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Effect of different mycobionts on growth parameters of *Dactylorhiza hatagirea* (D. Don) Soo: implications on conservation strategies

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Dactylorhiza hatagirea maintain a symbiotic relationship with rhizospheric fungi in their lifecycle. Rhizospheric fungi have different roles during its growth and development. Although various rhizospheric fungi have been isolated from *D. hatagirea*, little is known about their specific effects on its growth and development. To understand the role of fungal species on growth parameters of *D. hatagirea*, we compared the effect of eight fungal species on growth parameters of *D. hatagirea*. viz. *Trichoderma asperellum*, *Talaromyces falvus, Aspergillus candidus, Circinella muscae, Aspergillus flavus, Aspergillus niger, Cephalosporium acremonium* and *Trichoderma harzianum* in the form of three treatments. Treatment (T1) comprised the combined application of *Trichoderma asperellum*, *Talaromyces falvus, Aspergillus candidus* and *Circinella muscae*. Treatment (T2) comprised the combined application of *Aspergillus flavus, Aspergillus niger, Cephalosporium acremonium* and *Trichoderma harzianum*. Another treatment (T3) comprised the combined application of T1 and T2. A separate set of plants which were un-treated with any fungal isolated served as control. Our results revealed that the tubers inoculated with T3 conferred the highest shoot length, tuber length, optimal fresh and dry matter yield, and greatly enhanced other growth parameters, length of inflorescence, number of flowers and specific leaf area. Treatment (T3) has a discernible impact on plant growth compared to the T1, T2 and control. The results revealed that these fungal species we used in the presented study of tested plant *D. hatagirea* promoted growth with different efficiencies. Our results also revealed that rhizospheric fungal associations with *D. hatagirea* showed development-dependent preference and hence may provide the basic knowledge for use of different fungal species in conservation of *D. hatagirea* at different elevations.

KEYWORDS

***Dactylorhiza*, fungal species, growth parameters, tuber, treatments**

Introduction

A well-documented example of mutualism is the symbiotic relationship between fungi and land plants, where fungi facilitate the acquisition of essential mineral nutrients from the soil and deliver them to plants, while plants reciprocate by supplying photosynthates to fungi (Smith and Read, 2008). In the case of orchids, a compatible fungal association is critical for seed germination in natural habitats (Rasmussen, 1995). During the germination of orchid seeds and the early development of protocorms, the fungi transfer mineral nutrients to the orchid, a phenomenon termed "mycoheterotrophy" (Leake, 1994). Upon successful fungal colonization, hyphae invade and form coiled structures known as pelotons within the cortical cells of the orchid (Smith and Read, 2008). These pelotons undergo degradation and senescence, releasing nutrients into the orchid cells (Kuga et al., 2014). The cyclical formation and breakdown of pelotons are essential processes for the nutrient exchange between the orchid and its fungal symbiont (Dearnaley and Cameron, 2017; Fochi et al., 2017). Orchids display varying degrees of specificity in their fungal associations. Some mycoheterotrophic orchids, such as those in the Corallorhiza striata complex (Barrett et al., 2010) and Hexalectris species (Kennedy and Watson, 2010), exhibit a narrow fungal partner range. In contrast, other orchids engage with a broader spectrum of fungal taxa. For example, *Cypripedium californicum* forms associations with fungi from the families Tulasnellaceae, Ceratobasidiaceae, and Sebacinales (Sheferson et al., 2007). Similarly, fungal taxa such as Tulasnellaceae, Telephoraceae, Ceratobasidiaceae, Sebacinales, Russulaceae, and Clavulinaceae have been detected in *Cymbidium goeringii* and *Cymbidium lancifolium* (Ogura-Tsujita et al., 2012). Despite the broad fungal colonization observed in orchids, the effects of these associations on orchid growth and development can vary. For instance, in *Vanilla*, different fungal isolates from the roots exhibit variable impacts on plant growth and survival (Porras-Alfaro and Bayman, 2007).

