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ISOLATION OF PLANT GROWTH PROMOTING ENDOPHYTIC FUNGI FROM DICOTS INHABITING COASTAL SAND DUNES OF KOREA

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Abstract

We investigated plant growth promoting activity of roots inhabiting endophytic fungi in order to evaluate their role in the survival of host plants under extreme sand dune environment of coastal regions. For this purpose, 122 fungal isolates were collected from the roots of 9 sand dune plants and were screened for growth promoting secondary metabolites. Our results showed that 101 fungal isolates (82.7%) promoted plant height and shoot length of *Waito-C* rice, while 21 fungal isolates (17.2%) inhibited growth attributes. The fungal isolate *Gibberella fujikuroi* along with distilled water and Czapek broth medium were used as control during the experiment. It was concluded that a major proportion of endophytic fungi inhabiting sand dune plants produce metabolites, which are helpful in plant growth and development.

Introduction

Exposure of plants to continually changing environmental conditions had adapted them to develop symbiotic associations with such microbes that can help in their protection and survival, 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 (Rodriguez *et al.*, 2004). 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 (Rodriguez & Redman, 1997; Clay & Holah, 1999, Hong *et al*., 2011). In fact, symbiotic fungi contribute to and may be responsible for the adaptation of plants to environmental stresses (Morton, 2000; Redman *et al.*, 2002). These fungi express a variety of symbiotic lifestyles including mutualism, commensalism, and parasitism (Lewis, 1985).

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 (Seigel & Bush, 1997), growth promoters (Bacon & White, 2000) and competitors of microbial pathogens (Scannerini *et al.,* 2001). 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 (Belesky & Malinowski, 2000; Hill *et al*., 1996). 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 (Hamayun *et al*., 2010[a]).

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 (Girard *et al.,* 2002; Kim, 2005). 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 fungi 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.

Materials and Methods

We visited the sand dunes located at Pohang beach, a famous recreational spot in Korea. We collected root samples from 9 sand dune plants and analyzed them for fungal endophytes.

Plants, fungal strains, culture medium and growth conditions: The thirteen plants selected for isolation of endophytic fungi were *Vitex rotundifolia, Calystegia soldanella, Lathyrus littoralis, Polygonum convolvulus, Oxalis corniculata, Lathyrus japonica, Ixeris repenes, Glehnia littoralis and Salsola komarovi.* Screening and isolation of plant root fungi was carried out on Hagem minimal medium plates supplemented with 80 ppm Streptomycine (Yamada *et al.,* 2001). For storage, PDA plates and slants were used, while Czapek broth medium containing 1% glucose and peptone was used for Gibberellin production (Hasan, 2002) by incubating strain at 30ºC 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 25ºC until emergence of fungi from inside of root pieces (Bayman *et al*., 1997; Vazquez *et al*., 2000). 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 30ºC and 120rpm. 40ml of culture fluid was harvested through centrifugation at 5000*xg* at 4ºC for 15 min. The harvested pellet and supernatant were immediately stored at -70ºC and later lyophilized. The lyophilized supernatants were mixed with 1 ml autoclaved distilled water. Seeds of *Waito-C* rice were surface sterilized (Mineo, 1990) and treated with 20 ppm uniconazol for 24 h, 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. 10µl 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 distilled water or *G. fujikuroi* treated *Waito-C* rice seedlings.

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 (Cragg *et al*., 1997; Higgs *et al*., 2001). 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 122 fungal isolates from 9 plant species. Maximum of 28 fungi were isolated from *Vitex rotundifolia*, while the least i.e., 7 fungi were isolated from *Polygonum convolvulus*. Out of the total isolated fungi, 101 isolates promoted growth of *Waito-C* rice, while 21 isolates inhibited it. The growth promoting fungi constitute 82.7%, while the inhibitors constituted 17.2% of the total.

