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Research Paper

A Study of Plant Growth Promoting Diversity of Fungi

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***Abstract:** Plant growth-promoting fungi (PGPF) have attracted considerable interest as bio-fertilisers due to their multiple beneficial effects on plant quantity and quality and their positive relationship with the ecological environment. Progress has been made towards PGPF production for crops and economic crops, but such changes for PGPF use with common medicinal herbs, such as Salvia miltiorrhiza, are uncommon. Waito-C rice (WR) seedling for plant growth-promoting verification was handled with Fungal culture filtrates (FCF). The Sj-2-2 fungal strain has been reported to promote plant growth (PGP) in WR. Then, Sj-2-2 culture filtrate treatment confirmed PGP from Suaeda japonica. Consequently, it has been reported that Sj-2-2 culture filtrate is better than positive control in S. Japanese. HPLC analysis and GC-MS SIM analysis identified the secondary metabolites involved in cultivation filtrate of Sj-2-2. It was detected that the Sj-2-2 culture was filtered with the presence of physiologically bioactive gibberellins (GAs) and other inactive GA. The similitude to penicillussp was shown in the molecular sequence study of Sja-2-2 of homology of 99 percent. The PGP of Sj-2-2 was reported, along with the symbiosis among endophytic fungi and the halophytes naturally cultivated in salt marsh. Sj-2-2 has been identified by molecular sequence analysis as a new fungal strain generating GAs. Therefore, Penicilliumsp was referred to as the Sj-2-2 fungal strain. D-2-2. D-2. The study confirmed the diversity and the PGP of the new gibberellin-producing fungal strain isolated from the roots of halophytes in marsh salt.*

***Keywords:** Endophytic fungi, fungal diversity, plant growth promotion, gibberellin, halophyte, salt marsh.*

I. INTRODUCTION

The marshes are populated by diverse biospecies and functions as ecotones among terrestrial and water ecosystems in environmental purity, surface water sources, flow control and underground storage and recharge. Salt marshes harbour, as well as some hydrophytes, a high diversity of microorganisms. The cluster study of soil isolates was used to test for microbial abundance and diversity. However in salt marshes there has been no relevant studies with endophytic fungi connected to wild halophytes. Endophytic fungi live with plants in symbiosis and play a major role in plant growth. Fungi contain a wide range of herbal hormone products, including gibberellin (GAs), abscisic acid (ABA), and auxine (IAA). Research on the use of these hormones in biological regulation has evolved. The phytohormone GA, a diterpenoid complex, regulates plant growth and encourages flora, stem stretching, germination and maturation. The bakana-producing Gibberellafujikurui was found in the 1920s and was in 1935 isolated from its triggered portion. GA is rendered with G. G. Phaeosphaeriasp, fujikuroi. It is formed by bacteria, plants and fungi, amongst other things: L487, Neurosporacrassa, Pseudomonas sp., Pacelomamanihoticola, Aspergillus sp.) and Penicillium sp. About 136 kinds of GA have been found to be secondary metabolites; PGP activity is associated with GA1, GA3, GA4 and GA7. Various plant growth experiments in conjunction with GAs have been performed.

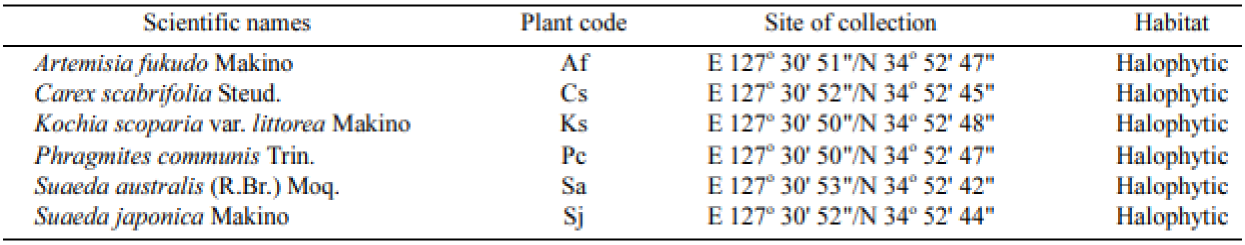
Since the metabolites of S are very much interested. As the raw material for pharmaceuticals, Miltiorrhiza roots studies have increasingly concentrated on ways of enforcing the production of certain active metabolites, such as the creation of new strains that can produce active compounds[7] and the use of biotic and abiotics elicitors in cell or S-root crops that can stimulate the accumulation of active metabolites. [4,8 – 10]. [4,8–12]. But these solutions have not been fully developed and used in large-scale processing and field-cultivated S extraction. The primary source of bioactive compounds is still roots of miltiorrhiza. So then, S. The cultivation of miltiorrhiza urgently needs new methods for improving measures of field management to increase the accumulation of this plant growth and secondary metabolite. Plants benefit significantly from symbiotic systems based on plant/microbes and some plant-wide growth-promoting endophytes are being used as potential bio-fertilizers for crop grows[11] such as Pseudomonas stutzeri A15 inoculated rice seedlings[12] and inoculated with Paénibacilluspolymyxa CR1[13]. Plant breeding plants are being used as potential bio-fertilizers. Plant growth-promoting fungi (PGPF) to increase yields, and contribute to the accumulation of secondary metabolites are ideal for herbal medicinal plants. Asian production in tissue culture seedlings of Centellaasiatica has been enhanced with Live piriformosporaindica [14] while Mycenasp lives. F-23 was used in pot cultures to stimulate the Anoectochilusformosanus content of kinsenoside and flavonoids[15]. The application of PGPF as a biofertilizer is thus promising and has advantages including improving product quantity, consistency and reducing emissions due to chemical fertiliser in the agriculture environment. The successful components of the S was found by Wang[16]. The roots of miltiorrhiza are connected to the variety and the amount of endophytic fungi used by the plant. Therefore, microorganisms originating from S are endophytic or rhizosphere. Miltiorrhizes cultivated in native manufacturing areas can be excellent plant growth-fostering S microbes. Cultivation of miltiorrhizes. In most previous studies, however, growth of hairy-root cultures was fostered[8,17]. PGPF implementation study in S. Research. The cultivation of miltiorrhiza is very small. Diversity study of S-derived endophytic fungi. Miltiorrhiza grown in the native manufacturing areas[18] demonstrated the dominant fungus of Alternaria in line with Yan's findings[19]. Many strains of the Alternaria genus also develop the same secondary metabolite as their host plant, for example, paclitaxel in Alternaria, alternate TPF6, podophyllotoxin and analogues in AlternarianeesexTy[21], Alternariasp, vinblastine. In AlternariaTenuissima Y2-3, 97CG1 and flavonoids. The endophytic fungi of the Alternaria genus are therefore screened isolated from S. A reliable and efficient way to identify PGPFs that encourage the development of the host medicinal plants and the secondary build-up of metabolites, Miltiorrhiza cultivated in native production areas.

