

## Isolation of Endophytic Fungi Capable of Plant Growth Promotion from Monocots Inhabited in the Coastal Sand Dunes of Korea

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Endophytic fungi predominantly inhabit grasses, and produce a variety of beneficial metabolites for plant growth, as well as help their hosts against pathogens and herbivores. Current study was focused on plant growth promoting activity of endophytic fungi inhabited in the roots of sand dune grasses. We collected 49 fungal isolates from the roots of four most common sand dune grasses and screened them for their growth promoting capacity. Results showed that 37 fungal isolates (75.5%) promoted plant height and shoot length of waito-c rice, 11 fungal isolates (22.5%) suppressed it, while 1 fungus (2%) showed no effect on the growth attributes. The fungal strain *Gibberella fujikuroi*, along with distilled water and Czapek broth medium, were taken as control for this experiment. It was concluded that a major proportion of endophytic fungi inhabited in the sand dune plants produce metabolites, and thus help in growth and development of the host plant.

**Key words** : Secondary metabolites, sand-dunes, endophytic fungus, plant growth, waito-c rice

### Introduction

Throughout the evolutionary time, plants have been exposed to changing environmental conditions, forcing them to adapt or succumb to selective pressures such as extreme temperatures, insufficient water and toxic chemicals. Plants have thus evolved complex biochemical/genetic systems to perceive stresses, transmit stress-activated signals to different tissues and activate cellular responses to avoid detrimental effects [18]. Most plant studies do not consider the fact that plants in natural ecosystems have symbiotic associations with fungi. These fungi are important to the structure, function, and health of plant communities [5,17]. In fact, symbiotic fungi contribute to and may be responsible for the adaptation of plants to environmental stresses [14,15]. These fungi express a variety of symbiotic lifestyles including mutualism, commensalism, and parasitism [6].

Endophytes constitute a major portion of fungal symbionts associated with plants, reside entirely within plant

tissues, and may be associated with roots, stems and/or leaves. These fungi can act as defenders against predators [20], growth promoters [1] and competitors of microbial pathogens [19]. According to some researchers, the vegetative growth enhancement shown by many grass species in the presence of their fungal symbionts has been principally attributed to increased plant fitness [3,10]. However, recent studies have shown that plant growth promotion may be attributed to the secretion of plant growth promoting secondary metabolites (gibberellins, auxin, cytokinin) by the endophytic fungi in the rhizosphere [16].

Coastal regions of the world are of immense importance as they offer high economic returns and recreational opportunities and are therefore subjected to more anthropogenic stress. The sand dunes in coastal areas of the world in general and that of Korea in particular are on verge of destruction due to excessive loss of native species as the efficiency of conservation and re-vegetation has been slowed by intensive human activities [7,11]. The sand dune flora is always subjected to stress due to nutrient deficiency, fluctuating water levels and high salinity. Under such adverse climatic conditions, the role of symbiotic fun-

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gi in plant growth and conservation cannot be overlooked. During current study an effort was made to investigate the role of indigenous endophytic fungi in the growth promotion of the sand-dune flora and their possible role in any future conservation strategy. For this purpose 4 most common dune grasses were selected for study.

### Materials and Methods

We visited the sand dunes located at Pohang beach, a famous recreational spot in Korea, and collected root samples from 4 sand dune grasses for isolation of fungal endophytes.

Plants, fungal strains, culture medium and growth conditions

The 4 plants selected for the isolation of endophytic fungi were *Digitaria sanguinalis*, *Carex kobomugi*, *Carex pumila* and *Elymus mollis*. Screening and isolation of plant root fungi was carried out on Hagem minimal medium plates supplemented with 80 ppm Streptomycin [23]. For storage, PDA plates and slants were used, while Czapek broth medium containing 1% glucose and peptone was used for Gibberellin production [8] by incubating strain at 30°C and 120 rpm for 7 days. We used wild type *Gibberella fujikuroi* as control during the experiment.

Isolation of endophytic fungi from roots of sand dune flora

The root samples were washed with tap water to remove sand particles and other debris, treated with Tween 80 solution and surface sterilized using perchloric acid (1%) solution. The surface sterilized roots were then cut into 0.5 cm pieces in laminar hood, cultured on Hagem media plates and, incubated at 25°C until emergence of fungi from inside of root pieces [2,22]. The isolated pure cultures of root fungi were stored on PDA plates and slants.