Screening bioassay of fungal isolates from *Vitex rotundifolia*: *V. rotundifolia* is native to Asia. They are also termed as beach vitex, as they are found frequently in sand-dunes of coastal regions and belong to family Lamiaceae. They are good sand binders due to their extensive root system, and thus serve as plants of choice for re-vegetation of eroded beaches. They are resistant to salt spray and drought conditions of coastal zones (Fujiki *et al.*, 2001). Bioassay was carried out on Waito-c rice seedlings to check growth promotion activity of fungal culture filtrates. 28 fungi were checked, of which 21 were found as growth promoters (Fig. 1).

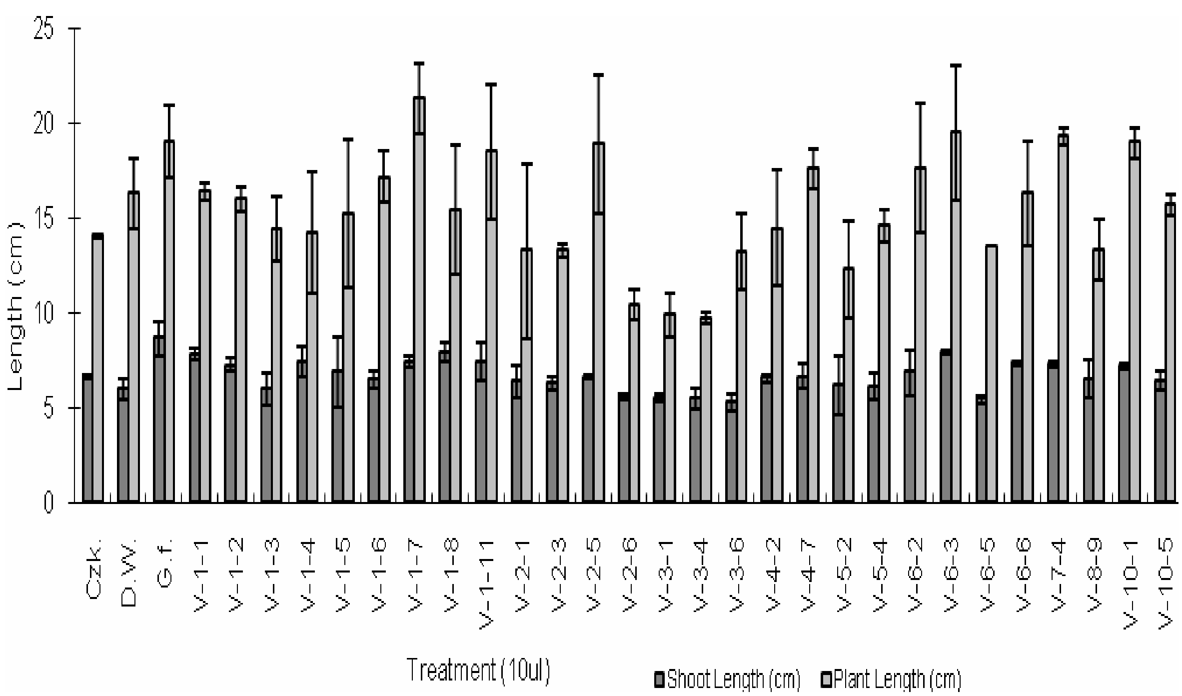

Fig. 1. Growth promotion effect on *Waito-C* rice seedlings treated with culture filtrates of fungi isolated from roots of *Vitex rotundifolia.* Czk = Czapek broth medium; DW = Distilled water; G.f = *G. fujikuroi*.

Screening bioassay of fungal isolates from *Calystegia soldanella*: *C. soldanella* is a perennial vine of family Convolvulaceae, which grows in beach sand and other coastal habitats. It is commonly termed as seashore false bindweed. They are common inhabitants of North America, Europe and Asian coastal sand dunes (Asuka *et al*., 2008). Bioassay was carried out on *Waito-C* rice seedlings to check growth promotion activity of fungal culture filtrates. 19 fungi were checked, of which 14 were found as growth promoters (Fig. 2).