II. MATERIALS AND METHODS

Plant Materials

Healthy roots of halophytes were collected from a salt marsh of Suncheon Bay in South Korea. The local sites and scientific names of six halophytes are listed in Table 1.

Table 1. Details of sampling sites and halophytes species collected in this study



Scientific names	Plant code	Site of collection	Habitat
Artemisia fukudo Makino	Af	E 127° 30' 51"/N 34° 52' 47"	Halophytic
Carex scabrifolia Steud.	Cs	E 127° 30' 52"/N 34° 52' 45"	Halophytic
Kochia scoparia var. *littorea* Makino	Ks	E 127° 30' 50"/N 34° 52' 48"	Halophytic
Phragmites communis Trin.	Pc	E 127° 30' 50"/N 34° 52' 47"	Halophytic
Suaeda australis (R.Br.) Moq.	Sa	E 127° 30' 53"/N 34° 52' 42"	Halophytic
Suaeda japonica Makino	Sj	E 127° 30' 52"/N 34° 52' 44"	Halophytic

Preparation and Growth Conditions of the Sample

Halophytes were treated for root samples with a 10 min Tween 80 solution, and a 1% (w/v) perchloric acid solution was added to the surface through bleaching. The sterilised roots were cut into pieces of 3 cm (25^{o}C) and incubated until the development, during culture, of fungi from the rootstock in hagem minimal streptomycin-containing medium. PDA has been subcropted for fungal strains isolated from the root sections. For development of secondary metabolites, Czapek Broth Medium (CBM) containing 1% (w/v) glucose and peptone was used[7]. Power G wild-type. The Fujikuroi established as positive control in this study by the Korean Cultural Center of Microorganisms (KCCM).

DNA Extraction, PCR Amplification, and Identification of Fungal Strains

The subculture of fungal strains was incubated in PDA for 7-10 days. The mycelia of fungal strains were incubated into an Erlenmeyer flask containing 50 ml of potato dextrose broth medium in a shaking incubator for 7 days at 120 rpm at 28o C. The lyophilized fungal strains were obtained by vacuum filtration. The fungal genomic DNA was extracted with a DNeasy plant mini kit (QIAGEN, Valencia, CA, USA). The fungal strains were identified by sequencing the ITS region with universal primers ITS-1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS-4 (5'-TCC TCC GCT TAT TGA TAT GC-3'). Fifty microliters of a total PCR mixture was used. The mixture contained 5 µl (v/v) of dNTPs and Ex-Taq buffer, 4 µl (v/v) of each primer, 1 µl (v/v) of DNA sample, 0.25 µl (v/v) of Ex-Taq polymerase, and 30.75 µl (v/v) of autoclaved deionized distilledwater. The reaction cycles consisted of 2 min of initial denaturation (95o C), 35 cycles by 2 min of denaturation (95o C), 1 min of annealing (55o C), 1 min of extension (72o C), and 7 min of final extension (72o C). The 5 µl (v/v) PCR products were observed by agarose gel electrophoresis with ethidium bromide (EtBr) staining. The products were purified and sequenced with a QIAquick PCR purification kit (QIAGEN), ABI PRISM BigDye Terminator Cycle Sequencing Kit (PE Biosystems, Foster, CA, USA), and ABI 310 DNA sequencer (Perkin Elmer).

Phylogenetic Analysis of Fungal Strains

The BLAST software is checked for the rDNA-ITS regions' nuclear sequence. ClustalW was matched to the MEGA 4.0 software programme for the obtained sequences. To align the obtained sequences and generate the neighborjoining (NJ) tree, ClustalW was used. Bootstrap replication (1,000 replications) provided statistical support for the nodes in the phylogenetic tree.

