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RESEARCH ARTICLE

Endophytes *Aspergillus caespitosus* LK12 and *Phoma* sp. LK13 of *Moringa peregrina* produce gibberellins and improve rice plant growth

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Two new strains of endophytic fungi were isolated from the bark of *Moringa peregrina* and identified as *Aspergillus caespitosus* LK12 and *Phoma* sp. LK13. These endophytes were identified through amplifying polymerase chain reaction (PCR) and sequencing the 18S internal transcribed spacer of DNA extracted from both endophytes. Pure cultures of endophytic fungi were subjected to extract and isolate gibberellins (GAs). Deuterated standards of [17,17-$^2H_2$]-GA_1, [17,17-$^2H_2$]-GA_3, [17, 17-$^2H_2$]-GA_4 and [17, 17-$^2H_2$]-GA_7 were used to quantify the endophytic fungal GAs. The analysis revealed that both the endophytes are producing bioactive GAs in various quantities (ng mL^{-1}). *A. caespitosus* LK12 was producing GA_1 (54.51 ± 1.23), GA_4 (26.5 ± 0.65), and GA_7 (2.87 ± 1.23) while *Phoma* sp. LK13 was secreting GA_1 (4.8 ± 0.12), GA_3 (8.65 ± 0.21), GA_4 (23.7 ± 0.98), and GA_7 (22.7 ± 0.73). The culture filtrate (CF) of *A. caespitosus* and *Phoma* sp. significantly increased the shoot length of GAs-deficient mutant *waito-c* and normal Dongjin-beyo rice seedlings as compared to control. Application of such growth-promoting and GAs-producing endophytes can ameliorate poorly growing crop plants.

Keywords: endophytes; gibberellins; rice growth; *Moringa peregrina*

Introduction

Endophytic fungi, residing in the host tissues without causing symptoms of disease, have been regarded a pivotal source of bioactive secondary metabolites (Zhou et al. 2014). Various classes of biologically active molecules including flavonoids, peptides, alkaloids, steroids, phenolics, terpenoids, lignans, and volatile organic compounds with many of them biologically active have been found to be produced by endophytes (Schulz & Boyle 2005; Hamilton & Bauerle 2012). In fact, a previous study by Schulz et al. (2002) showed that about 51% of biologically active metabolites originate from endophytes compared to only 38% of novel substances originating from other soil microflora. In the past two decades, many novel bioactive compounds with antimicrobial, insecticidal, cytotoxic, and anticancer properties have been successfully isolated and characterized from endophytic fungi. The role of endophytic fungi in the production of plant growth-promoting substances (gibberellins [GAs]) is unexplored whilst identifying such microorganisms can extend greater benefits in improving agricultural productivity and human food security.

GAs are ubiquitous plant hormones that elicit various metabolic functions required for the growth of a plant, its seed germination, stem elongation, sex expression, flowering, formation of fruits, and senescence (Lee et al. 1998). Till now, more than 136 GAs have been identified (Bomke et al. 2008) which are named based on their discovery (http://www.plant-hormones.info/gibberellin_nomenclature.htm). Many fungal species, for example *Gibberella fujikuroi*, *Sphaceloma manihoticola*, *Phaeosphaeria* sp., *Neurospora crassa*, have been found to produce GAs (Rademacher 1994). Among fungi, endophytes are not only associated with plants but also in most cases they live inside the plant, and this can either be the aerial parts of the plant or the root. This symbiosis is without causing any disease symptoms to the host (Schulz & Boyle 2005; Hyde & Soytong 2008; Kumar et al. 2012; Waqas et al. 2014). Endophytes have been mostly reported to improve plant growth (Schulz & Boyle 2005). They can influence key aspects of plant physiology and increase host plant fitness against biotic and abiotic stresses (Khan et al. 2011a, 2011b). It has also been recently shown that strains of *Penicillium commune* (Choi et al. 2005), *Phaeosphaeria* sp. L487 (Kawaide 2006), *Chrysosporium pseudomerdarium* (Hamayun et al. 2009), *Aspergillus fumigatus* (Khan et al. 2011a), and *Penicillium funiculosum* (Khan et al. 2011b) can produce GAs. Previously some strains of *Cephalotheca sulfurea* and *Phoma* sp. have been reported to secret GAs, suggesting that these might have an active GAs biosynthesis pathway (Hamayun et al. 2010, 2012).

