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A meta-analysis of the dark septate endophytic fungal role in the plant host adaptability to drought stress

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Abstract

Drought stress is a major concern for global agriculture, reducing plant growth and productivity. Recent research has demonstrated the potential of dark septate endophytic fungi (DSE) in increasing plant resilience to drought. This meta-analysis brings together findings from several research papers to better understand the role of DSE fungi in plant host adaptation to drought stress. We conducted a comprehensive assessment and analysis of data from 19 peer-reviewed studies published between 2018 and 2023, with an emphasis on major morphological, physiological, and plant nutrient responses of plants colonized by DSE fungi during drought. Our findings show that DSE fungi considerably improve plant growth (plant height, root and shoot biomass, total biomass, and root length), improve root colonization, increase total chlorophyll, superoxide dismutase (SOD), and indole-3-acetic acid (IAA), decrease proline and malondialdehyde (MDA), and enhance N availability. The meta-analysis also found that the significance of these beneficial effects varied by species and environmental context, highlighting the importance of host-fungus compatibility and local adaptability. This study suggests that the use of DSE fungi as a biological agent has potential to mitigate and adapt drought stress in plants. Future studies should focus on understanding the underlying molecular mechanisms and investigating practical applications of DSE fungi in sustainable agriculture, particularly in arid and semi-arid regions.

Keywords – plant growth – morphology – physiology – Indole-3-acetic acid – Superoxide dismutase

Introduction

Drought stress is one of the most important environmental variables restricting agricultural productivity globally (Li et al. 2022). Climate change-induced droughts are becoming more frequent and severe, necessitating the development of new strategies to improve plant resilience (Gehring et al. 2020). Traditional breeding and genetic engineering have had some success; nevertheless, they frequently demand significant time and resources (Liu et al. 2022). As a result, there is a growing interest in studying symbiotic connections between plants and microbes including dark septate endophytic fungi (DSE) as a potential option for increasing plant tolerance to abiotic challenges.

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Dark septate endophytes are common root-associated fungi distinguished by melanized septate hyphae and production of melanized microsclerotia (Jumpponen & Trappe 1998, Melati et al. 2023a). Compared to arbuscular mycorrhizal fungi, which have been intensively investigated, DSE fungi received less attention, despite their widespread occurrence across many environments and plant species (Gong et al. 2023). Recent research indicates that DSE fungi play an important role in improving plant response to diverse environmental stressors, including drought (Liu et al. 2022).

DSE fungi can colonize plant roots without inflicting noticeable damage, developing mutualistic interactions that benefit their hosts in a variety of ways (Knapp et al. 2015). These advantages include better nutrient availability and acquisition (He et al. 2021a, b), higher root growth (Li et al. 2019a), and increased production of secondary metabolites (He et al. 2020, Dalimunthe et al. 2023), which help in stress tolerance. Furthermore, DSE fungi have been demonstrated to cause physiological and biochemical changes in host plants, such as the buildup of osmoprotectants and the activation of antioxidant defense mechanisms, both of which are critical for dealing with water deficiency conditions (Liu et al. 2022). These benefits include improved rhizosphere nutrient availability and root development, which may facilitate nutrient acquisition by the host plant (He et al. 2021a, b).

Despite DSE fungi's intriguing potential, the extent and mechanisms of their contribution to plant drought tolerance are still unclear (Hou et al. 2021). Given the variety of experimental methods, plant species, fungal strains, and environmental variables used in different investigations, we synthesized existing data in meta-analysis data to summarize their contribution to drought tolerance. This meta-analysis study seeks to thoroughly analyze and quantify the impacts of DSE fungi on plant adaptation to drought stress, thus providing a better understanding of their role and identifying factors that determine their efficacy.

This meta-analysis study aims to answer the following main questions by combining and evaluating data from multiple studies: (1) How can DSE fungi influence plant morphological, physiological and nutritional availability responses during drought? (2) How does DSE fungi's efficacy vary between plant species and drought stress contexts? (3) What are the possible pathways for the positive effects of DSE fungi on drought-stressed plants? Addressing these issues will not only increase our scientific understanding but will also pave the road for practical applications of DSE fungi in sustainable agriculture, particularly in drought-prone regions.