Data Analysis with Statistical Treatment

The diversity of endophytic fungi isolated from six halophytes was calculated by the Shannon diversity index (H') following to the formula

$$H' = -\sum_{i=1}^{k} P_i \ln P_i$$

where k is the total number of fungal species encountered and Pi is the proportion of all individuals in a sample that species i contributes to the total. The data of all graphs were analyzed statistically for standard deviation using Microsoft Excel. Statistical analysis of PGP was conducted using one-way analysis of variance (ANOVA). A Duncan’s multiple range test (DMRT) was conducted to analyze significant differences ($P < 0.05$) among the different groups (using SPSS 18.0 for Windows; SPSS, Chicago, IL, USA).

Screening of FCFs for Plant Growth Promotion on WR Seedling

The fungal strains in 25oC and 180 rpm in CBM were incubated for 7 days. The mycelia and FCFs were processed and lyophilized at -70 degrees C. The FCFs have been lyophilized with 1 ml of distilled, autoclaved water. The sterilised WR seeds were treated with 20 ppm uniconazol at notice in order to reduce activity in the GA seed coat (Sumitomo Heavy Chemical Co., Takarazuka, Japan). The WR seeds were washed and immersed into the sprout sterile distilled water. WR plants were transplanted into 0.6% water-agar tubes and cultivated in the growth cell. In the apical meristem of WR seedlings at the two-leaf level, ten microliters of a concentrated FCF solution were added. A week after the seedlings were handled with G distilled water, CBM, and culture filtrate, the shoot length (SL) and plant length (PL) of WR sowings have been observed. Used fodder Controls to cause SL and PL of seedlings were viewed as negative and positive controls.

Bioassay on S. japonica Seedling

The seeds of S. japonica were collected from Suncheon Bay in South Korea. The surface-sterilized S. japonica seeds were soaked in distilled water for germination and then treated with uniconazol. S. japonica seedlings were cultivated in a growth chamber at 25o C. The two-leaves stage of S. japonica seedlings were treated with lyophilized FCF (10 µl) of fungal strain Sj-2-2. The SL and PL were measured after 14 days and compared with control treatments.

Extraction and Quantification of GAs from FCFs

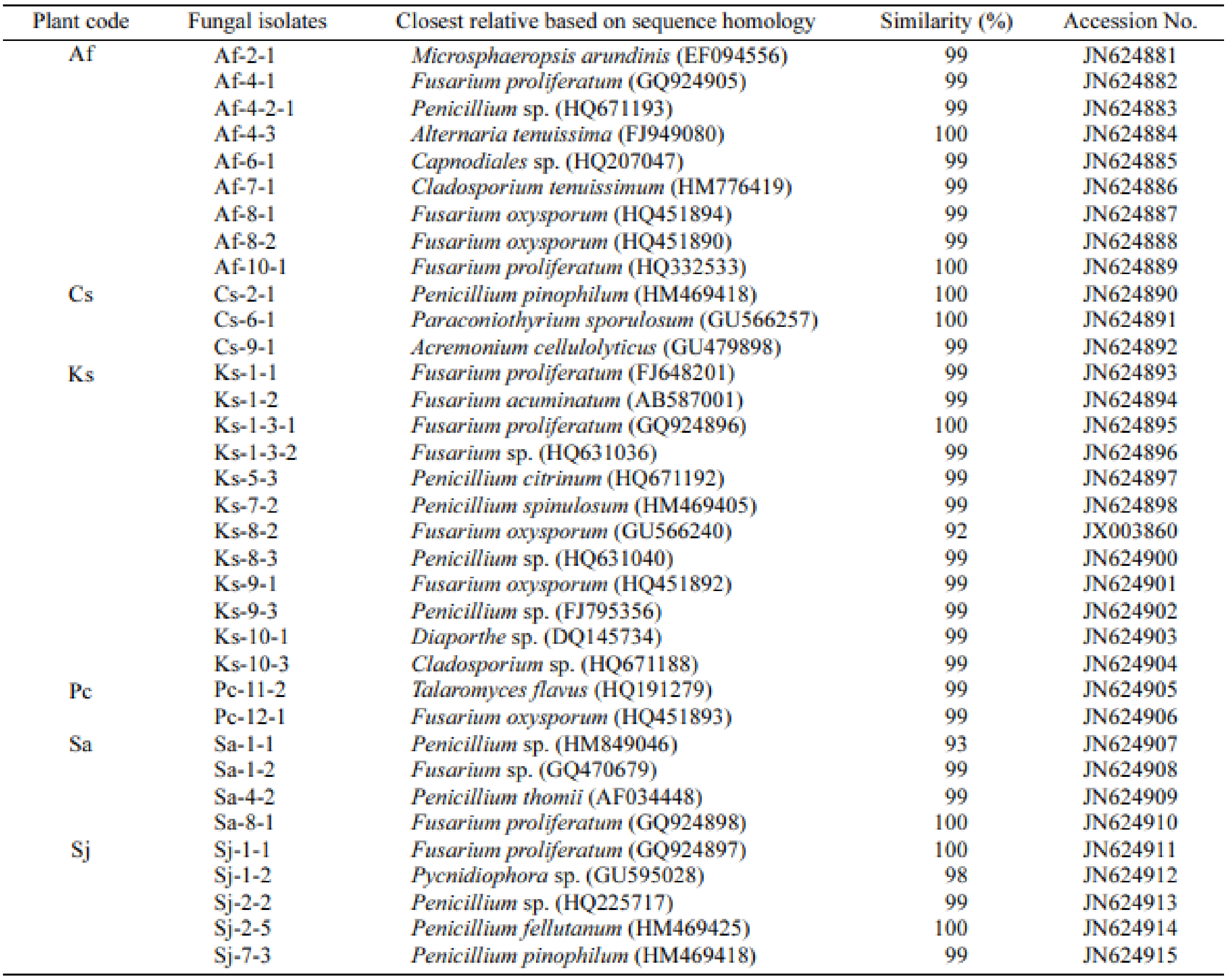
GA was removed from FCF by an existing protocol for 7 days after incubation[18]. Reverse Phase C18 HPLC was used to analyse the GAs collected and prepare the fractions to a GC-MS with a selected GC system (GC network 6890N and selective network mass detector 5973, Agilent Technologies, Palo Alto, CA, USA). GC-MS information has been obtained and analysed. The three main ions of GA internal standards augmented [2 H2] were simultaneously controlled by Professor Lewis N. Mander, Australian National University, Canberra, Australia and GAs. In the measurement of retained periods, hydrocarbon standards were used to measure the Kovats Retention index (KRI) value and GA peak area ratios were used to quantify GAs [3, 8, 15, 16].