Moringa peregrina Fiori (Moringaceae), a deciduous tree, is an economically important plant of the Arabian and North African region (Hegazy et al. 2008; Zaghloul et al. 2011). Besides its ecological importance, it is mainly used in folk medicine and eaten as a vegetable (Marwah et al. 2007; Hegazy et al. 2008; Goma & Picó 2011; Zaghloul et al. 2011). It is effective for abdominal pain, burns,

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constipation, and headache while its bark has been used to remove freckles (Miier & Morris 1989). Its associated microflora has been poorly known. Looking at the importance of its bark and the potential of the tree to withstand extreme environmental conditions, in the present study, we aimed to assess the endophytes present in the bark.

Materials and methods

Fungal endophyte isolation

The bark of *M. peregrina* Fiori (Moringaceae), growing in the wild mountains of Jabal Al-Akhdar (23°04′ 22.00 N″; 57°40′ 07.00 E″), Sultanate of Oman, was detached from the tree. The climatic condition of the region is arid and *M. peregrina* trees are growing in extreme water-deficient conditions ($\Psi = -4.23$ hPa). About 20 samples of bark randomly collected from different position of the stem, i.e. above the soil surface and below the twigs of four trees, were stored in a sterilized polythene zip-bag during transportation. The samples were thoroughly washed in running tape water. To isolate endophytic microbes, the method of Arnold et al. (2007) was adopted. Briefly, the 20 bark samples were further sliced into 60 pieces (0.5 cm) and surface sterilized with 2.5% sodium hypochlorite (30 min in a shaking incubator at 120 rpm) and washed with autoclaved double distilled water (DDW) to remove contaminants and microorganisms. The bark pieces were carefully placed in petri-plates containing Hagem media (0.5% glucose, 0.05% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% NH_4Cl, 0.1% $FeCl_3$, 80 ppm streptomycin, and 1.5% agar, pH 5.6 ± 0.2). The sterilized bark parts were also imprinted on separate Hagem plates to ensure the effectiveness of the surface sterilization (Arnold et al. 2007). The newly emerged fungal spots from the bark were isolated and grown on potato dextrose agar (PDA) medium under sterile conditions. A total of eight different fungal strains were isolated and grown on PDA media. Rate of prevalence was calculated as the average % frequency of isolates per bark samples of stem. These strains were inoculated in Czapek broth (50 ml; 1% glucose, 1% peptone, 0.05% KCl, 0.05% $MgSO_4 \cdot 7H_2O$, and 0.001% $FeSO_4 \cdot 7H_2O$; pH 7.3 ± 0.2) and grown for 7 days (shaking incubator – 120 rpm; temperature 30°C) to separate the liquid culture medium and the fungal mycelia (centrifugation 2500×*g* at 4°C for 15 min). The culture medium (culture filtrate [CF], 50 ml) and mycelium were immediately shifted to −70°C freezer and then freeze-dried (Virtis Freeze Dryer, Gardiner, NY, USA) for 4–7 days. The lyophilized CF was diluted with 1 ml of autoclaved DDW, while the mycelia were used for genomic DNA extraction.

GAs screening using mutant rice

Presence or absence of GAs in the pure culture of endophytes was analyzed by performing screening bioassay on (1) *waito-c* and (2) *Oryza sativa* L. cv. Dongjin-byeo. *Waito-c* is a GAs biosynthesis *dy* mutant with dwarf phenotype while Dongjin-beyo has active GAs biosynthesis pathway with a normal growth pattern. Rice seeds were surface sterilized with 2.5% sodium hypochlorite for 30 min, rinsed with autoclaved DDW and incubated for 24 h with 20 ppm uniconazole (except Dongjin-byeo) to obtain equally germinated seeds. The mutant was treated with uniconazole to further inhibit the GAs pathways. For providing axenic conditions, the pre-germinated *waito-c* and Dongjin-byeo seeds were transferred to autoclaved small plastic pots containing 0.8% water: agar medium (Khan et al. 2011a, 2011b; Redman et al. 2011). After attaining two leaves stage, the CF (20 µl) harvested from endophytes was applied at the apex of rice seedlings. After 7 days, the rice growth was recorded and compared with CF of medium and *G. fujikuroi* KCCM12329-treated rice plants.