D. hatagirea, also known by its common names such as salam panja in Hindi and Pahari, Panchangle in Nepali, Narmad in Shina and Kashmiri, is a perennial herb that thrives in sub-alpine to alpine regions. This plant has gained global recognition due to its numerous advantageous properties. It has been found effective in treating various disorders related to the urinary system (Ballabh et al., 2008), respiratory system, nervous system (Khajuria et al., 2017), digestive system (Tsering et al., 2017), skeletal system (Giri and Tamata, 2010), and reproductive system (Chauchan, 1990). Additionally, it has the ability to enhance the immune system to combat infectious diseases. The roles of various fungal species isolated from rhizosphere of *D. hatagirea* have never been tested systematically with *in vivo* methods. To understand the effects of various fungal species on ontogenetic stages in orchid, we compared the effect of eight fungal species of *Trichoderma asperellum, Talaromyces falvus, Aspergillus candidus, Circinella muscae, Aspergillus flavus, Aspergillus niger, Cephalosporium acremonium* and *Trichoderma harzianum* on their ability to promote growth by examining the growth parameters. Knowledge of rhizospheric fungal association in medicinal important *D. hatagirea* species would be helpful for propagation, commercial cultivation and conservation.

Materials and methods

For the fungal inoculum, the fungal species were cultured in Potato dextrose agar (PDA for 5 days at 28°C. After this incubation period, the actively growing fungal plate was flooded with sterile water, and gentle agitation was employed to liberate the spores from the mycelia. The liberated spores were then carefully separated from the mycelial debris by passing them through a sieve and were then collected in a beaker. The spore concentration was then adjusted by dilution with sterile water to a final concentration of 10^4 to 10^6 spores/ml. The concentration of fungal spores was selected based on an OD reading of 0.1 of the suspension at 600 nm using a spectrophotometer (Figure 1).

Preparation of potting mixture

The potting mixture used in the experiment was carefully prepared to provide an optimal environment for seedling growth. It was composed of sterile soil and sand, combined in a 3:1 ratio. To ensure the absence of any living microorganisms or contaminants that could interfere with the experiment, the potting mixture was autoclaved thrice. Each autoclave cycle involved subjecting the potting mixture to high-pressure steam of 15 pascals at a temperature of 121°C for

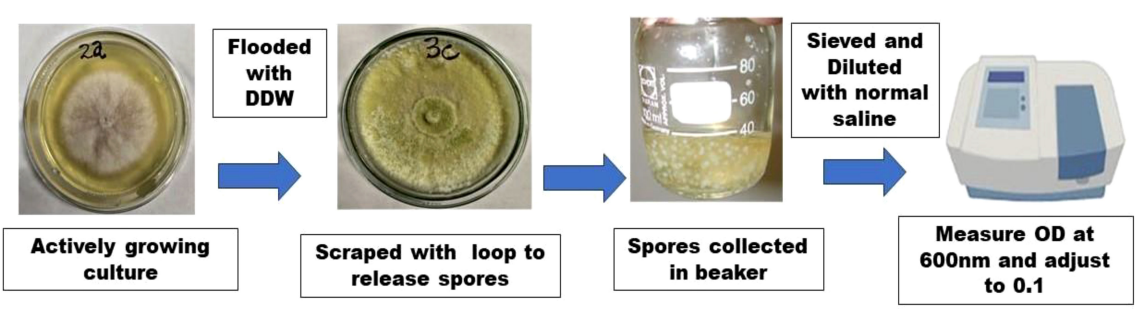

FIGURE 1
Preparation of fungal inoculum.

20 minutes. Following the first autoclave cycle, the material was autoclaved again the following day. This allowed any remaining heat-resistant spores within the material to potentially germinate overnight and subsequently be eradicated. The third autoclave cycle was performed on the third day to ensure thorough eradication of any potential pathogens, including bacteria, fungi, or weed seeds. Tubers were sterilized in (2% NaOCl for 10 min followed by streptomycin (100 µg/ml) + cycloheximide (100 µg/ml) for 12 hrs. (Schmidt et al., 2021). After sterilization, the inoculated tubers were planted in pots (4 replicates for each treatment) filled with triple autoclaved soil and sand in a ratio of 3:1. The pots were placed in a green house and the pots were irrigated with double distilled water every alternate day (Figures 2, 3). Plants were grown in the green house for 3 months during which various observations like tuber length, tuber fresh mass, tuber dry mass, shoot lengths, shoot dry mass, shoot fresh mass, specific leaf area were recorded. The plants were allowed to reach the flowering stage, and the length of inflorescence and number of flowers were recorded before the termination of the experiment. Three treatments (T1, T2, T3) and Control were employed to investigate the effect of rhizospheric fungi on the growth characteristics.