III. RESULTS

Identification and Phylogenetic Analysis of Endophytic Fungi

The nucleotide sequences of endophytic fungi were registered in the GenBank database of the National Center forBiotechnology Information (Accession numbers JN624881- JN624898, JX003860, JN624900-JN624915) (Table 2). Thirtyfive endophytic fungi isolated from the halophytes were confirmed to belong to Ascomycota. It was confirmed that Penicillium sp. and Fusarium sp. were the main endophytic fungi; then phylogenetic analysis was carried out (Fig. 1).

Table 2. Identification of endophytic fungi isolated in this study



Plant code	Fungal isolates	Closest relative based on sequence homology	Similarity (%)	Accession No.
Af	Af-2-1	*Microsphaeropsis arundinis* (EF094556)	99	JN624881
	Af-4-1	*Fusarium proliferatum* (GQ924905)	99	JN624882
	Af-4-2-1	*Penicillium* sp. (HQ671193)	99	JN624883
	Af-4-3	*Alternaria tenuissima* (FJ949080)	100	JN624884
	Af-6-1	*Capnodiales* sp. (HQ207047)	99	JN624885
	Af-7-1	*Cladosporium tenuissimum* (HM776419)	99	JN624886
	Af-8-1	*Fusarium oxysporum* (HQ451894)	99	JN624887
	Af-8-2	*Fusarium oxysporum* (HQ451890)	99	JN624888
	Af-10-1	*Fusarium proliferatum* (HQ332533)	100	JN624889
Cs	Cs-2-1	*Penicillium pinophilum* (HM469418)	100	JN624890
	Cs-6-1	*Paraconiothyrium sporulosum* (GU566257)	100	JN624891
	Cs-9-1	*Acremonium cellulolyticus* (GU479898)	99	JN624892
Ks	Ks-1-1	*Fusarium proliferatum* (FJ648201)	99	JN624893
	Ks-1-2	*Fusarium acuminatum* (AB587001)	99	JN624894
	Ks-1-3-1	*Fusarium proliferatum* (GQ924896)	100	JN624895
	Ks-1-3-2	*Fusarium* sp. (HQ631036)	99	JN624896
	Ks-5-3	*Penicillium citrinum* (HQ671192)	99	JN624897
	Ks-7-2	*Penicillium spinulosum* (HM469405)	99	JN624898
	Ks-8-2	*Fusarium oxysporum* (GU566240)	92	JX003860
	Ks-8-3	*Penicillium* sp. (HQ631040)	99	JN624900
	Ks-9-1	*Fusarium oxysporum* (HQ451892)	99	JN624901
	Ks-9-3	*Penicillium* sp. (FJ795356)	99	JN624902
	Ks-10-1	*Diaporthe* sp. (DQ145734)	99	JN624903
	Ks-10-3	*Cladosporium* sp. (HQ671188)	99	JN624904
Pc	Pc-11-2	*Talaromyces flavus* (HQ191279)	99	JN624905
	Pc-12-1	*Fusarium oxysporum* (HQ451893)	99	JN624906
Sa	Sa-1-1	*Penicillium* sp. (HM849046)	93	JN624907
	Sa-1-2	*Fusarium* sp. (GQ470679)	99	JN624908
	Sa-4-2	*Penicillium thomii* (AF034448)	99	JN624909
	Sa-8-1	*Fusarium proliferatum* (GQ924898)	100	JN624910
Sj	Sj-1-1	*Fusarium proliferatum* (GQ924897)	100	JN624911
	Sj-1-2	*Pycnidiophora* sp. (GU595028)	98	JN624912
	Sj-2-2	*Penicillium* sp. (HQ225717)	99	JN624913
	Sj-2-5	*Penicillium fellutanum* (HM469425)	100	JN624914
	Sj-7-3	*Penicillium pinophilum* (HM469418)	99	JN624915