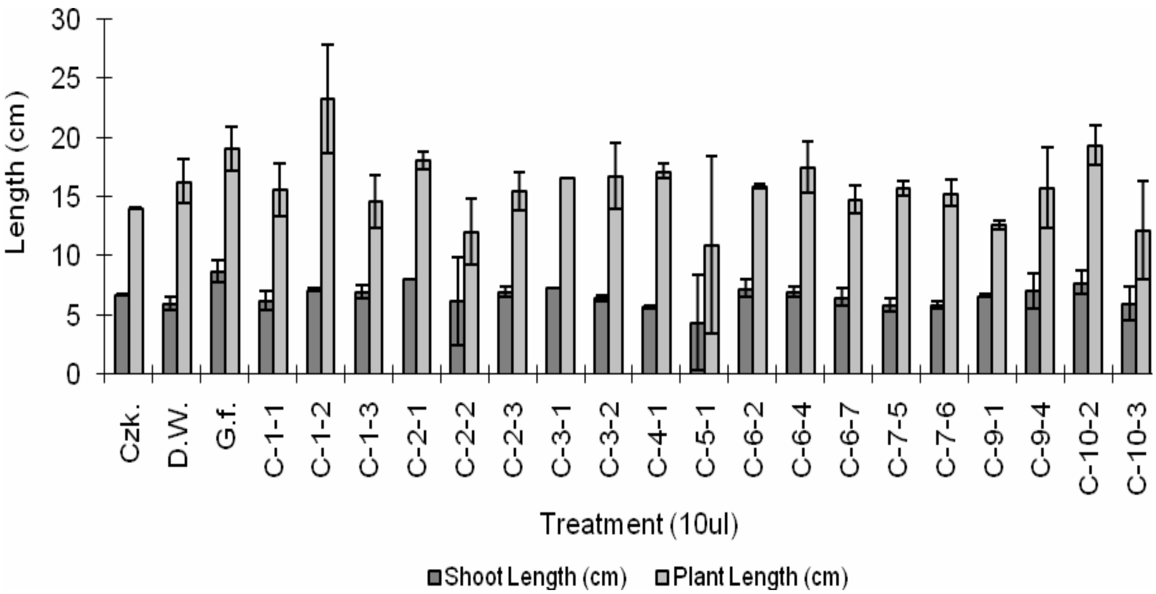

Fig. 2. Growth promotion effect on *Waito-C* rice seedlings treated with culture filtrates of fungi isolated from roots of *Calystegia soldanella.* Czk = Czapek broth medium; DW = Distilled water; G.f = *G. fujikuroi.*

Screening bioassay of fungal isolates from *Lathyrus littoralis*: *L. littoralis* is a species of wild pea, and is known by common name of silky beach pea. This is a perennial herb of family Fabaceae, which grow in patches of hairy grey-green stems along the sandy ground or slightly upright. It is a resident of beaches and dunes, spread mostly in western American coast line, with less frequent distribution in Asian dunes (Gegory *et al*., 2005). Bioassay was carried out on *Waito-C* rice seedlings to check growth promotion activity of fungal culture filtrates. 9 fungi were checked, of which 8 were found as growth promoters (Fig. 3).