Materials & Methods

Literature search and selection criteria

A database was developed from several types of literature which reported the effect of utilization of dark septate endophytes on plant performance under drought environments. The literature search was conducted using Scopus and PubMed and the keywords used were ‘drought’, and ‘dark septate endophyte’ in May 2024. The initial search resulted in 68 articles with the selection criteria were: (a) English-language articles; (b) direct comparison between using DSE and control without DSE; (c) the studies were conducted on drought environment; (d) comparison on plant morphology, physiology and nutrition uptake and; (e) replication and variance were reported (standard deviation (SD) or standard error or means (SEM)). These criteria followed the Preferred Reporting Item for Systematic Reviews and Meta-Analysis (PRISMA) protocol.

The selection process is shown in Figure 1. Concisely, the initial search was screened based on the title. A total of 19 articles were excluded for several reasons (non-related title, review articles or conference proceedings). In abstract screening, the useful literature consisted of 53 articles, and the rest were excluded because of duplication and irrelevant content or variables. Hence, the full text evaluation resulted in 22 articles while 31 articles were excluded due to lack of comparison (n=1), conference and review articles (n=5), irrelevant contents or variables (n=25). The final articles (n=19) after assessment are considered as databases in meta-analysis (Table 1).

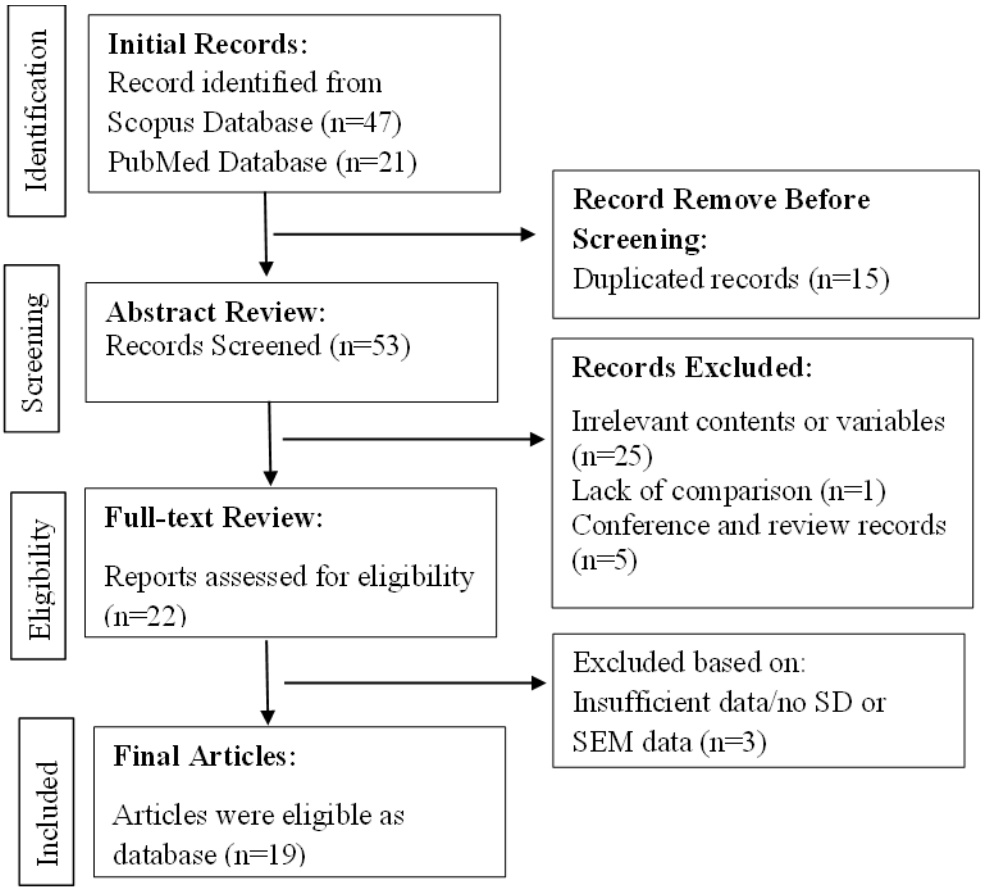


Fig. 1 – PRISMA flow diagram of study identification, screening, eligibility, and inclusion for the meta-analysis. Diagram adapted from PRISMA 2020 guidelines (Page et al. 2021) and formatted following examples in Risyahadi et al. (2023).