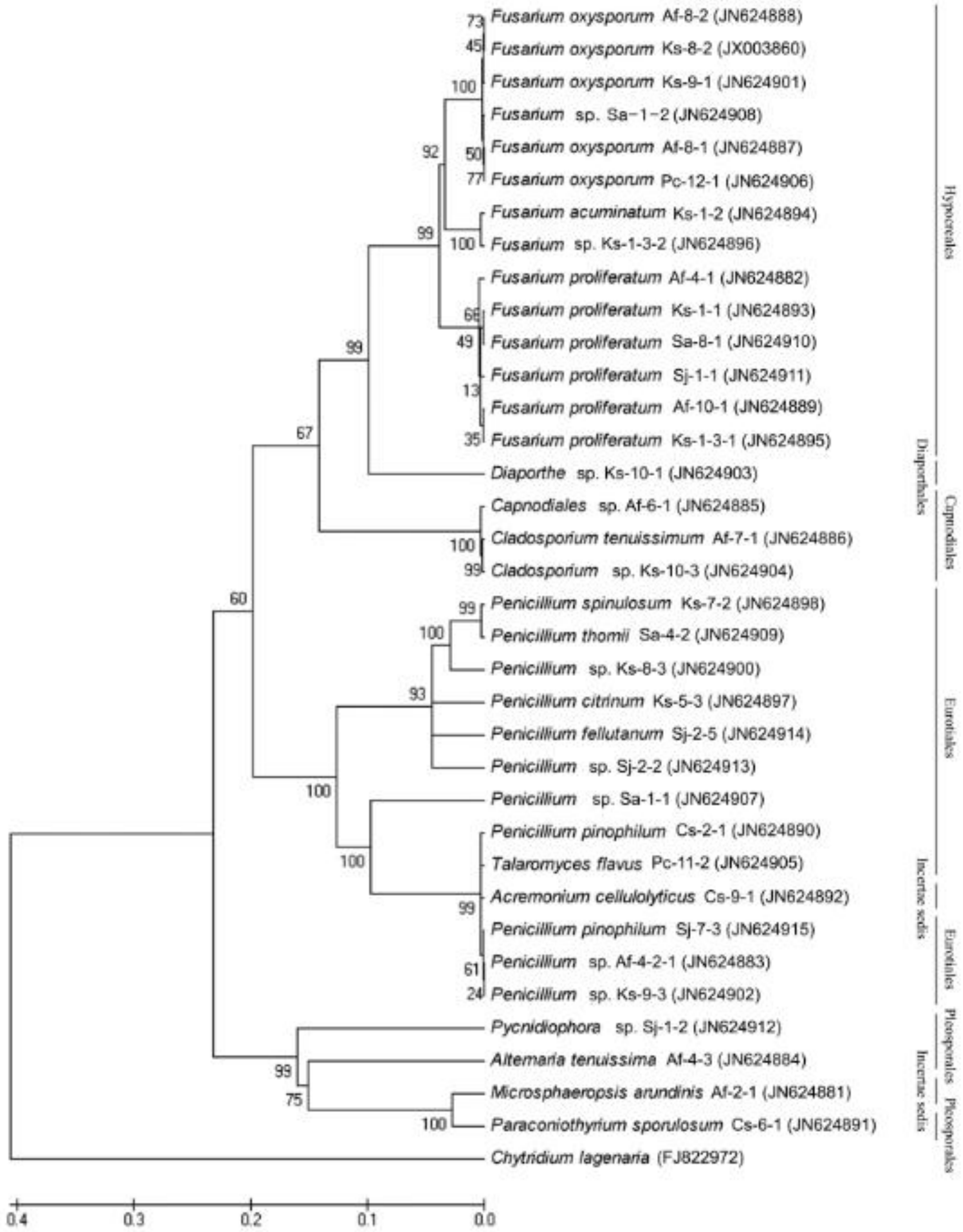


Fig. 1. Phylogenetic analysis of endophytic fungi using rDNA-ITS sequences

Fungal Diversity of Endophytic Fungi Isolated from Halophytes

Thirty-five culturable fungal strains were isolated from roots of six halophytes based on colony morphologies. All endophytic fungi from halophytes belonged to 11 genera based on molecular identification. The Shannon's diversityindices of endophytic fungi were greater in A. fukudo(1.5811), and lowest in P. communis and S. australis (0.6932). The endophytic fungi showed different relative frequencies in all halophytes (Table 3).

Table 3. Distribution and diversity index of endophytic fungi isolated from six halophytes in Suncheon Bay

Fungal taxonomy	Af	Cs	Ks	Pc	Sa	Sj	Total
Acremonium		1					1
Alternaria	1						1
Capnodiales	1						1
Cladosporium	1		1				2
Diaporthe			1				1
Fusarium	4		5	1	2	1	13
Microsphaeropsis	1						1
Paraconiothyrium		1					1
Penicillium	1	1	5		2	3	12
Pycnidiophora						1	1
Talaromyces				1			1
Total	9	3	12	2	4	5	35
Shannon's diversity index	1.5811	1.0986	1.1437	0.6932	0.6932	0.9503	

Screening for Plant Growth-Promoting Activity of FCFs on WR Seedling

The culture filtrates of all endophytic fungi were applied to WR seedlings to verify PGP activity. A result of the culture filtrate treatment of the Sj-2-2 fungal strain indicated 13.033 cm of SL and 21.7 cm of PL and confirmed PGP activity. The culture filtrate treatment of the Sj-2-2 fungal and G. fujikuroi strains confirmed the similarity of PGP between these two strains (Table 4).

