学位論文要旨

The role of *Phialocephala fortinii* on promoting *Asparagus officinalis* growth under various stressed conditions

様々なストレス条件下でのアスパラガスの生育における *Phialocephala fortinii* の役割に関する研究

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Besides mycorrhizal fungi, recently dark septate endophytic (DSE) fungi attract more attention to be investigated especially regarding their ecological role either in forest or agriculture ecosystems. Several DSE fungal species have been isolated and identified but their role in the promoting agricultural crops such as *Asparagus officinalis* under various conditions is still limited. DSE are a group of fungi that generally have melanized hyphae, form dark colonies on agar media, and are able to colonize plant roots both inter- and intracellularly without causing any typical disease symptoms.

There is limited information as to whether DSE fungi are able to degrade organic carbon, nitrogen and phosphorus compounds in soil and if these fungi have a significant role in nutrient cycles in nature, especially under organic nutrient conditions. In order to understand further knowledge in this area, 25 DSE fungal isolates were obtained and tested for their promotion of *A. officinalis* seedling growth. Three isolates of *Phialocephala fortinii* were found to be most effective in increasing the growth of *A. officinalis* plants compared with uninoculated controls. These isolates had the ability to degrade all carbon and nitrogen compounds tested except

for lignin. Using organic phosphorus and nitrogen sources, three *P. fortinii* isolates were able to promote the growth of *A. officinalis* compared with control plants. These isolates were also able to promote the growth of *A. officinalis* seedlings on semi-organic and organic conditions. Our findings demonstrate that *P. fortinii* has a role in the promotion of *A. officinalis* growth under organic nutrient conditions, possibly by decomposing organic phosphorus and nitrogen compounds in soil.

The association of DSE fungi with agricultural plants such as *A. officinalis* in acidic conditions is still infrequently to be investigated. Thus, this study was conducted to confirm the promoting of selected *P. fortinii* on *A. officinalis* seedling growth under various acidic conditions using agar media in vitro and organic acidic soils. Three *P. fortinii* isolates promoted *A. officinalis* growth under acidic conditions with pH values 3, 4, and 5. Three *P. fortinii* isolates promoted *A. officinalis* growth better than control plants in acidic media using various inorganic and organic P and in agar media contained 30 µM $AlCl_3.6H_2O$. In the organic condition that used 0.1% corn steep liquor with low pH, three *P. fortinii* isolates promoted *A. officinalis* growth compared with control plants. *Phialocephala fortinii* CKG.I.11 effectively promoted the growth of *A. officinalis* seedling in organic extremely and slightly acidic soils at nursery setting.

We demonstrated that five of eight selected DSE fungal isolates have the ability to inhibit the growth of *Fusarium oxysporum* f. sp. *asparagi* under dual culture test. In this study, three of DSE fungal isolates positively degraded colloidal chitin in the agar media. We demonstrated that *A. officinalis* inoculated with DSE fungi could survive to grow healthy without any typical disease symptoms under *Fusarium* disease challenge in inorganic or organic conditions. *Phialocepahal fortinii* CKG.I.11 most effectively promoted the growth of *A. officinalis* under *Fusarium* disease either in inorganic or organic conditions.

We demonstrated that the cellulolytic index of three *P. fortinii* isolates compared with *Pleurotus eryngii* which are known as white rot fungi as higher in the CMC agar media. Three cellulolytic *P. fortinii* associated with non-mycorrhizal plants, e.g., *Brassica oleracea var. capitata* and *Spinacia oleracea*.

We succeeded obtain DSE fungal species from *A. officinalis* root by using *A. officinalis* baiting method. The results of combined ITS and LSU sequences of the four selected DSE fungal isolates showed that isolates D1.1 had 100% similarity to *Exophiala* sp., isolate D1.2 had 99% similarity to *Exophiala* sp., isolate D1.3 had 100% similarity to *Cladophialophora chaetospira* and isolate D1.4 had 98% similarity to species of *Cladophialophora*. Those DSE fungi degraded several carbon and nitrogen compounds, and improved *A. officinalis* growth under acidic and organic conditions with heavily colonization.

In conclusion, the findings suggest that DSE fungi, especially *P. fortinii* CKG.I.11 promote *A. officinalis* in various conditions such as on organic nitrogen and phosphorus, acidic and *Fusarium* disease conditions. Besides that, DSE fungi can be isolated from *A. officinalis* roots and promote *A. officinalis* under acidic and organic conditions. Therefore, the selected DSE fungi in this study have potential to be used in the organic agriculture system in the future.