Endophyte identification by polymerase chain reaction (PCR)

An efficient method was developed for the isolation of genomic DNA from endophytic fungi, because usual Cetyltrimethylammonium bromide (CTAB) extraction method and mycelial grinding were causing DNA shearing. Rich mycelial culture was obtained by growing fungus in Czapek culture broth (supplemented with 1% glucose and peptone) for 7 days on rotary shaking incubator (120 rpm and 28°C) and lyophilized for 24 hrs. A 0.5 g of lyophilized sample was broken carefully in 2 ml eppendorf, with the blunt end spatula or with a glass rod. Double volume of lysis buffer (20 mM Tris–HCL, pH 8.0; 10 mM EDTA; 1% SDS) containing 1% of 2-mercaptoethanol was added. The mixture was vortexed briefly (30 sec) to obtain homogeneity and left to incubate for 2 hr in water bath set at 55°C. Pre-heated 4% CTAB extraction buffer (250 µl/ml) was added to lysed cells mixture and incubated further at 65°C for 1 hr. Chloroform extraction followed by iso-propanol precipitation yielded condensed strand of nucleic acid, which was cleaned from RNA using 10 µl of RNase A for 2 hr of incubation at 37°C. The isolated DNA was suspended in 50 µl of autoclaved DDW and tested for purity (Hamayun et al. 2009).

The fungal isolate was identified through sequence analysis of the internal transcribed region (ITS) of 18S rDNA, using universal primers ITS1 (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) (Taylor & Bruns 1999). A 25 µl of PCR mixture contained 2.5 µl of dNTPs and Ex-Taq buffer, 2 µl of each primer, 0.5 µl of DNA sample, and 0.2 µl of Ex-Taq polymerase. The remaining volume was adjusted with 15.3 µl of autoclaved DDW. For the amplification of ITS1 and ITS4 regions of 18S rDNA, the reaction cycle consisted of initial denaturation (95°C) for 2 min, 35 cycles of denaturation (95°C) for 30 s, annealing (55°C) for 60 s, extension (72°C) for 30 s and a final extension time for 5 min (72°C). The resultant products were gene cleaned using a Nucleogen gene clean kit, ligated in T-vector using Takara Perfect T-cloning kit, and then inserted into DHa *Escherichia coli* competent cells (RBC)

by overnight incubation (37°C). Transformed cells were selected, grown overnight (37°C) in LB broth, and their plasmids were extracted using SolGent Plasmid mini-prep kit, which were later sequenced.

The BLAST search program (http://www.ncbi.nlm.nih.gov/BLAST) was used to compare the sequence homology of nucleotide of 18S ITS region of fungi. The closely related sequences obtained were aligned through CLUSTAL W using MEGA version 5.1 software (Tamura et al. 2011), and the maximum parsimony (MP) tree was constructed using the same software. The bootstrap replications (1K) were used as a statistical support for the nodes in the phylogenetic tree.