Results

Effect of rhizospheric fungi on growth parameters of *D. hatagirea*

The effect of various treatments on growth parameters is shown in (Figure 4). Mycobial inoculants significantly improved different growth parameters. Tuber length was highest (6.52 cm) under T3 and lowest (4.21 cm) under control treatment (Table 1, Figure 4). ANOVA and *post-hoc* analysis revealed that the increase in tuber length under the T3 treatment is significantly higher compared to other treatments. Shoot length varied from a highest of 3.7 cm under T3 and lowest of 25.27 cm under control treatment. Like tuber and shoot length, tuber fresh mass was also highest (3.56 g/plant) under T3 and lowest (1.4 g/plant) under the control treatment (Table 2, Figure 4). Shoot fresh mass also differed under different treatments and was significantly higher (17.54 g/plant) under T3 and was lowest (8.3 g/plant) under control treatment (Table 2, Figure 4). ANOVA and *post-hoc* analysis revealed significant differences among all the treatment (Tables 2, 3). Further the number of flowers per plant ranged from 40 under T3 to 24.5 under control (Table 1, Figure 4). ANOVA and *post-hoc* analysis revealed that treatments such as T3 and T2, T1 and control were not significantly different from one another. Moreover, the specific leaf area was highest in T3 (2.32 cm^2/gm) and lowest in control (1.56 cm^2/gm). There were significant differences between T3 and control treatments, however no significant differences were found between T1 and T2. The effects of different treatments (T1, T2, T3) and Control, on tuber length, tuber fresh mass, tuber dry mass, shoot length, shoot fresh mass, shoot dry mass, specific leaf area (SLA), number of flowers, and length of inflorescence revealed significant differences in these growth parameters across the treatments, suggesting that the rhizospheric fungi have a discernible impact on plant growth compared to the control group (Table 1). Each parameter was measured in four replicates (n=4), and the results are presented as mean values with their respective standard deviations (SD).

ANOVA tests conducted on various plant growth parameters, including tuber length, fresh and dry mass, shoot length, fresh and dry mass, specific leaf area (SLA), number of flowers, and length of inflorescence. The Table 3 displays the degrees of freedom (Df), sum of squares (Sum sq), mean squares (Mean sq), F values, and the p-values (Pr(>F)). Significant results are denoted by asterisks, with *** indicating $p < 0.001$, ** indicating $p < 0.01$, and * indicating $p < 0.05$. These results highlight where treatment effects are statistically significant, indicating a notable impact of the treatments on the respective growth parameters. Tukey's pairwise comparisons for several plant growth parameters (Table 4), including tuber length, fresh and dry mass, shoot length, fresh and dry mass, specific leaf area (SLA), number of flowers, and length of inflorescence. The comparisons are made between different

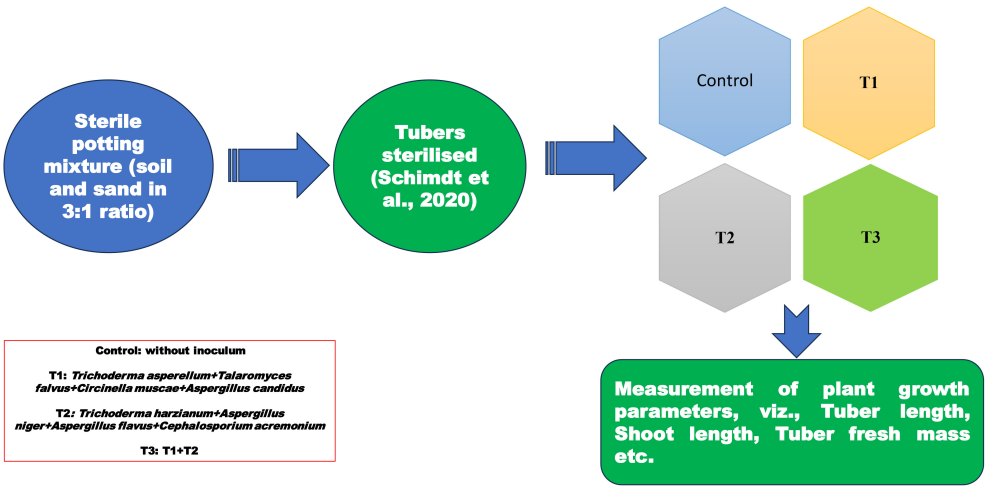

FIGURE 2
Representation of methodology for inoculum potential.