Screening of fungal culture filtrates for plant growth promoting metabolites on rice

The culture filtrates of fungal isolates were bioassayed on waito-c rice seedlings for their plant growth promoting capacity. The fungal isolates were grown on Czapek broth medium, on a shaking incubator for 7 days at 30°C and 120 rpm. 40 ml of culture fluid was harvested through centrifugation at 5,000× g at 4°C for 15 min. The harvested pel-

let and supernatant were immediately stored at -70°C and later lyophilized. The lyophilized supernatants were mixed with 1 ml autoclaved distilled water. Seeds of waito-c rice were surface sterilized [13] and treated with 20 ppm uniconazol for 24 hr, in order to further minimize gibberellin (GA) biosynthesis. The treated seeds were washed thoroughly and soaked in autoclaved distilled water until radical emergence. The young seedlings were transplanted in glass tubes containing 0.8% water-agar medium and grown in a growth chamber. 10 µl of supernatant solution of each fungal culture filtrate was applied on apical meristem of rice seedlings at two leaves stage. The shoot and plant length of waito-c rice was observed after a week of culture filtrate application and compared with waito-c rice seedlings treated with distilled water or *G. fujikuroi*.

### Results and Discussion

Microbial extracts had been and will continue to be a productive source of biologically active compounds. Screening microbial secondary metabolites is an established method to identify novel biologically active molecules [6,9]. In current study, the presence of plant growth promoting metabolites in culture filtrates of our fungal strains were determined through a primary screening experiment on waito-c rice seedlings. We isolated 49 fungi from the roots of 4 plant species i.e. 20 fungal isolates from *Digitaria sanguinalis*, 11 fungal isolates from *Elymus mollis*, while 9 fungal isolates from each of *Carex kobomugi* and *Carex pumila*. Out of the total isolated fungal endophytes, 37 isolates promoted growth of waito-c rice, 11 isolates inhibited it, while 1 fungus produced no effect on plant growth. The growth promoting fungi constitute 75.5%, while the inhibitors constituted 22.5% of the total.

Screening bioassay of fungal isolates from *Digitaria sanguinalis*

*D. sanguinalis* is very common in disturbed areas of prairies, weedy meadows, edges of degraded wetlands, areas along roads and coastal zones. It belongs to family Poaceae, and thus is categorized as true grass. Culture filtrates of endophytic fungal isolates from roots of *D. sanguinalis* were bioassayed on waito-c rice seedlings to check their plant growth promotion activity. 20 fungi were checked, of which 16 were found as growth promoters (Fig. 1).

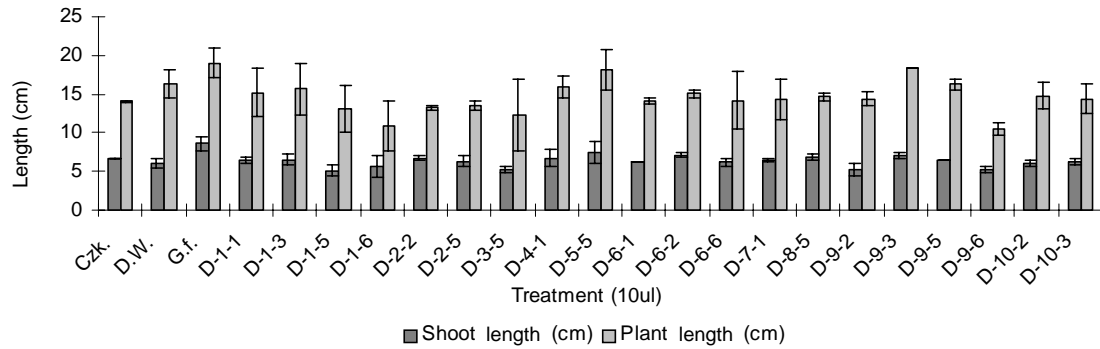


Fig. 1. Growth promotion effect on waito-c rice seedlings treated with culture filtrates of fungi isolated from roots of *Digitaria sanguinalis*.