Database development

The bibliography, DSE species, host plant, and treatment were inputted into Microsoft Excel spreadsheet. Response variables included in database consisted of three groups, including morphological (plant height, total biomass, root biomass, shoot biomass, root length, and root diameter), physiological (root colonization, proline, total chlorophyll, indole-3-acetic acid (IAA), malondialdehyde (MDA), and superoxide dismutase (SOD)), and nutrition absorption (available N and P). All variables were converted into the same units of measurements. The database of descriptive statistics is presented in Table 2.

Data analysis

All data were analyzed using the random effects meta-analysis method. The calculation was based on the standardized mean difference of Hedges' where the mean value of without DSE inoculation was grouped into control group (X^C) and the mean value of with DSE inoculation was the experimental group (X^E). The calculation is as follows:

$$d = \frac{(X^E - X^C)}{S} J$$

J was the correction factor for the small sample size while *S* was the pooled standard deviation. The equation was derived from Sánchez-Meca & Marín-Martínez (2010) with 95% confidence interval. The data were analyzed using the OpenMEE application, encompassing 14 response variables across 19 studies, grouped as morphological (6 parameters), physiological (6 parameters), and nutrient absorption (2 parameters).

Table 1 Articles included in meta-analysis.

No	DSE species	Host plant	Treatment	Reference
1	*Chalastospora* sp. *Clarea lozoyensis* *Helotiales* sp. *Lasiosphaeriaceae* sp. *Ophiosphaerella* sp. 1 *Ophiosphaerella* sp. 2 *Paraphoma* sp. *Pleosporales* sp. *Preussia terricola* *Sordariomycetes* sp.	*Pinus edulis*	Heat, drought, drought and heat	Gehring et al. (2020)
2	*Exophiala pisciphila* *Alternaria chartarum*	*Zea mays*	Well-watered, drought stress	Gong et al. (2023)
3	*Acrocalymma vagum* *Paraboeremia putaminum* *Fusarium acuminatum*	*Glycyrrhiza uralensis*	Drought stress, normal	He et al. (2019)
4	*Acrocalymma vagum* *Paraphoma chrysanthemicola* *Alternaria longissima* *Darksidea alpha* *Alternaria chlamydospora* *Acremonium nepalense* *Preussia terricola* *Alternaria chartarum*	*Glycyrrhiza uralensis*	Drought stress, well-watered	He et al. (2021a)
5	*Acrocalymma vagum* *Paraboeremia putaminum*	*Glycyrrhiza uralensis*	Drought stress, well-watered	He et al. (2021b)
6	*Acrocalymma vagum* *Paraboeremia putaminum*	*Glycyrrhiza uralensis*	Drought stress, well-watered	He et al. (2021b)
7	*Alternaria chlamydospora* *Neocamarosporium phragmitis* *Microascus alveolaris*	*Lycium ruthenicum*	Drought stress, well-watered	He et al. (2022a)
8	*Acrocalymma vagum* *Paraboeremia putaminum*	*Astragalus mongholicus*	Drought stress, well-watered	He et al. (2022b)
9	*Acrocalymma vagum* *Paraboeremia putaminum*	*Astragalus mongholicus*	Drought stress, well-watered	He et al. (2022b)
10	*Paraboeremia putaminum* *Alternaria chlamydosporigena* *Paraphoma chrysanthemicola* *Bipolaris sorokiniana*	*Artemisia ordosica*	Drought stress	Hou et al. (2021)
11	*Paraconiothyrium* sp. *Paraconiothyrium* sp. *Darksidea* sp. *Phialophora* sp. *Darksidea* sp. *Knufia* sp. *Leptosphaeria* sp. *Embellisia chlamydospora*	*Ammopiptanthus mongolicus*	Drought stress	Li et al. (2018)
12	*Paraphoma* sp. *Embellisia chlamydospora* *Cladosporium oxysporum*	*Hedysarum scoparium*, *Glycyrrhiza uralensis*, *Zea mays*	Wild drought stress, extreme drought stress	Li et al. (2019a)
13	*Alternaria alternata* *Paraphoma pye* *Paraphoma radicina*	*Triticum* sp.	Drought stress, well-watered, severe drought stress	Li et al. (2022a)

Table 1 Continued.