Quantification of GAs from bioactive endophyte

To characterize GAs secreted in the pure culture, endophytes were cultivated in Czapek broth for 7 days at 30°C (120 rpm) in 500 ml Erlenmeyer flask (150 ml liquid medium) as described previously (Waqas et al. 2014). The culture medium (CF) was used to extract and purify GAs as described by Lee et al. (1998) and Hamayun et al. (2009). Briefly, the CF (pH 2.5) was partitioned with ethyl acetate (EtOAc) and the organic layer was vacuum dried. It was added with 60% methanol (MeOH) while the pH was increased to 8.0 ± 0.3 by adding 2N NH_4OH. Before column chromatography, deuterated GAs internal standards ([17, 17-$^2H_2$] GA_1, GA_3, GA_4, and GA_7 were added to the CF. The quantification of GAs was performed according to the method of Lee et al. (1998). The extracts were passed through a Davisil C18 column (90–130 µm; Alltech, Deerfield, IL, USA). The eluent was reduced to near dryness at 40°C in vacuum. The sample was then dried onto Celite and loaded onto SiO_2 partitioning column (deactivated with 20% water) to separate the GAs as a group from more polar impurities. GAs were eluted with 80 ml of 95:5 (v/v) EtOAc: hexane saturated with formic acid. This solution was dried at 40°C in vacuum, redissolved in 4 ml of EtOAc, and partitioned three times against 4 ml of 0.1 M phosphate buffer (pH 8.0). Dropwise addition of 2 N NaOH was required during the first partitioning to neutralize residual formic acid. One gram polyvinylpolypyrrolidone (PVPP) was added to the combined aqueous phases, and this mixture was slurried for 1 h. The pH was reduced to 2.5 with 6 N HCl. The extract was partitioned three times against equal volumes of EtOAc. The combined EtOAc fraction was dried in vacuum, and the residue was dissolved in 3 ml of 100% MeOH. This solution was dried on a Savant Automatic Environmental Speedvac (AES 2000, Madrid, Spain). The extract of fungal CF was subjected to high performance liquid chromatography (HPLC) using a 3.9×300 m Bondapak C18 column (Waters Corp., Milford, MA, USA) and eluted at 1.0 ml/min with the following gradient: 0–5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5–35 min, linear gradient from 28% to 86% MeOH; 35–36 min, 86–100% MeOH; 36–40 min, isocratic 100% MeOH. Forty-eight fractions of 1.0 ml each were collected (Supplementary information 1[1]). The fractions were then prepared for gas chromatography/mass spectrometry (GC/MS) with selected ion monitoring (SIM) system (6890N Network GC System, and 5973 Network Mass Selective Detector; Agilent Technologies, Palo Alto, CA, USA). For each GAs, 1 µl of sample was injected in GC/MS (Supplementary information 1). Full-scan mode (the first trial) and three major ions of the supplemented [17-$^2H_2$] GAs internal standards and the fungal GAs were monitored simultaneously. The fungal CF GAs (GA_1, GA_3, GA_4, and GA_7) were calculated from the peak area ratios. The retention time was determined using hydrocarbon standards to calculate the Kovats retention index (KRI) value (Supplementary information 1). The data were calculated in nano-grams per milliliter (for fungal CF) while the analyses were repeated three times.

Statistical analysis

The data were analyzed statistically for standard deviation and error by using GraphPad Prism (version 5.0; CA USA). The mean values were compared using Duncan's multiple range tests at $P < .05$ (SAS, Cary, NC, USA).

Results and discussion

Endophytes isolation

We isolated 11 fungal endophytes from 20 bark explants, which were further partitioned into 60 pieces (0.5 cm) randomly. These endophytes were grouped into eight different morpho-types on the basis of colony shape, thickness, color of aerial hyphae, colony reverse color, growth rate and pattern, margin characteristics, surface texture, and growth depth into medium (Tehler 1995). The rate of prevalence was 18.61%. Among eight endophytes (Table 1), MPB-1 and MPB-2 endophytes were found to be more abundant in bark samples.

Bioactivity of endophytes

The endophytes were screened for their role in promoting the growth of GAs-deficient mutant *waito-c* and wild-type Dongjin-beyo rice seedlings. The activation of suppressed GAs biosynthesis in *waito-c* would reveal the presence of bioactive secondary metabolites while the effects were rectified using normal rice (Khan et al. 2011a, 2011b; Waqas et al. 2014). The screening results showed that CF application of endophytic strains MPB-1 and MPB-2 significantly increased the mutant and normal rice growth (Table 1). The shoot length, chlorophyll contents, and shoot fresh weight of *waito-c* and normal rice seedlings were significantly enhanced by the CF application of MPB-1 and MPB-2 as compared to the CF of *G. fujikuroi* and DDW applied to rice. The growth-promoting effects of MPB-1 and MPB-2 were not significantly different from each other (Table 1).

Dwarf rice (*waito-c*) is a GAs biosynthesis mutant line having passive *dy* gene which synthesizes bioactive GAs through C13 hydroxylation pathway (Ikeda et al.

Table 1. Effect of cultural filtrates of isolated endophytes on growth of GA mutant *Waito*-C and wild-type rice seedlings.