Table 4. Screening for PGP of WR seedling with FCFs of endophytic fungi isolated from halophytes

Fungal isolates	SL	PL	Fungal isolates	SL	PL
D. water*	$6.07^{klm} \pm 0.32$	$13.57^{no} \pm 0.85$	Ks-5-3	$8.83^{bcd} \pm 0.45$	$17.43^{bcd} \pm 0.78$
Medium	$7.10^{fghijk} \pm 0.66$	$14.70^{ijklmn} \pm 0.40$	Ks-7-2	$9.17^{b} \pm 0.45$	$17.17^{bcde} \pm 0.25$
*G. fujikuroi***	$12.83^{a} \pm 0.42$	$21.57^{a} \pm 0.71$	Ks-8-2	$6.50^{ijklm} \pm 0.79$	$16.27^{cdefghi} \pm 0.51$
Af-2-1	$7.93^{cdefg} \pm 0.67$	$15.47^{ghijkl} \pm 0.83$	Ks-8-3	$8.13^{bcdef} \pm 0.25$	$15.70^{efghijk} \pm 1.10$
Af-4-1	$7.67^{defghi} \pm 0.73$	$16.20^{cdefghij} \pm 0.46$	Ks-9-1	$6.00^{klm} \pm 0.62$	$14.77^{ijklmn} \pm 1.01$
Af-4-2-1	$8.07^{bcdef} \pm 0.51$	$15.67^{efghijk} \pm 0.72$	Ks-9-3	$7.90^{cdefgh} \pm 0.30$	$15.30^{ghijklm} \pm 0.87$
Af-4-3	$7.87^{cdefgh} \pm 0.42$	$16.10^{defghij} \pm 0.66$	Ks-10-1	$7.53^{efghij} \pm 0.71$	$16.17^{defghij} \pm 0.81$
Af-6-1	$7.30^{efghij} \pm 0.75$	$14.67^{jklmn} \pm 0.81$	Ks-10-3	$9.00^{bc} \pm 0.66$	$17.67^{bc} \pm 0.40$
Af-7-1	$7.30^{efghij} \pm 0.20$	$14.10^{lmn} \pm 0.46$	Pc-11-2	$9.07^{bc} \pm 0.51$	$17.07^{bcdef} \pm 0.42$
Af-8-1	$6.57^{ijklm} \pm 0.64$	$14.70^{ijklmn} \pm 0.62$	Pc-12-1	$6.60^{ijklm} \pm 0.70$	$15.13^{hijklm} \pm 0.40$
Af-8-2	$7.43^{efghij} \pm 0.49$	$15.17^{hijklm} \pm 0.59$	Sa-1-1	$8.27^{bcdef} \pm 0.51$	$15.83^{efghijk} \pm 1.82$
Af-10-1	$5.87^{lm} \pm 0.49$	$12.40^{o} \pm 0.70$	Sa-1-2	$8.43^{bcde} \pm 0.55$	$16.67^{bcdefgh} \pm 1.05$
Cs-2-1	$6.33^{jklm} \pm 0.71$	$15.17^{hijklm} \pm 0.56$	Sa-4-2	$5.47^{m} \pm 0.60$	$13.87^{mn} \pm 0.35$
Cs-6-1	$6.53^{ijklm} \pm 1.06$	$14.37^{klmn} \pm 1.25$	Sa-8-1	$6.83^{ghijkl} \pm 0.93$	$15.00^{ijklmn} \pm 0.44$
Cs-9-1	$6.80^{ghijkl} \pm 0.36$	$15.73^{efghijk} \pm 0.61$	Sj-1-1	$7.13^{fghijk} \pm 0.45$	$15.63^{fghijkl} \pm 0.45$
Ks-1-1	$8.20^{bcdef} \pm 0.50$	$15.77^{efghijk} \pm 0.60$	Sj-1-2	$6.70^{hijkl} \pm 0.36$	$15.40^{ghijklm} \pm 0.78$
Ks-1-2	$5.63^{lm} \pm 0.95$	$14.43^{klmn} \pm 1.25$	Sj-2-2	$13.03^{a} \pm 0.42$	$21.70^{a} \pm 0.82$
Ks-1-3-1	$7.47^{efghij} \pm 0.73$	$15.53^{ghijkl} \pm 0.78$	Sj-2-5	$8.50^{bcde} \pm 0.53$	$17.80^{b} \pm 0.36$
Ks-1-3-2	$7.43^{efghij} \pm 1.16$	$15.40^{ghijklm} \pm 0.30$	Sj-7-3	$7.37^{efghij} \pm 0.55$	$16.83^{bcdefg} \pm 1.24$

Bioassay of Sj-2-2 Strain for Plant Growth-Promotion on S. japonica

The SL and PL of S. japonica seedlings treated with Sj-2-2 FCF were measured after 14 days of culture filtrate application. The PGP capacity of S. japonica seedling bythe Sj-2-2 fungal strain was compared with that of concentrated culture filtrate of CBM and that of wild-type G. fujikuroi, respectively. As CBM contains nutrients, its control was used to observe if the nutrients affects shoot elongation. The seedlings treated with the SL of Sj-2-2 culture filtrate were 8.74 cm, which were higher than those with G. fujikuroi culture filtrate (7.76 cm) and those with Czapek broth (5.53 cm) (Fig. 2).