No	DSE species	Host plant	Treatment	Reference
14	*Alternaria chartarum* *Paraphoma chrysanthemicola* *Acrocalymma vagum*	*Artemisia ordosica*	Drought stress, well-watered	Li et al. (2022b)
15	*Macrophomina pseudophaseolina* *Paraphoma radicina*	*Astragalus mongholicus*	Drought stress, well-watered	Li et al. (2022)
16	*Phialophora* sp. *Knufia* sp. *Leptosphaeria* sp. *Embellisia chlamydospora*	*Hedysarum scoparium*	Drought stress, well-watered	Li et al. (2019b)
17	Unidentified DSE strain (HB3-1)	*Coelogyne viscosa*	Drought stress	Liu et al. (2022)
18	*Acrocalymma vagum*	*Ormosia hosiei*	Well-watered, moderate watered, low water	Liu & Wei (2019)
19	*Acrocalymma vagum*	*Ormosia hosiei*	Well-watered, moderate watered, low water	Liu & Wei (2021)

Table 2 Descriptive statistics of database.

No	Parameter	NC	Do c	Mean		Min		Max		SD	
				DSE	nonDSE	DSE	nonDSE	DSE	nonDSE	DSE	nonDSE
1	Height	518	11	18.247	15.034	3.600	1.500	100	95	13.939	12.003
2	Root biomass	508	10	0.293	0.248	0.000	0.010	3.380	2.110	0.525	0.440
3	Root diameter	258	4	0.344	0.347	0.105	0.113	0.668	0.560	0.127	0.134
4	Root length	383	12	286.280	237.166	1.192	1.690	1390	1136	325.223	273.595
5	Shoot biomass	230	9	0.249	0.226	0.023	0.028	1.430	1.430	0.361	0.350
6	Total biomass	383	10	1.367	1.063	0.094	0.1	6.030	4.150	1.487	1.067
7	Colonization	60	3	30.344	0	0.380	0	78	0	33.842	0
8	Proline	133	4	45.499	47.247	0.570	0.540	100	130	32.130	31.155
9	Chlorophyll	108	4	22.713	20.574	0.013	0.018	49	37	17.998	15.835
10	IAA	125	4	5.173	4.010	0.076	0.074	48	37	13.683	10.878
11	MDA	123	4	3.492	4.160	0.013	0.020	13	13	4.286	5.048
12	N available	150	3	24.847	18.557	2.562	5.730	82	43	24.215	15.325
13	P available	210	4	5.870	6.034	0.369	0.360	11	11	2.697	2.808
14	Superoxide dismutase (SOD)	173	6	157.882	154.324	21	20	618	452	3.021	32.393

Notes: SD (standard deviation), SEM (standard error of mean), NC (number of comparisons), and MDA (malondialdehyde)

Table 3 Effects of dark septate endophytic fungi (DSE) on morphology, physiology and nutrition uptake on host plant.

No	Variables	NC	Estimate	Lower bound	Upper bound	Std Error	p-Value	τ^2	Q	Het. p-value	I^2
Morphology											
1	Height	114	0.144	0.021	0.266	0.063	0.022	0	20.409	1	0
2	Root length	85	1.724	0.95	2.499	0.395	< 0.001	10.176	830.527	< 0.001	89.886
3	Root diameter	54	-0.078	-0.564	0.407	0.248	0.751	2.636	291.735	< 0.001	81.833
4	Root biomass	124	1.729	1.213	2.245	0.263	< 0.001	6.588	974.606	< 0.001	87.38

Table 3 Continued.