	Shoot length (cm)		Shoot biomass (gm)		Chlorophyll contents (SPAD)	
Treatment	*Waito-c*	Dongjin-beyo	*Waito-c*	Dongjin-beyo	*Waito-c*	Dongjin-beyo
Control DDW	4.31 ± 0.17c	6.1 ± 0.02d	0.23 ± 0.02c	0.41 ± 0.01c	14.23 ± 1.07d	16.2 ± 1.04d
G. fujikuroi	5.12 ± 0.21b	7.01 ± 0.43c	0.31 ± 0.04b	0.52 ± 0.02b	16.70 ± 2.14c	17.2 ± 1.07c
MPB-1	6.23 ± 0.12a	9.1 ± 0.34a	0.41 ± 0.03a	0.68 ± 0.05a	17.1 ± 1.86a	21.0 ± 1.86a
MPB-2	6.17 ± 0.62a	9.23 ± 1.01a	0.49 ± 0.02a	0.67 ± 0.02a	17.9 ± 0.21a	20.9 ± 1.01a
MPB-3	5.16 ± 0.56b	8.1 ± 0.98b	0.36 ± 0.05b	0.61 ± 0.01a	17.1 ± 1.03a	19.5 ± 1.43a
MPB-4	5.57 ± 0.48b	7.8 ± 1.23b	0.38 ± 0.05ab	0.58 ± 0.02b	16.8 ± 0.77bc	20.3 ± 0.87a
MPB-5	5.80 ± 0.72b	7.9 ± 1.87b	0.34 ± 0.01b	0.57 ± 0.01b	15.9 ± 0.23c	19.9 ± 0.98a
MPB-6	5.87 ± 0.32b	7.21 ± 0.21c	0.36 ± 0.02b	0.57 ± 0.02b	15.2 ± 0.83c	18.8 ± 0.33b
MPB-7	5.56 ± 0.86b	7.6 ± 0.87bc	0.30 ± 0.05b	0.51 ± 0.01b	15.3 ± 0.46c	17.9 ± 0.92bc
MPB-8	6.0 ± 0.88ab	7.8 ± 0.74b	0.32 ± 0.025b	0.60 ± 0.02ab	15.9 ± 0.23c	19.3 ± 0.88b

SPAD = soil plant analysis development, DDW = double distilled water.
Note: Values within the same column followed by different letter(s) are significantly different at ($P < .05$) levels as estimated by Duncan's Multiple Range Test (DMRT).

2001). The *waito-c* rice seeds were treated with uniconazole to further suppress the GAs biosynthesis pathway (Ikeda et al. 2001). The use of *waito-c* rice can help detecting very small amounts of GAs if present in the sample (Hamayun et al. 2010). Agar and water, on the other hand, were used as growing medium for rice. During the bioassay, the rice seeds were fully grown on agar water media till the harvest in order to accurately measure the sole effect of fungal CF (Khan et al. 2011a, 2011b). *Fusarium fujikuroi* produces a wide array of diterpenoids including a group of phytohormones mainly GAs (Albermann et al. 2013). We compared the effect of CF of wild-type *F. fujikuroi* with that of our endophytes and found a similar growth-promoting behavior (Hamayun et al. 2010; Khan et al. 2011a, 2011b, 2013). The results of bioassay showed that MPB-1 and MPB-2 have significantly higher growth stimulatory effects; therefore, the CF of the strain might contain biologically active secondary metabolites.

GAs production by endophytes

The CF of MPB-1 and MPB-2 was subjected to chromatography and GC/MS SIM analysis for the quantification of GAs. For comparison, the wild type of *G. fujikuroi* was also subjected to GA analysis (Bomke et al. 2008). The results showed that the CF of both the bioactive endophytes contains GAs. The results of GC/MS SIM analysis evidenced the present of GA ion signals in correlation with [$^2H_2$] GA standards. The GA produced by both the endophytes has been given in Table 2. Total four different GAs (GA_1, GA_3, GA_4, and GA_7) in various amount (ng mL^{-1}) were detected in the CF of MPB-1 (GA_1 – 54.51 ± 1.23; GA_4 – 26.5 ± 0.65; GA_7 – 2.87 ± 1.23) and MPB-2 (GA_1 – 4.8 ± 0.12; GA_3 – 8.65 ± 0.21; GA_4 – 23.7 ± 0.98; GA_7 – 22.7 ± 0.73) (Supplementary information 1). The detection of bioactive GAs gives us the insight of GA production biosynthesis pathway in our isolated endophytes. The presence of GA_4 in CF of both endophytes shows the subsequent conversion of GA_{14} and GA_4 on desaturation then yields GA_3 and GA_7. While GA_1 production is different from them, which is the minor product of 13-hydroxylation of GA_4 and does not convert into GA_3 (Albermann et al. 2013; Waqas et al. 2014).

Table 2. GAs quantification of the CFs of fungal endophytes.