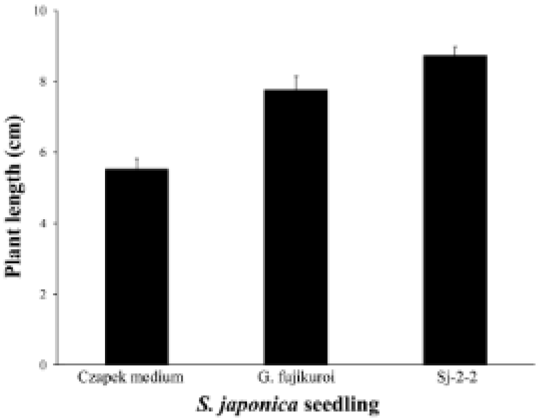


Fig. 2. Bioassay of FCFs on S. japonica seedlings of endophytic fungi

IV. DISCUSSION

Thirty-five endophytic fungi have been isolated from 6 halophytes' root and classified by ITS1, 5.8s, and ITS2 phylogenetic sequences. All endogenous fungi, containing Acremonium, Alternaria, Capnodiales, Diaporium, Fusarium (Gibberellla), Paraconiothyrium, Penicillium, Pycnidiophora and Talaromyces were described in the genus. All fungi were found at the level of genus. The Fusarium and Penicillium genus were the more frequently distributed in six halophytes of all the endophytic fungi isolated. Ascomycota has been reported to contain phylum from various endophytic fungi. It showed that endophytic fungi are present in salt marshes at the roots of various halophytes. A screening of philtres of microbial crops to identify biologically active molecules was used to confirm the existence of secondary metabolites. In the culture filtrates of endophytic fungi isolated from halophytic roots with WR seedlings were identified in this analysis, PGP hormones. The use of WR was highly effective [8, 14, 15], a dwarf rice mutant with GA reduced biosynthesis. Uniconazol was currently treated for seeds of WR as a retardant of GA biosynthesis. Use WR seedlinge, a strain of fungal Sj-2-2, which has strain of PGP activity. S was then verified for the PGP behaviour of halophyte. Seedling Japanese. The fungal strain Sj-2-2 demonstrated the advanced WR and S PGP operation. Seedling from Japan. For recent studies, DNA sequence analysis provides diverse and significant details. Many genes of rDNA are strongly preserved for identification on the taxonomic side. In order to classify fungal strains, The genes are generally added. On the basis of these evidence, 5.8S gene, ITS1 and ITS2 areas have been identified [8, 15, 16]. For the identification and monitoring of genetic evolution, phylogenetic classification analysis with BLAST database search has become very relevant. This approach is based on statistical analysis such as bootstrap tests and offers the most useful knowledge about maximum DNA sequence homology. The consensus bootstrap is capable of constructing a phylogenetic tree with maximal sequence replication. In order to classify different endophytic fungi, bootstrapping can be used on a NJ tree [8, 15]. Psylogenetic analyses with different endophytic fungi have verified homology and relationship. Penicilliumsp was the name of the fungal strain Sj-2-2 with phylogenetic analysis. (JN 624913). Sj-2 -2. The Sim GC-MS is capable of simultaneously analysing highly complex blends and detecting compound in various groups [3, 8, 12, 17] for the study of cultural filtrate fungal trains Sj-2-2. GC-MS SIM is useful for analysis and is used in plant research [8, 16], in a variety of compounds. GC-MS SIM has been used in various phytohormones due to its reliability.

V. CONCLUSION

In this study, many endophytic fungi were isolated from the roots and their relationships were confirmed by the spontaneous growth of six halophytes in a salt marsh. Penicillium sp. PGP behaviour analysis. It was carried out. Our recent study reports on Penicilliumsp's ability. GAs provided by Sj-2-2. The importance of using Penicilliumsp is stressed in this review. In the salt stress environment for plant growth and production. Additional field test trials are recommended for the latest strain of Penicillium sp. The fungus was stored at the General Collection Center for Microbiological Culture (CGMCC, No. 6380). This is why S is used as an effective bio-fertilizer. Cultivation of miltiorrhiza to increase quality and yield of plants. The excellence of its broad growth opportunities as well as the importance of raw herbal materials is the solution. The fungus increases the range and size of S. Miltiorrhiza growing in non-original areas in particular, with good plant quality preserved.