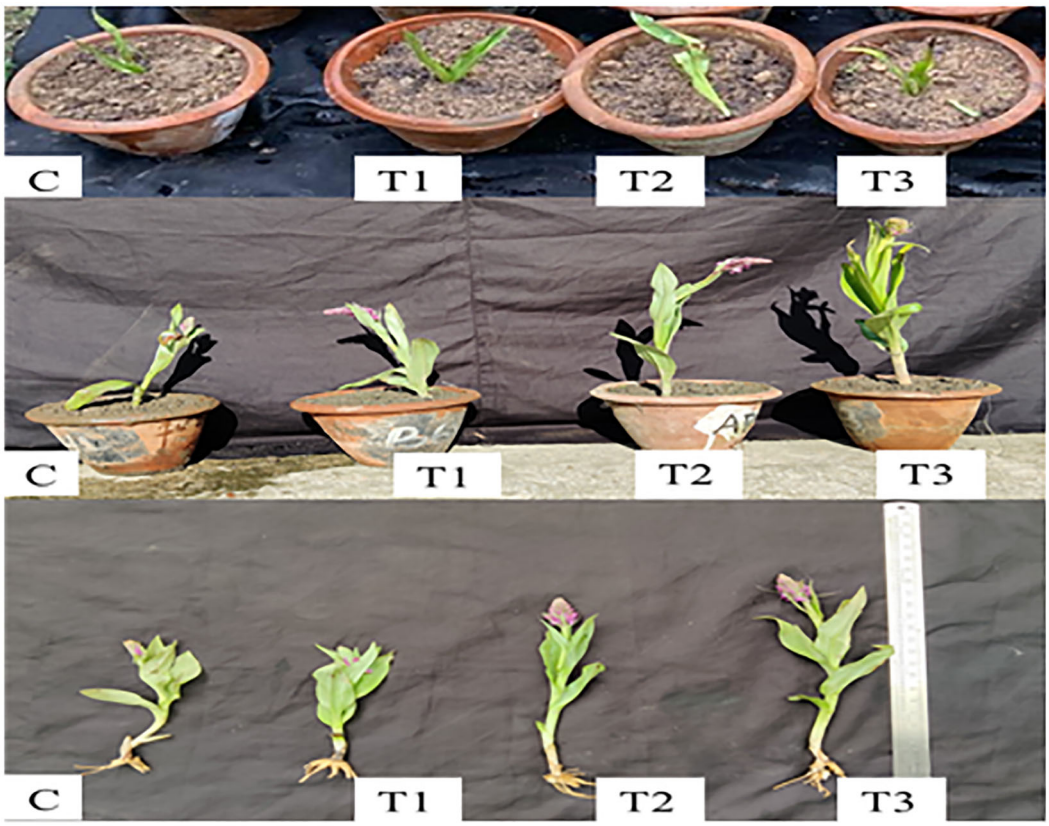

FIGURE 3
Effect of fungal species on plant growth parameters of *Dactylorhiza hatagirea*.

treatment groups (T1, T2, T3) and a control group. The columns show the differences (diff), lower and upper confidence limits (lwr, upr), and the adjusted *p*-values (*p* adj). Significant differences (*p* adj < 0.05) are highlighted to indicate where the treatments had a notable effect compared to the control or other treatments.

Discussion

Fungi play a critical role in plant growth and development, particularly through symbiotic interactions where they supply essential soil minerals, such as nitrogen and phosphorus, in exchange for carbon-based compounds produced by the plant. These symbiotic fungi, particularly mycorrhizal fungi, form associations with the roots of over 90% of plant species and can fulfill up to 80% of the plant's mineral nutrient requirements. Orchids, a diverse and ecologically significant family, are known for their complex fungal associations, which often involve clades such as Serendipitaceae (Sebacinales), Tulasnellaceae, and Ceratobasidiaceae (Batty et al., 2006; Valadares et al., 2011). These associations can vary in specificity, ranging from highly specialized relationships with a narrow group of fungal partners to more generalized interactions with multiple fungal taxa (Otero et al., 2002; Ma et al., 2003; McCormick et al., 2004; Sheferson et al., 2005; Suárez et al., 2006; Li et al., 2021). In orchids, fungi play a fundamental role in seed germination, protocorm development, and overall growth. Previous studies have demonstrated that specific fungal species can stimulate key growth parameters, including seed germination and early developmental stages of orchids (Wang et al., 2011; Zhao et al., 2013; Tian et al., 2022). This is particularly relevant for species like *D. hatagirea*, an orchid of high conservation and medicinal value, which forms specific associations with certain fungal species. In this study, we found that the tubers of *D. hatagirea* are compatible with eight fungal species, suggesting a specialized yet flexible symbiotic relationship.