No	Variables	NC	Estimate	Lower bound	Upper bound	Std Error	p-Value	τ^2	Q	Het. p-value	I^2
5	Shoot biomass	113	0.808	0.421	1.194	0.197	< 0.001	3.313	635.615	< 0.001	82.379
6	Total biomass	79	1.358	0.775	1.941	0.298	< 0.001	5.731	650.075	< 0.001	88.001
Physiology											
7	Colonization	12	4.61	2.734	6.486	0.957	< 0.001	7.021	79.055	< 0.001	86.086
8	Proline	26	0.328	-1.778	2.434	1.075	0.76	17.941	365.746	< 0.001	93.165
9	Chlorophyll	24	3.095	1.407	4.783	0.861	< 0.001	12.071	254.866	< 0.001	90.976
10	IAA	25	2.155	1.163	3.146	0.506	< 0.001	4.706	179.745	< 0.001	86.648
11	MDA	27	-1.712	-2.825	-0.599	0.568	0.003	6.78	232.532	< 0.001	88.819
12	Superoxide dismutase SOD	34	2.921	1.734	4.108	0.606	< 0.001	9.773	359.923	< 0.001	90.831
Nutrition uptake											
13	N available	30	1.483	0.075	2.891	0.719	0.039	13.256	364.971	< 0.001	92.054
14	P available	42	-0.321	-1.094	0.543	0.395	0.417	5.421	355.62	< 0.001	88.471

Results

This meta-analysis study used Q statistics test, τ^2 and I^2 to examine heterogeneity. The *Q*-statistics was the weighted sum of the squared values of each study that effect size's deviation from the mean effect size of all studies. The estimate of the population variable tau (τ) was the standard deviation of the overall effect size and τ^2 represents the variance of the overall effect size. The I^2 index was a measure of the proportion of unexplained heterogeneity. Based on the Heterogeneity Q statistics test, τ^2 and I^2 showed that some variables were categorized in high heterogeneity and others were low heterogeneity. In terms of morphological, root length, root diameter, root biomass, shoot biomass and total biomass had excess heterogeneity when Q was higher than degrees of freedom (NC-1) meanwhile plant height was categorized in low or no excess heterogeneity when Q was lower than degrees of freedom (NC-1). For physiological and nutrient intake, all the variables were high heterogeneity. Heterogeneity was impacted by several factors: the number of studies in the meta-analysis, how much the study effect sizes varied from each other (between studies variance) and how much variance existed in the observed effect size for each study (within-study variance). Heterogeneity of this study was high due to DSE species, host plant and treatment watering. This observed heterogeneity also reflects biological variation among DSE fungal strains and host plant combinations. The magnitude and direction of effects varied across studies, suggesting that certain DSE taxa or strain–host pairings may confer stronger drought tolerance than others. These strain-specific responses highlight the importance of matching compatible DSE–host systems when evaluating drought adaptation potential.

Table 3 shows the detailed meta-analysis results of morphological and physiological results according to Cohen's methodology. In comparison to non DSE, DSE inoculation significantly increased plant height, root length, root biomass, shoot biomass and total biomass ($P<0.05$) while the root diameter was unaffected by DSE inoculation. On the physiological aspect, DSE increased colonization, chlorophyll, IAA, SOD, but significantly decreased MDA. For proline, no significant effect of DSE inoculation was observed. In terms of nutrient uptake, DSE inoculation significantly contributed to increasing N available ($P<0.05$). On the contrary, the P available was not affected in DSE inoculations ($P>0.05$).

Discussion

The findings of this meta-analysis highlight the critical function that DSE fungi play in improving plant tolerance to drought stress. Our analysis of 19 peer-reviewed papers demonstrates

consistent patterns in physiological, morphological, and nutrient availability responses, indicating DSE fungi's potential as a biological agent for enhancing drought tolerance in plants. DSE fungi have been shown to improve their host plants' tolerance and growth to biotic (Surono & Narisawa 2018, Dalimunthe et al. 2023) and abiotic (Marfuah et al. 2024, Melati et al. 2023b) stress conditions.

Increased plant growth and nutritional availability in drought stress conditions

One of the most significant findings in this meta-analysis is that plants colonized by DSE fungi have improved plant drought tolerance. This improvement can be attributable to the investigated various elements, including root biomass, which facilitates improved water and nutrient absorption from soil. Dark septate endophytic fungi form symbiotic relationships with plant roots, improving nutrient uptake and stress resistance by producing melanized hyphae and microsclerotia inside the host plant root tissues, which supply water and nutrients to plant root tissues, especially during drought (Santos et al. 2017). Dark septate endophytic fungi promote root growth and development by increasing root surface area, root volume, and the number of root tips. This improves the plant's ability to absorb water and nutrients, which is especially beneficial under stressful conditions like drought (Surono & Narisawa 2017, Santos et al. 2017). This is consistent with previous research demonstrating that DSE fungi can alter root architecture, promote extensive root growth and development, increase root surface area, root volume, and the number of root tips, and thus improve water and nutrient uptake, which is critical during drought conditions (Li et al. 2022a).