GAs	*A. caespitosus* LK12	*Phoma* sp. LK13
GA_1	54.51 ± 1.23a	4.8 ± 0.12b
GA_3	ND	8.65 ± 0.21a
GA_4	26.5 ± 0.65a	23.7 ± 0.98a
GA_7	2.87 ± 1.23b	22.7 ± 0.73a

ND: not detected.
Note: The error bars refer to mean ± SD of three replications. The different letters in row show values are significantly different ($P < .05$) as evaluated by DMRT.

GC/MS SIM is an established method to identify targeted novel secondary metabolites (Supplementary information 1) and the absence of the GA in the fungal-free culture broth supported our results. The repetition of the experiment and correlation with deuterated GA standards further confirmed our findings. Many fungal/endophytes species for example *G. fujikuroi, S. manihoticola, Phaeosphaeria* sp., *N. crassa* (Rademacher 1994), *P. commune* (Choi et al. 2005), *Phaeosphaeria* sp. L487 (Kawaide 2006), *Penicillium citrinum*, and *P. funiculosum* (Khan et al. 2011a) have been known to produce GAs. Previously *A. fumigatus* LHL02 (Khan et al. 2011c), *Phoma* sp. GAH7, and *Phoma herbarum* (Hamayun et al. 2009) were reported for GA production. The detection of these GAs suggests the existence of a GA gene cluster as reported for *G. fujikuroi*, *Phaeospheria* sp. L487, and *Sphaceloma* sp. (Bomke et al. 2008). Endophytes have been mostly reported for their behavior to stimulate plant growth. They can influence key aspects of plant physiology and host's protection against biotic and abiotic stresses (Schulz & Boyle 2005). Secretion of bioactive metabolites such as phytohormones by endophytes can extend greater benefits to the host plants even during extreme environmental conditions (Khan et al. 2011a, 2011b) while on the other hand, being eco-friendly, these can be extradited for commercial application and use at industrial levels.