REFERENCES

1. Shen Nong's Herbal Classic. Available online: http://www.theqi.com/cmed/oldbook/sn_herb/ (accessed on 30 August 2015).
2. WHO Statistics. Available online: www.who.int/cardiovascular_diseases (accessed on 12 November 2016).
3. Bomme, U.; Bauer, R.; Friedl, F.; Heuberger, H.; Heubl, G.; Torreslondoño, P.; Hummelsberger, J. Cultivating Chinese medicinal plants in Germany: A pilot project. J. Altern. Complement. Med. 2007, 13, 597–601.
4. Chen, H.; Chena, F.; Chiu, F.C.; Lo, C.M. The effect of yeast elicitor on the growth and secondary metabolism of hairy root cultures of Salvia miltiorrhiza. Enzyme Microb. Tech. 2001, 28, 100–105.
5. Ling, H.; Lu, X.; Zhao, Y.; Zhang, S. The survey on the research of water-soluble components of Chinese medicine Danshen. Natl. Prod. Res. Dev. 1999, 11, 75–81.
6. Petersen, M.; Abdullah, Y.; Benner, J.; Eberle, D.; Gehlen, K.; Hücherig, S.; Janiak, V.; Kyunghee, K.; Sander, M.; Weitzel, C. Evolution of rosmarinic acid biosynthesis. Phytochemistry 2009, 70, 1663–1679.
7. Ming, Q.; Han, T.; Li, W.; Zhang, Q.; Zhang, H.; Zheng, C.; Huang, F.; Rahman, K.; Qin, L. Tanshinone IIA and tanshinone I production by Trichoderma atroviride D16, an endophytic fungus in Salvia miltiorrhiza. Phytomedicine 2012, 19, 330–333.
8. Ming, Q.; Su, C.; Zheng, C.; Jia, M.; Zhang, Q.; Zhang, H.; Rahman, K.; Han, T.; Qin, L. Elicitors from the endophytic fungus Trichoderma atroviride promote Salvia miltiorrhiza hairy root growth and tanshinone biosynthesis. J. Exp. Bot. 2013, 64, 5687–5694.
9. Wu, J.Y.; Ng, J.; Shi, M.; Wu, S.J. Enhanced secondary metabolite (tanshinone) production of Salvia miltiorrhiza hairy roots in a novel root–bacteria coculture process. Appl. Microbiol. Biotechnol. 2007, 77, 543–550.
10. Zhao, J.L.; Zhou, L.G.; Wu, J.Y. Effects of biotic and abiotic elicitors on cell growth and tanshinone accumulation in Salvia miltiorrhiza cell cultures. Appl. Microbiol. Biotechnol. 2010, 87, 137–144.
11. Mahanty, T.; Bhattacharjee, S.; Goswami, M.; Bhattacharyya, P.; Das, B.; Ghosh, A.; Tribedi, P. Biofertilizers: A potential approach for sustainable agriculture development. Environ. Sci. Pollut. Res. Int. 2017, 24, 3315–3335.
12. Pham, V.T.; Rediers, H.; Ghequire, M.G.; Nguyen, H.H.; De Mot, R.; Vanderleyden, J.; Spaepen, S. The plant growth-promoting effect of the nitrogen-fixing endophyte Pseudomonas stutzeri A15. Arch. Microbiol. 2017, 199, 1–5.
13. Weselowski, B.; Nathoo, N.; Eastman, A.W.; Macdonald, J.; Yuan, Z.C. Isolation, identification and characterization of Paenibacilluspolymyxa CR1 with potentials for biopesticide, biofertilization, biomass degradation and biofuel production. BMC Microbiol. 2016, 16, 244.
14. Satheesan, J.; Narayanan, A.K.; Sakunthala, M. Induction of root colonization by Piriformosporaindica leads to enhanced asiaticoside production in Centellaasiatica. Mycorrhiza 2012, 22, 195–202.
15. Zhang, F.S.; Lv, Y.L.; Zhao, Y.; Guo, S.X. Promoting role of an endophyte on the growth and contents of kinsenosides and flavonoids of Anoectochilusformosanushayata, a rare and threatened medicinal orchidaceae plant. J. Zhejiang Univ. Sci. B 2013, 14, 785–792.
16. Wang, M.; Dai, G.J.; Yun-Tong, M.A.; Zhang, Q.; Chen, X.; Wan, D.G.; Yan, Z.Y. Correlation between Active Components and Endophytic fungi in Salvia miltiorrhiza. Chin. J. Exp. Tradit. Med. Formulae 2013, 23, 66–73.
17. Zhao, J.L.; Zhou, L.G.; Wu, J.Y. Promotion of Salvia miltiorrhiza hairy root growth and tanshinone production by polysaccharide–protein fractions of plant growth-promoting rhizobacterium Bacillus cereus. Process Biochem. 2010, 45, 1517–1522.
18. Tang, K.; Biao, L.I.; Guo, S.X. Analysis of Diversity of Endophytic Fungi in Salvia miltiorrhiza from Henan in China. Chin. Pharm. J. 2015, 50, 570–573.
19. Yan, Q.; Shi, M.; Ng, J.; Wu, J.Y. Elicitor-induced rosmarinic acid accumulation and secondary metabolism enzyme activities in Salvia miltiorrhiza, hairy roots. Plant Sci. 2006, 170, 853–858.
20. Tian, R.; Yang, Q.; Zhou, G.; Tan, J.; Zhang, L.; Fang, C. Taxonomic study on a taxol producing fungus isolated from bark of Taxuschinensis var. mairei. J. Wuhan Botanical Res. 2006, 24, 541–545.