Furthermore, the colonization of host plants by DSE fungi has a substantial impact on nutrient availability during drought stress conditions, notably nitrogen (N), which is required for plant growth and development (Li et al. 2018). Our meta-analysis reveals that DSE fungi play an important role in increasing nitrogen uptake in host plants by utilizing both organic and inorganic nitrogen sources (Usuki & Narisawa 2007, Surono & Narisawa 2017). The symbiotic relationship between DSE fungi and plant roots encourages the formation of longer, more widespread root systems. These improved root structures allow for wider exploration of the soil profile, allowing plants to utilize nitrogen supplies more efficiently, especially in drought-stressed conditions. As a result, higher nitrogen availability promotes greater shoot and overall plant biomass, which is critical for sustaining growth and production under adverse conditions. N is crucial for plant growth and development, and its availability can significantly impact plant performance under drought stress (He et al. 2019). Although DSE fungi significantly enhanced nitrogen uptake, their effect on phosphorus availability under drought was not significant (He et al. 2021a). Research by Gaber et al. (2023) showed that the P content in the shoots of plants inoculated with DSE was not significantly different compared to plants inoculated in all treatments. Phosphorus is an essential macronutrient, but under drought stress, DSE inoculation did not significantly improve its uptake, indicating that drought may limit fungal facilitation of P availability. This study found that DSE inoculation had variable effects on soil available P and on plant responses under drought stress, with some effects being significant and others non-significant (He et al. 2022a, Gaber et al. 2023).

According to a meta-analysis in this study, DSE colonization was substantially higher in drought-stressed host plants compared to non-drought-stressed controls. DSE fungi have a remarkable ability to colonize host plants, especially under drought stress, which is critical for their beneficial impacts on plant resilience (Gehring et al. 2020). The DSE colonization involves melanized hyphae and microsclerotia that may enhance nutrient exchange and stress resilience, although the extent of nutrient uptake enhancement remains debated among studies (Santos et al. 2017, He et al. 2021a). First, DSE fungi invade plant roots by specialized hyphae that penetrate the root tissues. These hyphae can enter intercellular gaps in the root cortex and form symbiotic relationships with plant cells. This close relationship enables DSE fungi to exchange nutrients and signals with the host plant, resulting in mutualistic benefits like improved nutrient absorption and stress tolerance (He et al. 2022b). Microsclerotia are structures formed by DSE fungi within root tissues. These are compact structures made up of fungal hyphae that can live in plant roots for long periods of time. Microsclerotia serve as a reservoir for fungal survival and multiplication, ensuring that the symbiotic association lasts even in severe environmental situations such as drought.

Asparagus officinalis colonized by the dark septate fungus *Phialocephala fortinii* accumulates significant levels of glycogen, protein, polyphosphate, and phosphorus in its microsclerotia (Li et al. 2018).

Colonization of plant roots by dark septate endophytic (DSE) fungi induces physiological responses in both partners, resulting in a complex interplay of interactions that significantly influence plant growth, development, and stress tolerance (He et al. 2019). For example, DSE fungi encourage the development of host plant root systems, encouraging root multiplication and increasing root biomass. Inoculation with dark septate endophytic (DSE) fungi significantly increased root length, surface area, and branch number, while having no effect on root diameter (He et al. 2019). This architectural change allows plants to explore a larger volume of soil, improving their ability to get water and nutrients required for growth and survival during conditions of drought. Furthermore, DSE fungi affect root morphology by causing alterations in root architecture. These changes include changes to root branching patterns and the production of lateral roots, which improve the efficiency of water and nutrient uptake. DSE fungi increase the absorptive surface area of roots, increasing the plant's ability to endure water scarcity circumstances. DSE fungi successfully colonize host plants by a complex process of hyphal penetration, microsclerotia production, and physiological adaptation. He et al. (2019) reported DSE colonization on licorice roots, expanding earlier records of strong DSE colonization known since the 1980s in other plant hosts (Jumpponen & Trappe 1998). These systems not only aid in the formation of symbiotic relationships, but also considerably improve plant resilience to drought stress. Understanding the processes of DSE colonization and their effects on plant physiology is critical for capitalizing on these beneficial interactions to increase agricultural sustainability and ecosystem resilience in the face of environmental challenges.

