DSE on diseases caused by nematodes has been described. Chu et al. (2021), reported that the exogenous inoculation of the ectomycorrhizal fungi *Suillus bovinus* and *Amanita vaginata* with the DSEs *Gaeumannomyces cylindrosporus* and *Paraphoma chrysanthemicola* reduced the severity of the disease caused by the nematode *Bursaphelenchus xylophilus*, the causal agent of pine wilt. The authors refer to the fact that the synergy between microorganisms improved and stabilized the microbial biomass, which is postulated as the mechanism of action.

Conclusions

Although originally considered an elusive group of endophytes with unclear classification and forming ambiguous associations with plants, current research has shown their occurrence, significance, and benefits to host plants. DSEs show high tolerance to abiotic stresses, which would explain their frequent presence in the roots of plants inhabiting harsh environments. DSEs also provide higher bioavailability of nutrients and protection against several stressors to their symbiotic host plants. Despite the inexistence of an intimate relationship between DSEs and their host plants, the endohytic fungi have been shown to modulate the plant metabolism and physiology to a great extent. Although DSEs are considered to be of low specificity, based on their positive effect on plants, it is possible that a certain degree of compatibility might be required, which might be limited under severe stress and low soil organic matter content. There exists a clear potential for application of DSEs in phytoremediation and revegetation of degraded lands. Nevertheless, further research is needed to ascertain their potentials as bioinoculants as well as to better understand the DSEs utility and further assess their environmental impact.

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