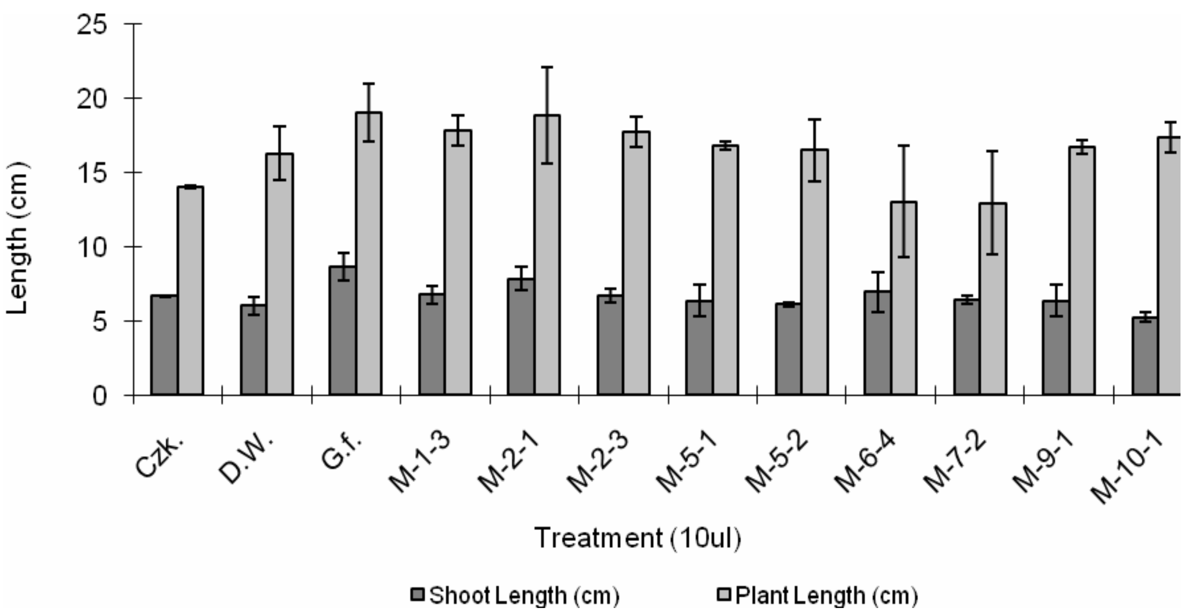

Fig. 3. Growth promotion effect on *Waito-C* rice seedlings treated with culture filtrates of fungi isolated from roots of *Lathyrus littoralis.* Czk = Czapek broth medium; DW = distilled water; G.f = *G. fujikuroi*

Screening bioassay of fungal isolates from *Polygonum convolvulus*: *P. convolvulus* is a perennial belonging to family Polygonaceae. It is regarded as weed among crop fields, and can exist in variety of environmental conditions rangiong from moist to dry environments. Most species of Polygonum genus have worldwide distribution, *P. convolvulus* being mainly found in North America and East Asian countries (Clinton, 1992). Bioassay was carried out on *Waito-C* rice seedlings to check growth promotion activity of fungal culture filtrates. 7 fungi were checked, of which 6 were found as growth promoters (Fig. 4).