The impact of DSE on the host physiology under drought stress

Dark septate endophytic (DSE) fungi have a substantial impact on host plant physiology by producing phytohormones, mainly indole-3-acetic acid (IAA), especially under drought stress circumstances (Li et al. 2018). This symbiotic interaction promotes plant growth and development by influencing important physiological processes. IAA and other phytohormones have important roles in controlling plant responses to environmental stressors, including drought. DSE fungi increase the synthesis and release of IAA in host plants, enhancing root and shoot growth. Specifically, IAA improves root system architecture by encouraging lateral root development and elongation. This structural improvement enables plants to explore deeper soil layers, increasing water and nutrient uptake efficiency during droughts. In addition to root development, IAA stimulates shoot growth by stimulating cell elongation and division. These mechanisms are critical for sustaining plant growth and biomass production under water-stress conditions. By controlling these growth processes, DSE fungi assist plants in maintaining physiological functions and growth, hence improving overall plant performance in comparison to non-colonized equivalents. The production of IAA by DSE fungi is a result of the intricate interactions between fungal hyphae and plant tissues (He et al. 2022a). These fungi can generate IAA directly or indirectly via enzymatic pathways inside plant cells. Upon colonization, DSE fungi activate molecular signaling pathways that upregulate genes involved in IAA production, raising hormone levels in the plant. In addition to the higher IAA/ABA ratio observed in DSE-inoculated seedlings compared to non-inoculated seedlings (Liu & Wei 2019). Understanding the role of DSE fungi-mediated IAA synthesis in increasing plant drought tolerance has important implications for agriculture and ecology. Harnessing these symbiotic relationships may lead to the development of microbial inoculants or biostimulants that improve crop resilience to water scarcity, lowering the demand for water and chemical inputs in agricultural techniques. Furthermore, improving the natural presence of DSE fungi in ecosystems may improve ecosystem resilience to environmental stressors, hence contributing to sustainable land management practices.

Drought stress causes a series of physiological responses in plants, including the overproduction of reactive oxygen species (ROS), which are highly reactive chemicals that can damage cellular components. Plant cells produce oxygen radicals and their derivatives, known as reactive oxygen species (ROS), during various processes associated with abiotic stress, and utilize

them as fundamental signaling molecules to respond to changing environmental conditions (Numponsak et al. 2018). ROS accumulation during dry circumstances causes oxidative stress, threatening plant health and productivity. Our meta-analysis shows that dark septate endophytic (DSE) fungi play an important role in oxidative damage mitigation by increasing the activity of superoxide dismutase (SOD) in host plants. The significant increase in SOD observed in DSE fungi under drought stress suggests their adaptation to mitigate oxidative damage by effectively removing reactive oxygen species (ROS) (Li et al. 2022b). Superoxide dismutase is a crucial antioxidant enzyme that converts superoxide radicals into less hazardous molecules like oxygen and hydrogen peroxide. The higher activity of SOD in DSE-colonized plants indicates a better ability to scavenge ROS, lowering oxidative stress and protecting cellular structures from damage. During drought stress, *A. ordosica* seedlings that were inoculated with DSE showed notable increases in both SOD activity and GSH levels (Li et al. 2022b). This improvement in antioxidant defense systems demonstrates the critical role of DSE fungus in increasing plant tolerance to environmental stressors, particularly drought. DSE fungi help host plants maintain cellular homeostasis and metabolic activities that are necessary for growth and survival under adverse conditions by increasing their antioxidant capacity. This defensive strategy not only aids plants in dealing with oxidative stress caused by drought, but it also increases their resilience to other environmental stresses. Furthermore, the symbiotic relationship between DSE fungi and host plants demonstrates a sophisticated adaptation mechanism that promotes plant health and productivity in a variety of ecological contexts. Understanding the molecular processes that drive DSE-mediated antioxidant improvement will help optimize plant-microbe interactions for agricultural and ecological uses. Using these insights may lead to the development of microbial inoculants or biostimulants that improve crop resilience to drought and other stressors, enabling sustainable agriculture and ecosystem stability.