Screening bioassay of fungal isolates from *Carex kobomugi* Ohwi

*C. kobomugi* is a common coastal dune grass, native to China, Japan and Korea. It belongs to family Cyperaceae, and thus is categorized as false grass. These plants appear grass-like, but are actually sedges, that do not follow true grass identification criteria. It is adapted to high concentrations of salt and wind along the coast and therefore thrives well in the shoreline environment ([www.hort.uconn.edu/cipwg/invader\\_month/invader\\_Carexkobomugi.pdf](http://www.hort.uconn.edu/cipwg/invader_month/invader_Carexkobomugi.pdf)). Culture filtrates of endophytic fungal isolates from roots of *C. kobomugi* were bioassayed on waito-c rice seedlings to check their plant growth promotion activity. Total 9 fungi were checked, of which 6 were found as growth promoters (Fig. 2).

Screening bioassay of fungal isolates from *Carex pumila*

*C. pumila* is native to Asia, and spread widely on sand

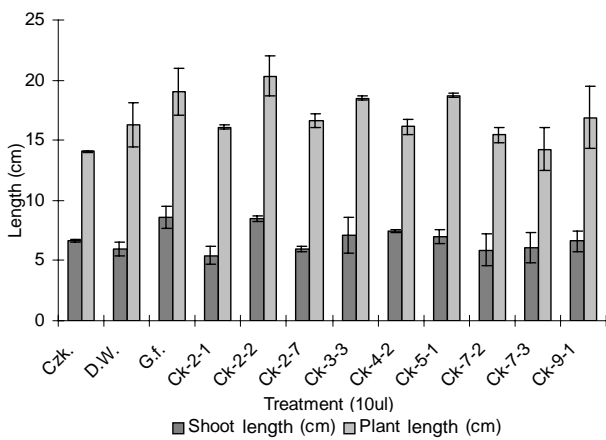


Fig. 2. Growth promotion effect on waito-c rice seedlings treated with culture filtrates of fungi isolated from roots of *Carex kobomugi* Ohwi.

dunes and coastal zones of Australia, Russia, and East Asia including China, Japan and Korea. All *Carex* spp. are sedges, and thus belong to family Cyperaceae, instead of Poaceae, and lack many characteristic features of true grass members ([www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?9108](http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?9108)). Culture filtrates of endophytic fungal isolates from roots of *C. pumila* were bioassayed on waito-c rice seedlings to check their plant growth promotion activity. 9 fungi were checked, of which 5 were found as growth promoters (Fig. 3).

Screening bioassay of fungal isolates from *Elymus mollis*

*E. mollis* is aggressive, perennial upright grass that is salt and drought tolerant, and grows well on sandy shores. It belongs to family Poaceae, and thus are characterized as true grass. It has world wide distribution, and usually out number other native plants and weeds. Most of the Korean coastal zones are covered by this grass in the form of distributed patches. Culture filtrates of endophytic fungal isolates from

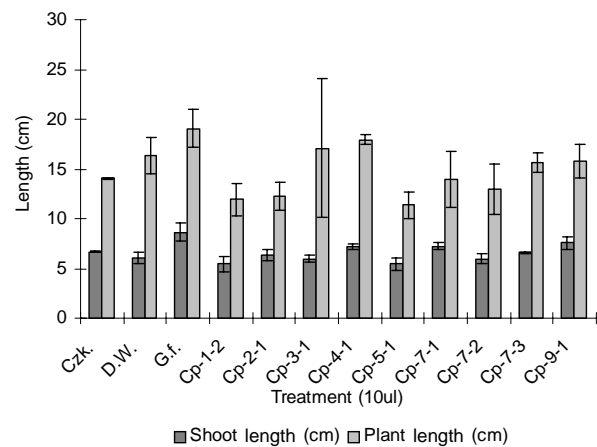


Fig. 3. Growth promotion effect on waito-c rice seedlings treated with culture filtrates of fungi isolated from roots of *Carex pumila*.

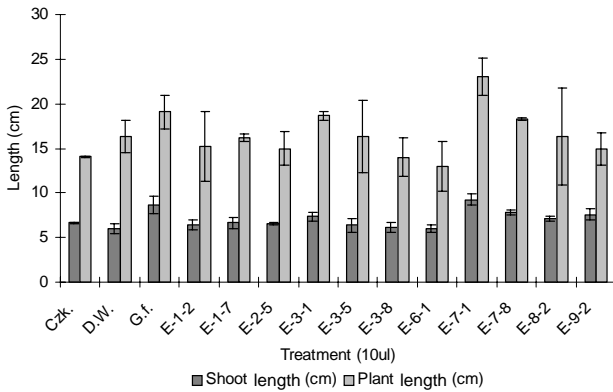


Fig. 4. Growth promotion effect on waito-c rice seedlings treated with culture filtrates of fungi isolated from roots of *Elymus mollis*.

roots of *E. mollis* were bioassayed on waito-c rice seedlings to check their plant growth promotion activity. 11 fungi were checked, of which 10 were found as growth promoters (Fig. 4).