In the context of plant growth enhancement, the addition of fungal inoculants such as *Trichoderma asperellum, Talaromyces flavus, Aspergillus candidus, Circinella muscae, Aspergillus flavus, Aspergillus niger, Cephalosporium acremonium*, and *Trichoderma harzianum*. these has been shown to significantly improve various growth parameters of *D. hatagirea*. Treatments involving these fungi (T1, T2, and T3) were evaluated for their effects on tuber length, tuber fresh and dry mass, shoot length, shoot fresh and dry mass, specific leaf area (SLA), number of flowers, and inflorescence length. The results revealed that all treatments led to substantial improvements in plant growth compared to the untreated control, with treatment T3 consistently showing the most pronounced effects across all measured parameters. Specifically, treatment T3 led to the greatest increases in tuber length, fresh and dry tuber mass, shoot length, fresh and dry shoot mass, SLA, number of flowers, and inflorescence length. Statistical analysis using ANOVA confirmed the significance of these effects, with p-values less than 0.001

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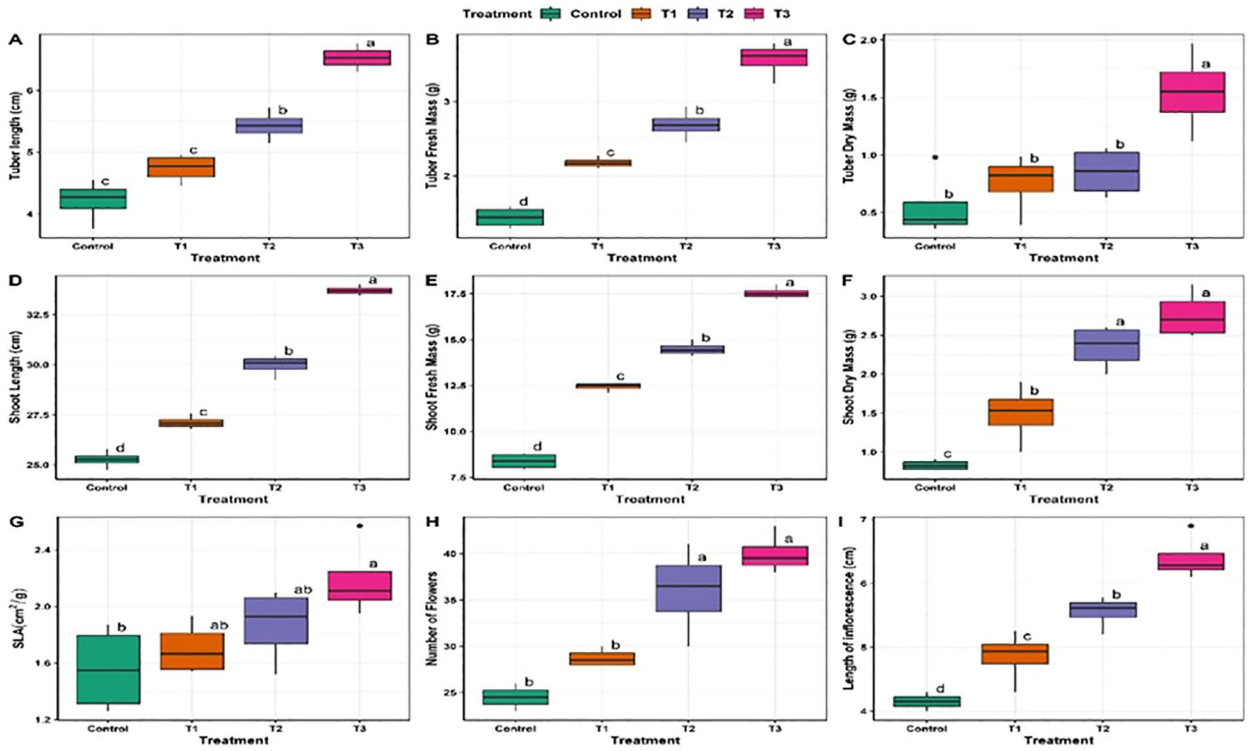