This meta-analysis study demonstrates that DSE fungal colonization has different impacts on plant stress responses, especially under drought. Specifically, we found that DSE colonization does not result in significant changes in proline content when compared to non-colonized or control plants. Proline is an important osmoprotectant in plants, accumulating during stress to maintain cellular integrity and reduce damage caused by drought-induced osmotic stress. DSE-colonized plants had steady proline levels, implying that they face lower overall stress levels than non-colonized counterparts. Furthermore, our findings show a considerable reduction in malondialdehyde (MDA) levels in plants colonized by DSE fungi under drought stress conditions. Under drought stress, the malondialdehyde (MDA) content of DSEs was significantly higher compared to the control treatment (Li et al. 2022b). MDA is a reliable measure of lipid peroxidation, which occurs when plant cells experience oxidative stress because of reactive oxygen species (ROS) buildup during drought conditions. Malondialdehyde (MDA), extensively studied as a marker for assessing the severity of lipid peroxidation in plant cells, is one of the main products of lipid peroxidation (Dvojković et al. 2023). Lower MDA levels suggest less oxidative damage to cellular membranes in DSE-colonized plants, indicating a better ability to handle oxidative stress than non-colonized plants. Under drought stress, dark septate endophytic (DSE) fungi actively contributed to reducing the malondialdehyde (MDA) content in the host plant (Li et al. 2022b). These findings imply that DSE fungi play an important role in increasing plant drought tolerance by modulating osmotic and oxidative stress responses. DSE fungi's ability to maintain proline levels and reduce MDA accumulation demonstrates their effectiveness in alleviating stress-induced damage in host plants. Inoculation with DSE fungi under drought stress led to increases in GSH content, SOD activity, and levels of soluble protein and proline (He et al. 2022a). This dual system demonstrates DSE fungi's potential as beneficial symbionts, contributing to better plant resilience and overall performance under harsh environmental conditions.

In conclusion, this meta-analysis study found that DSE fungi had a significant positive effect on enhancing host plant growth, particularly such as plant height, root length, root diameter, shoot biomass, total biomass, increased host root colonization, especially N availability, and improved plant physiology, including increased IAA and SOD content, under drought stress. The meta-analysis revealed that the efficacy of DSE fungi to enhance drought resistance differs depending on plant

species and environmental variables. This variation underscores the importance of host-fungus compatibility and adaptation locally. Some DSE fungal strains may be more effective with certain plant species, meaning that targeted fungal inoculation could optimize the benefits. Furthermore, environmental conditions such as soil type, temperature, and humidity may influence the interaction of DSE fungi with host plants, altering the outcome. The specific processes by which these fungi develop tolerance are complex and diverse. This meta-analysis pinpoints multiple essential routes, including enhanced morphology, physiology, and availability capability. However, additional research is needed to understand the biochemical and genetic underpinnings of this symbiotic connection under drought stress. Understanding the signaling pathways and regulatory networks involved will provide further insight into the symbiotic connection between DSE fungi and plants. This meta-analysis study demonstrated that DSE fungi are successful in improving plant response to drought stress and can be employed as an environmentally friendly approach to climate change mitigation and adaptation.

Author Contributions

All authors contributed to the study conception and design. MAF, STR, SR, WFS, SRW, ISS, contributed to performing all tests, conceptualization, methodology, investigation, visualization and writing the original draft. MAF, S, STR contributed to conceptualization, method optimization, investigation, funding acquisition, supervision, review and editing. MAF, S, STR, SR, WFS, SRW, ISS, contributed to conceptualization, methodology, formal analysis, review and editing. MAF, S, STR, SR, WFS, SRW, ISS, contributed to data curation, investigation, formal analysis, review, and editing. All authors read, commented, and approved previous versions of the manuscript.

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Conflict of interest

The authors state that they have no known competing financial interests or personal relationships that could have influenced the work described in this study.

Data Availability

Raw data will be made accessible upon request.

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