1. Use of rice seedlings is beneficial as they can easily grow under controlled and sterilized conditions, hydroponically, using autoclaved water-agar media. Since this media is devoid of any nutrient, the sole effect of culture filtrate can easily be estimated. Waito-c rice is a known dwarf rice cultivar with reduced GA biosynthesis. Treatment of its seeds with uniconazol, a GA biosynthesis retardant, further suppresses the endogenous GAs production by blocking its biosynthesis pathway in the plant. Shoot elongation of these seedlings can thus effectively be related to activity of plant growth promoting secondary metabolites from fungal culture filtrates applied [4,16]. Similarly, it has been reported the biotechnological application of *Piriformospora indica*, a culturable mycelium possessing growth promoting effects in a vast range of plant hosts [2].

The plant growth promoting ability of fungi may be due to their capacity to produce higher amounts of growth promoting metabolites. Fungal endophytes thus facilitate their host plants to survive under stress condition by secreting favourable secondary metabolites. These fungal endophytes may provide tools for the conservation and re-vegetation of the rapidly eroding sand dune flora of coastal regions of the world. Of the total 49 fungal isolates from the sand dune grasses of Korean coastal region, a vast majority (75.5%) promoted growth of waito-c rice thus indicating the production of plant growth promoting hormones by these fungi. Discovering growth promoting capacity of such a large number of fungal endophytes have

opened new aspects of research and investigations, while identification of these growth promoting hormones and their level of production by respective fungi will contribute hugely to the existing understanding of growth regulating hormones synthesis in fungi.

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#### References

- Bacon, C. W. and J. F. White Jr. 2000. Physiological adaptations in the evolution of endophytism in the Clavicipitaceae, pp. 237-261, In Bacon, C. W. and J. F. White Jr (eds.), Microbial endophytes. Marcel Dekker, Inc., New York.
- Bayman, B., L. L. Lebron, R. L. Tremblay and D. J. Lodge. 1997. Variation in endophytic fungi from roots and leaves of *Lepanthes* (Orchidaceae). *New Phytol.* **135**, 143-149.
- Belesky, D. P. and D. P. Malinowski. 2000. Abiotic stresses and morphological plasticity and chemical adaptations of Neotyphodium-infected tall fescue plants, pp. 455-484, In Bacon, C.W. and J. F. White Jr (eds.), Microbial endophytes. New York.
- Choi, W. Y., S. O. Rim, J. H. Lee, J. M. Lee, I. J. Lee, K. J. Cho, I. K. Rhee, J. B. Kwon and J. G. Kim. 2005. Isolation of gibberellins producing fungi from the root of several *Sesamum indicum* plants. *J. Microbiol. Biotechnol.* **15**, 22-28.
- Clay, K. and J. Holah. 1999. Fungal endophyte symbiosis and plant diversity in successional fields. *Science* **285**, 1742-1744.
- Cragg, G. M., D. J. Newman and K. M. Snader. 1997. Natural products in drug discovery and development. *J. Nat. Prod.* **60**, 52-60.
- Girard, M., C. Lavoie and M. Theriault. 2002. The regeneration of a highly disturbed ecosystem: a mined peat land in southern Quebec. *Ecosystems* **5**, 274-288.
- Hasan, H. A. H. 2002. Gibberellin and auxin production plant root fungi and their biosynthesis under salinity-calcium interaction. *Rostlinna vyroba* **48**, 101-106.
- Higgs, R. E., A. Z. James, D. G. Jeffrey and D. H. Matthew. 2001. Rapid method to estimate the presence of secondary metabolites in microbial extracts. *Appl. Environ. Microbiol.* **67**, 371-376.
- Hill, N. S., J. G. Pachon and C. W. Bacon. 1996. *Acremonium coenophialum*- mediated short- and long-term drought acclimation in tall fescue. *Crop Sci.* **36**, 665-672.
- Kim, K. D. 2005. Invasive plants on disturbed Korean sand dunes. *Est. Coast. Shelf Sci.* **62**, 353-364.