FIGURE 4
Effect of fungal species on plant growth parameters of *D. hatagirea*. Letters represent the significant differences at $p < 0.05$. Where **(A)**: Tuber length, **(B)**: Tuber fresh mass, **(C)**: Tuber dry mass, **(D)**: Shoot length, **(E)**: Shoot fresh mass, **(F)**: Shoot dry mass, **(G)**: Specefic leaf area, **(H)**: Number of flowers, **(I)**: Length of Inflorescence.

for most parameters, indicating a strong correlation between fungal treatment and enhanced plant growth. Furthermore, Tukey's pairwise comparisons highlighted significant differences between the control and all treatments, with T3 being consistently superior, underscoring the remarkable efficacy of this particular treatment in promoting plant development. These findings provide valuable insights into the specific fungal interactions that underpin the growth and productivity of *D. hatagirea*. The variation in growth responses to different fungal species suggests a development-dependent specificity, where certain fungal strains are more effective at different stages of plant growth. These results not only contribute to our understanding of orchid-fungal symbioses but also have practical implications for conservation and cultivation strategies for *D. hatagirea*. The identification of effective fungal partners could be leveraged in conservation programs aimed at enhancing the survival and growth of this species, as well as in agricultural practices for its sustainable production. The study

TABLE 1 Effects of different treatments (Control, T1, T2, T3) on tuber length, tuber fresh mass, tuber dry mass, shoot length, shoot fresh mass, shoot dry mass, specific leaf area (SLA), number of flowers, and length of inflorescence.

Parameters	Treatment			
	Control	T1	T2	T3
Tuber length (cm)	4.22 ± 0.336	4.74 ± 0.233	5.43 ± 0.239	6.53 ± 0.198
Tuber Fresh Mass (g)	1.44 ± 0.143	2.18 ± 0.07	2.69 ± 0.198	3.56 ± 0.233
Tuber Dry Mass (g)	0.552 ± 0.288	0.758 ± 0.26	0.853 ± 0.214	1.55 ± 0.355
Shoot Length (cm)	25.3 ± 0.431	27.1 ± 0.325	29.30 ± 0.522	33.7 ± 0.237
Shoot Fresh Mass (g)	8.38 ± 0.443	12.4 ± 0.238	14.5 ± 0.381	17.5 ± 0.34
Shoot Dry Mass (g)	0.83 ± 0.067	1.49 ± 0.375	2.35 ± 0.284	2.76 ± 0.304
SLA (cm^2/g)	1.56 ± 0.307	1.7 ± 0.185	1.87 ± 0.265	2.18 ± 0.269
Number of Flowers	24.5 ± 1.29	28.8 ± 0.957	36 ± 4.69	40 ± 2.16
Length of inflorescence	4.15 ± 0.129	4.86 ± 0.4	5.55 ± 0.252	6.39 ± 0.351

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TABLE 2 ANOVA tests conducted on various plant growth parameters.