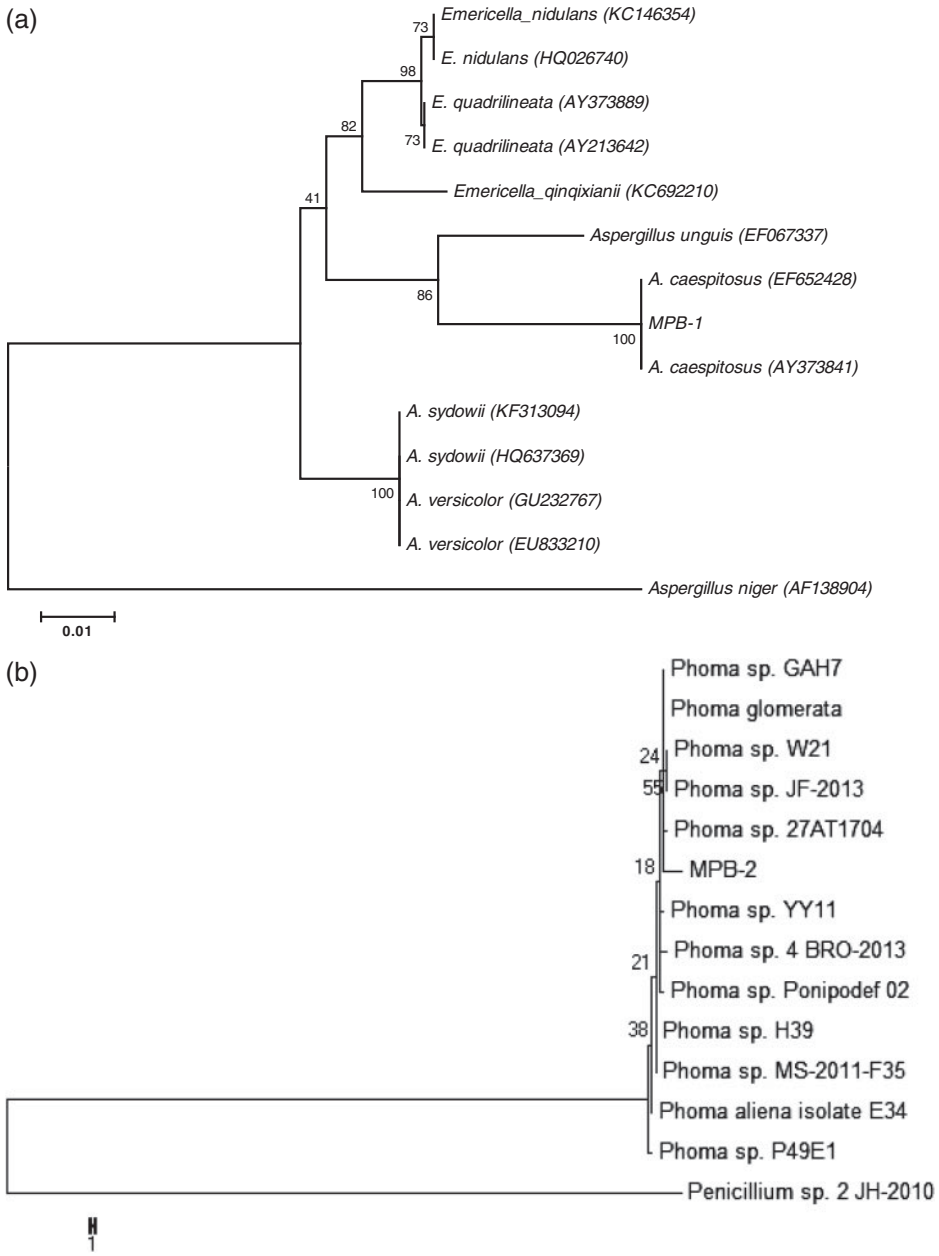


Figure 1. Phylogenetic tree constructed by MP method using 18S rDNA sequence (ITS region) of *A. caespitosus* LK12 (a) and *Phoma* sp. LK13. (b) MPB-1 and MPB-2 formed a clade (100% bootstrap support) with *Aspergillus* and *Phoma*. *Aspergillus niger* and *Penicillium* sp. were taken as an out group. The tree was prepared in MEGA 5.2.

Identification of endophytes

Fungal endophyte isolates were identified by PCR amplification and sequencing of ITS region. Phylogenetic analysis of the MPB-1 and MPB-2 was carried out through MEGA 5.1 using MP method. A consensus tree was made for MPB-1 from 13 (12 references and 1 clone) aligned ITS1 and ITS4 with 1000 bootstrap replications. The criteria for selection of strains to construct dendrogram were based on BLAST search showing maximum homology percentage, query coverage, and lowest *E* values with newly endophytes isolated in this study. Results of BLASTn search in National Center of Biotechnology Information (NCBI) revealed that MPB-1 has 100% sequence homology with *Aspergillus caespitosus*. In MP dendrogram, MPB-1 formed 100% bootstrap support with *A. caespitosus* (Figure 1). The phylogenetic tree of MPB-2 formed 55% clade with *Phoma* sp. Therefore, on the basis of sequence similarity and phylogenetic analysis, fungal isolates MPB-1 and MPB-2 were identified as the new strains LK12 and LK13 of *A. caespitosus* and *Phoma* sp., respectively. Previously these fungi were isolated and identified from various sources; however, these are reported first time from the bark of *Moringa*. El-Nagerabi et al. (2013) studied endophytic fungal population and isolated 80 different species including *A. caespitosus* and *Phoma sorghina* from the healthy leaves of *Ziziphus spina-christi* and *Ziziphus hajanensis* plants from the same study area over four years. They found that host-specific association with endophytes showed no seasonal

variation. Thus, our study suggests the ecological importance of isolated endophytes, which could help the host plant to adopt the particular harsh arid and semi-arid natural habitat (Arnold & Lutzoni 2007). This confirms the ecological niche that many fungal endophytes play a central role in maintaining a suitable adoptive response of plant hosts.

Conclusion

GAs-producing endophytes can be an essential arsenal of plant, which can help the host and other crop plants to improve its growth. A similar effect was observed in the present study. Two strains of fungal endophytes belonging to *A. caespitosus* and *Phoma* sp. increased the growth of dwarf mutant and wild-type rice seedlings. Upon chromatographic and spectroscopic analysis of the CF, it was found that both the endophytes are producing physiologically active GAs. Application of such GAs-producing plant growth-promoting endophytes can not only improve plants growth but also extend great help in enforcing plant responses/resistance to biotic and abiotic stresses. However, similar properties need further analysis at tissue culture level application to host and field level application to economically important crops. Additionally, the presence of GAs suggests the existence of GA biosynthesis pathway, which could be further analyzed by the molecular techniques.

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Note

1. Supplemental Content may be viewed online at http://dx.doi.org/10.1080/17429145.2014.917384

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