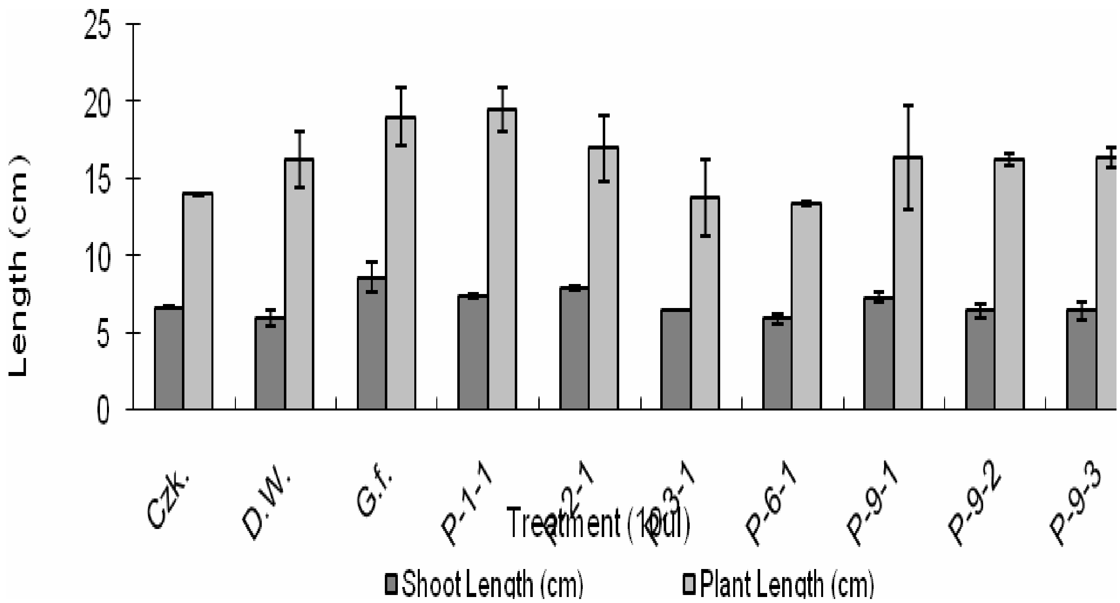

Fig. 4. Growth promotion effect on *Waito-C* rice seedlings treated with culture filtrates of fungi isolated from roots of *Polygonum convolvulus.* Czk = Czapek broth medium; DW = Distilled water; G.f = *G. fujikuroi*

Screening bioassay of fungal isolates from *Oxalis corniculata*: *O. corniculata*, also termed as wood sorrel, has a worldwide distribution. This creeper belongs to family Oxalidaceae. It usually prefers moist soils, but had been found inhabiting dry lands, or coastal zones frequently in East Asian countries (Kim, 2005). Bioassay was carried out on *Waito-C* rice seedlings to check growth promotion activity of fungal culture filtrates. 12 fungi were checked, of which 11 were found as growth promoters (Fig. 5).

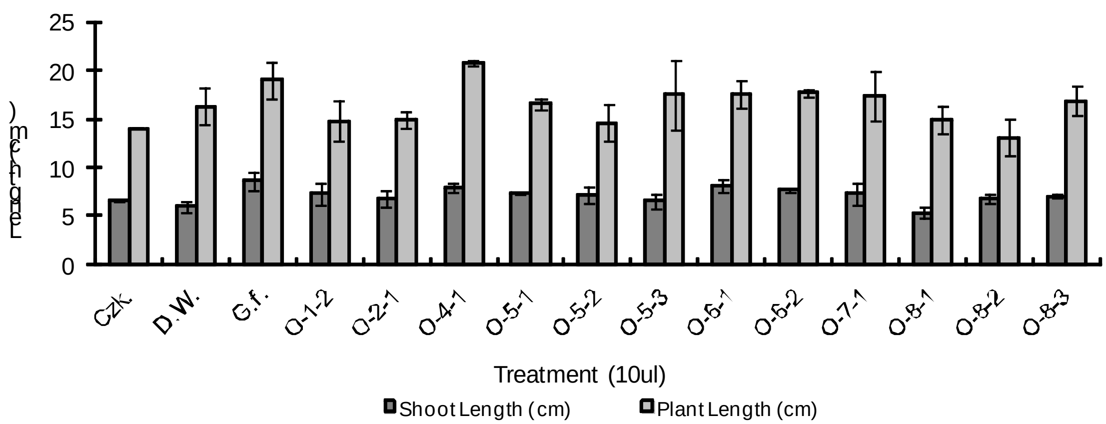

Fig. 5. Growth promotion effect on *Waito-C* rice seedlings treated with culture filtrates of fungi isolated from roots of *Oxalis corniculata.* Czk = Czapek broth medium; DW = distilled water; G.f = *G. fujikuroi.*

Screening bioassay of fungal isolates from *Lathyrus japonica*: *L. japonica* is a perrinial herbaceous plant belonging to family Fabaceae. This leguminous plant is commonly termed as sea pea or beach pea and is native to temperate coastal areas of Asia, Europe and North and South America (Gregory *et al*., 2005). Bioassay was carried out on *Waito-C* rice seedlings to check growth promotion activity of fungal culture filtrates. 11 fungi were checked, of which all were growth promoters (Fig. 6).