Variable	Treatment	N	mean	sd
Tuber length (cm)	Control	4	4.22	0.336
Tuber length (cm)	T1	4	4.74	0.233
Tuber length (cm)	T2	4	5.43	0.239
Tuber length (cm)	T3	4	6.53	0.198
Tuber Fresh Mass (g)	Control	4	1.44	0.143
Tuber Fresh Mass (g)	T1	4	2.18	0.07
Tuber Fresh Mass (g)	T2	4	2.69	0.198
Tuber Fresh Mass (g)	T3	4	3.56	0.233
Tuber Dry Mass (g)	Control	4	0.552	0.288
Tuber Dry Mass (g)	T1	4	0.758	0.26
Tuber Dry Mass (g)	T2	4	0.853	0.214
Tuber Dry Mass (g)	T3	4	1.55	0.355
Shoot Length (cm)	Control	4	25.3	0.431
Shoot Length (cm)	T1	4	27.1	0.325
Shoot Length (cm)	T2	4	30	0.522
Shoot Length (cm)	T3	4	33.7	0.237
Shoot Fresh Mass (g)	Control	4	8.38	0.443
Shoot Fresh Mass (g)	T1	4	12.4	0.238
Shoot Fresh Mass (g)	T2	4	14.5	0.381
Shoot Fresh Mass (g)	T3	4	17.5	0.34
Shoot Dry Mass (g)	Control	4	0.83	0.067
Shoot Dry Mass (g)	T1	4	1.49	0.375
Shoot Dry Mass (g)	T2	4	2.35	0.284
Shoot Dry Mass (g)	T3	4	2.76	0.304
SLA (cm2/g)	Control	4	1.56	0.307
SLA (cm2/g)	T1	4	1.7	0.185
SLA (cm2/g)	T2	4	1.87	0.265
SLA (cm2/g)	T3	4	2.18	0.269
Number of Flowers	Control	4	24.5	1.29
Number of Flowers	T1	4	28.8	0.957
Number of Flowers	T2	4	36	4.69
Number of Flowers	T3	4	40	2.16
Length of inflorescence	Control	4	4.15	0.129
Length of inflorescence	T1	4	4.86	0.4
Length of inflorescence	T2	4	5.55	0.252
Length of inflorescence	T3	4	6.39	0.351

highlights the critical role of fungal associations in the growth and development of *D. hatagirea*, demonstrating that the use of specific fungal strains, particularly in treatment T3, can substantially improve various growth parameters. These results lay the foundation for further research into the mechanisms of orchid-fungal interactions and offer practical applications in the conservation and propagation of *D. hatagirea*.

TABLE 3 The Table 4 displays the degrees of freedom (Df), sum of squares (Sum sq), mean squares (Mean sq), F values, and the p-values (Pr(>F)).

	Df	Sum sq	Mean sq	F value	Pr (>F)	
Tuber length (cm)						
Treatment	3	11.985	3.995	60.77	1.58E-07	***
Residuals	12	0.789	0.066			
Tuber Fresh Mass (g)						
Treatment	3	9.549	3.183	107.2	6.27E-09	***
Residuals	12	0.356	0.03			
Tuber Dry Mass (g)						
Treatment	3	2.2382	0.7461	9.267	0.0019	**
Residuals	12	0.9661	0.0805			
Shoot Length (cm)						
Treatment	3	162.15	54.05	348.8	6.20E-12	***
Residuals	12	1.86	0.15			
Shoot Fresh Mass (g)						
Treatment	3	177.48	59.16	460.9	1.18E-12	***
Residuals	12	1.54	0.13			
Shoot Dry Mass (g)						
Treatment	3	9.01	3.0032	37.78	2.16E-06	***
Residuals	12	0.954	0.0795			
SLA (cm2/g)						
Treatment	3	0.8751	0.29169	4.3	0.0281	*
Residuals	12	0.814	0.06783			
Number of Flowers						
Treatment	3	585.7	195.23	26.7	1.35E-05	***
Residuals	12	87.8	7.31			
Length of inflorescence						
Treatment	3	11.049	3.683	40.57	1.47E-06	***
Residuals	12	1.089	0.091			

Significance. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

Conclusion

Rhizospheric fungi establish mutualistic associations with plant roots known as root-fungal associations, playing a vital role in every aspect of plant growth and development stages while enhancing resistance against diseases and environmental stressors. The fungal rhizosphere is an intelligent and sentient system with inherent ecological healing properties that functions as a living Internet, facilitating nutrient transport and communication. Moreover, fungi

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TABLE 4 Tukey's pairwise comparisons for several plant growth parameters, including tuber length, fresh and dry mass, shoot length, fresh and dry mass, specific leaf area (SLA), number of flowers, and length of inflorescence.