12. Lewis, D. H. 1985. Symbiosis and mutualism: Crisp concepts and soggy semantics, . pp. 29-39, *In* Boucher, D. H. (ed.), *The Biology of Mutualism*, Croom Helm Ltd., London.
13. Mineo, L. 1990. Plant tissue culture techniques, *In* Tested studies in laboratory teachings *Proc ABLE*. **11**, 151-174.
14. Morton, J. B. 2000. Biodiversity and evolution in mycorrhizae in the desert. pp. 3-30, *In* Bacon, C. W. and J. F. J. White (eds.), *Microbial Endophytes*, Marcel Dekker, Inc., New York.
15. Redman, R. S., K. B. Sheehan, R. G. Stout, R. J. Rodriguez and J. M. Henson. 2002. Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis. *Science* **298**, 1581.
16. Rim, S. O., J. H. Lee, W. Y. Choi, S. K. Hwang, S. J. Suh, I. J. Lee, I. K. Rhee and J. G. Kim. 2006. *Fusarium proliferatum* KGL0401 as a new gibberellin-producing fungus. *J. Microbiol. Biotechnol.* **15**, 809-814.
17. Rodriguez, R. J. and R. S. Redman. 1997. Fungal life-styles and ecosystem dynamics: biological aspects of plant pathogens, plant endophytes and saprophytes. *Adv. Bot. Res.* **24**, 169-193.
18. Rodriguez, R. J., R. S. Redman and J. M. Henson. 2004. The Role of Fungal Symbioses in the Adaptation of Plants to High Stress Environments. *Mitigation and Adaptation Strategies for Global Change* **9**, 261-272.
19. Scannerini, S., A. Fusconi and M. Mucciarelli. 2001. The effect of endophytic fungi on host plant morphogenesis, pp. 427-447, *In* Seckbach, J. (ed.), *Cellular origin and life in extreme habitats. Symbiosis*.
20. Siegel, M. R. and L. P. Bush. 1997. Toxin production in grass/endophyte associations, pp. 185-207, *In* Carroll, G. C. and P. Tudzynski (eds.), *The Mycota*, Heidelberg, Springer-Verlag.
21. Varma, A., A. Singh, N. S. Sudha Sahay, J. Sharma, A. Roy, M. Kumari, D. Rana, S. Thakran, D. Deka, K. Bharti, T. Hurek, O. Blechert, K. H. Rexer, G. Kost, A. Hahn, W. Maier, M. Walter, D. Strack and I. Kranner. 2001. *Pyriiformospora indica*: an axenically culturable mycorrhizal-like endosymbiotic fungus, pp. 125-150, *In* Hock, B. (ed.), *The mycota IX. Fungal Assoc.* Berlin, Heidelberg, Germany.
22. Vazquez, M. M., S. Cesar, R. Azcon and J. M. Barea. 2000. Interaction between arbuscular mycorrhizal fungi and other microbial inoculants (*Azospirillum*, *Pseudomonas*, *Trichoderma*) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants. *Appl. Soil. Ecol.* **15**, 261-272.
23. Yamada, A., T. Ogura, Y. Degawa and M. Ohmasa. 2001. Isolation of *Tricholoma matsutake* and *T. bakamatsutake* cultures from field-collected ectomycorrhizas. *Mycoscience* **42**, 43-50.

#### 초록 : 사구에 서식하는 단자엽식물로부터 식물 생장 촉진 활성 내생 진균류의 분리

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내생성 진균류는 초본류의 식물체 내에 주로 서식하며, 식물의 병원균으로부터 숙주를 보호할 뿐만 아니라, 식물 생육에 유리하게 작용하는 다양한 대사 산물을 생산한다. 매우 흔히 접할 수 있는 사구식물의 뿌리로부터 49종의 균류를 분리하여 식물 생장 촉진 활성이 있는 균주를 선별하였다. 결과적으로 37균주(75.5%)는 awito-c 버의 생육을 촉진하였으며, 11균주(22.5%)는 생육을 저해하였으며, 1균주(2%)는 생육에 아무런 영향을 미치지 않았다. *Gibberella fujikuroi*와 증류수 및 Czapek broth 배지를 control 로 사용하여 실험을 수행하였다. 결론적으로 사구식물 내생균류의 많은 부분은 숙주식물의 생육과 발달을 조장하는 대사 산물을 생산함을 알 수 있었다.