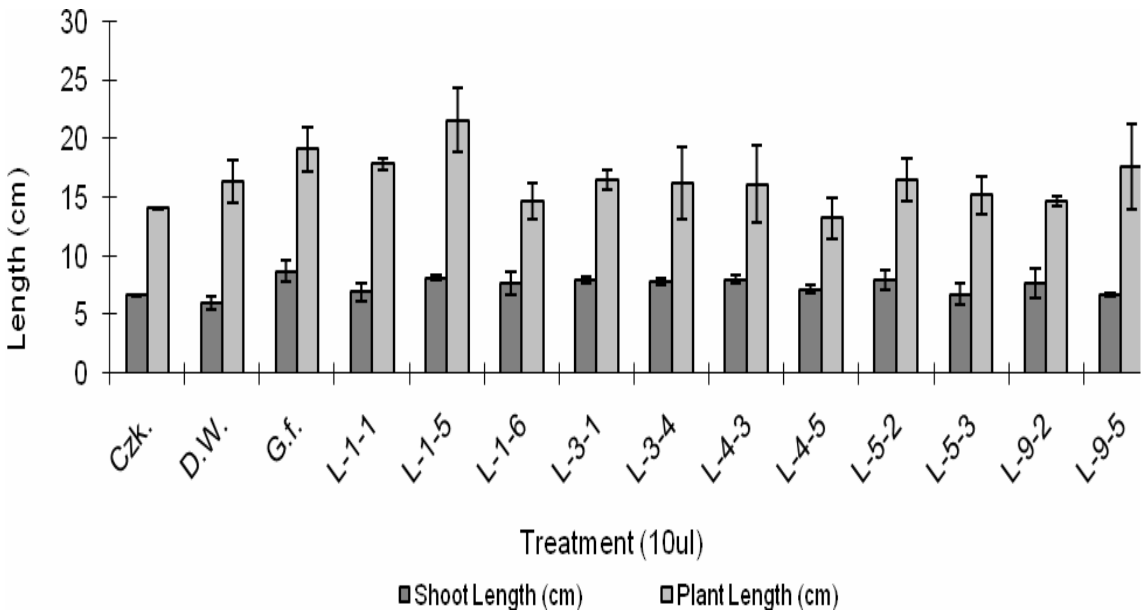

Fig. 6. Growth promotion effect on Waito-C rice seedlings treated with culture filtrates of fungi isolated from roots of *Lathyrus japonica.* Czk = Czapek broth medium; DW = distilled water; G.f = *G. fujikuroi.*

Screening bioassay of fungal isolates from *Ixeris repens*: *I. repens* is native to China, Japan and Korea, and is found inhabiting coastal zones. This perennial plant belongs to family Asteraceae (Kim, 2005). Bioassay was carried out on *Waito-C* rice seedlings to check growth promotion activity of fungal culture filtrates. 15 fungi were checked, of which all were growth promoters (Fig. 7).