Tuber length (cm)	*diff*	*lwr*	*upr*	*p adj*
T1-Control	0.5225	-0.01575408	1.060754	0.0580667
T2-Control	1.215	0.67674592	1.753254	0.0001118
T3-Control	2.3125	1.77424592	2.850754	0.0000001
T2-T1	0.6925	0.15424592	1.230754	0.0113097
T3-T1	1.79	1.25174592	2.328254	0.0000022
T3-T2	1.0975	0.55924592	1.635754	0.0002892
Tuber Fresh Mass (g)				
T1-Control	0.7375	0.3756953	1.099305	0.00029
T2-Control	1.2475	0.8856953	1.609305	0.0000015
T3-Control	2.1225	1.7606953	2.484305	0
T2-T1	0.51	0.1481953	0.871805	0.0059988
T3-T1	1.385	1.0231953	1.746805	0.0000005
T3-T2	0.875	0.5131953	1.236805	0.0000574
Tuber Dry Mass (g)				
T1-Control	0.205	-0.39066373	0.800664	0.7403435
T2-Control	0.3	-0.29566373	0.895664	0.4697402
T3-Control	0.995	0.39933627	1.590664	0.0016248
T2-T1	0.095	-0.50066373	0.690664	0.9635055
T3-T1	0.79	0.19433627	1.385664	0.0092097
T3-T2	0.695	0.09933627	1.290664	0.0210841
Shoot Length (cm)				
T1-Control	1.8525	1.026137	2.678863	0.0001195
T2-Control	4.7	3.873637	5.526363	0
T3-Control	8.4375	7.611137	9.263863	0
T2-T1	2.8475	2.021137	3.673863	0.0000015
T3-T1	6.585	5.758637	7.411363	0
T3-T2	3.7375	2.911137	4.563863	0.0000001
Shoot Fresh Mass (g)				
T1-Control	4.0575	3.305401	4.809599	0.00E+00
T2-Control	6.115	5.362901	6.867099	0.00E+00
T3-Control	9.165	8.412901	9.917099	0.00E+00
T2-T1	2.0575	1.305401	2.809599	1.67E-05
T3-T1	5.1075	4.355401	5.859599	0.00E+00
T3-T2	3.05	2.297901	3.802099	2.00E-07
Shoot Dry Mass (g)				
T1-Control	0.66	0.06811703	1.251883	0.0275872
T2-Control	1.52	0.92811703	2.111883	0.0000316
T3-Control	1.9325	1.34061703	2.524383	0.0000026
T2-T1	0.86	0.26811703	1.451883	0.0048077
T3-T1	1.2725	0.68061703	1.864383	0.0001773
T3-T2	0.4125	-0.17938297	1.004383	0.2177228
SLA (cm2/g)				
T1-Control	0.1425	-0.40425863	0.689259	0.8647646
T2-Control	0.3125	-0.23425863	0.859259	0.3667398
T3-Control	0.6275	0.08074137	1.174259	0.0232869
T2-T1	0.17	-0.37675863	0.716759	0.7932727
T3-T1	0.485	-0.06175863	1.031759	0.0885032
T3-T2	0.315	-0.23175863	0.861759	0.3603266
Number of Flowers				
T1-Control	4.25	-1.426932	9.926932	0.1722397
T2-Control	11.5	5.823068	17.17693	0.0003069
T3-Control	15.5	9.823068	21.17693	0.000017
T2-T1	7.25	1.573068	12.92693	0.0118789
T3-T1	11.25	5.573068	16.92693	0.0003744
T3-T2	4	-1.676932	9.676932	0.2104079
Length of inflorescence				
T1-Control	0.705	0.0724516	1.337548	0.0276656
T2-Control	1.4025	0.7699516	2.035048	0.0001326
T3-Control	2.2425	1.6099516	2.875048	0.0000011
T2-T1	0.6975	0.0649516	1.330048	0.029423
T3-T1	1.5375	0.9049516	2.170048	0.0000546
T3-T2	0.84	0.2074516	1.472548	0.0091287

produce extracellular enzymes essential for crucial soil processes such as the breakdown of organic matter, mineralization, and nutrient cycling. fungi are eco-friendly, have a crucial function in plant nutrient uptake, and bolster their capacity to adjust to environmental adversities. The utilization of fungal inoculants can be considered an eco-friendly method of improving plant fitness. The study also demonstrates that fungal mycobionts significantly enhanced growth parameters, including tuber and shoot mass, leaf area, and flower number. Statistical analysis (ANOVA and Tukey's tests) confirmed the efficacy of this fungal combination. The future implications of our study are conservation of endangered species like *D. hatagirea* and other rare or endangered plants, specific fungal associations can be leveraged to enhance growth, reproductive success, and *in-situ* or *ex-situ* conservation efforts.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

BD: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. RQ: Data curation, Methodology, Project administration, Software, Visualization, Writing – original draft. AW: Supervision, Investigation, Validation, Writing – original draft, Writing – review & editing. MYB: Supervision, Investigation, Validation, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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