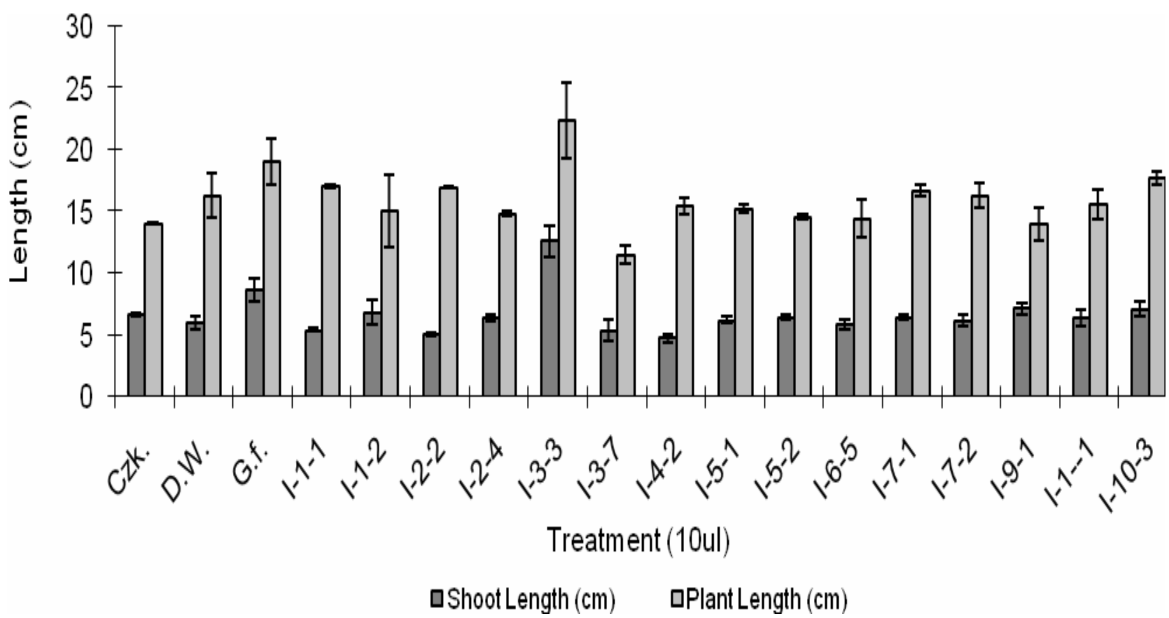

Fig. 7. Growth promotion effect on *Waito-C* rice seedlings treated with culture filtrates of fungi isolated from roots of *Ixeris repens.* Czk = Czapek broth medium; DW = distilled water; G.f = *G. fujikuroi*

Screening bioassay of fungal isolates from *Glehnia littoralis*: *G. littoralis* is a member of family Apiaceae, and is commonly named as beach silvertop. It is native to eastern Asia, particularly China, Japan, Korea and far-east Russia, as well as western North America, from Alaska to north California. The plant has medicinal value for curing cough (Kim, 2005). Bioassay was carried out on *Waito-C* rice seedlings to check growth promotion activity of fungal culture filtrates. 10 fungi were checked, of which all were growth promoters (Fig. 8).

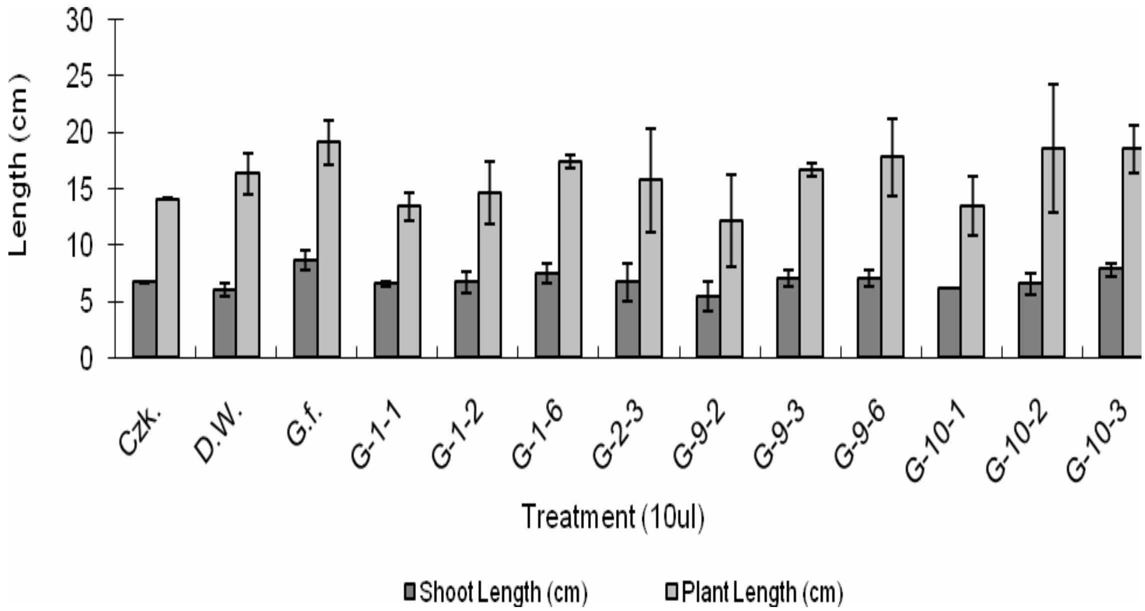

Fig. 8. Growth promotion effect on *Waito-C* rice seedlings treated with culture filtrates of fungi isolated from roots of *Glehnia littoralis.* Czk = Czapek broth medium; DW = distilled water; G.f = *G. fujikuroi*.

Screening bioassay of fungal isolates from *Salsola komarovi*: *S. komarovi* commonly called as saltwort, is herbaceous plant of family Chenopodiaceae. These are native to Africa, Asia and Europe, and typically grow on flat, often dry, saline soils (Tkeno *et al*., 1995). Bioassay was carried out on *Waito-C* rice seedlings to check growth promotion activity of fungal culture filtrates. 11 fungi were checked, of which all were growth promoters (Fig. 9).

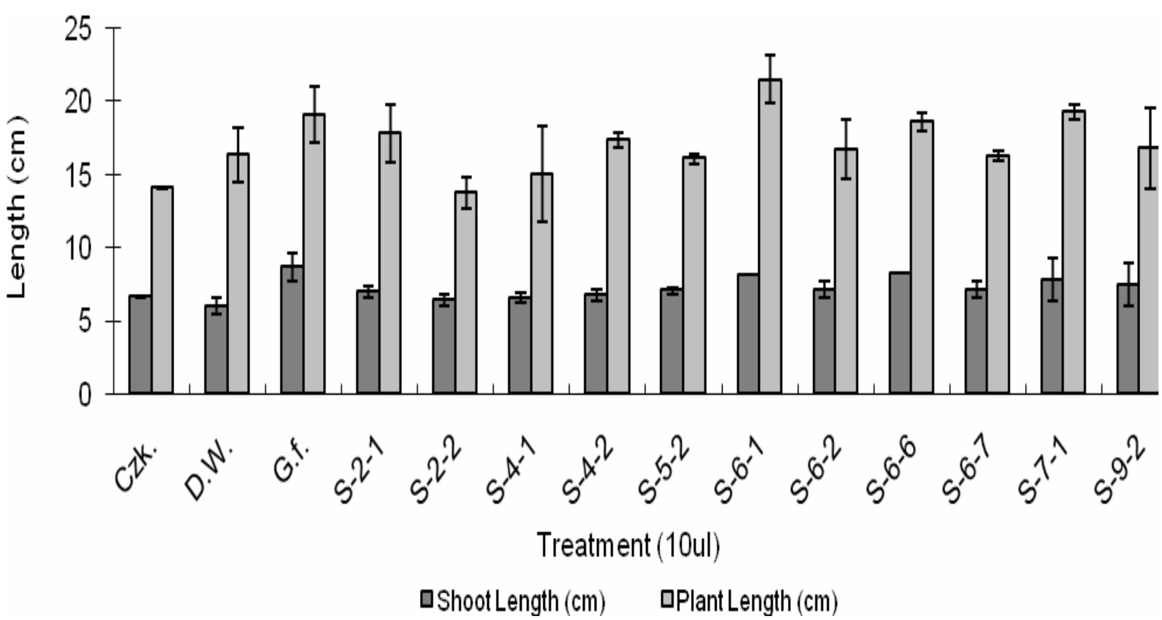

Fig. 9. Growth promotion effect on *Waito-C* rice seedlings treated with culture filtrates of fungi isolated from roots of *Salsola komarovi*. Czk = Czapek broth medium; DW = distilled water; G.f = *G. fujikuroi*.

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 (Hamayun *et al*., 2010[b], 2011; Khan *et al*., 2011[a,b]). 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 (Verma *et al*., 2001).

Conclusion

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 favorable 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 171 fungal isolates from the sand dune flora of Korean coastal region, a vast majority (80.7%) 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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