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ROLE OF ENDOPHYTE *CHAETOMIUM GLOBOSUM* LK4 IN GROWTH OF *CAPSICUM ANNUUM BY* PRODUCION OF GIBBERELLINS AND INDOLE ACETIC ACID

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Abstract

Endophytic fungi represent a trove of unexplored natural source of plant hormones like gibberellins (GAs) and indole-acetic acid (IAA). In present study, we isolated eight endophytes from the roots of drought stressed pepper (*Capsicum annuum* L.) plants. To assess phytohormones secreting potential, culture filtrates (CF) of endophytes were screened on GAs biosynthesis mutant *Waito-C* rice. Endophyte CAC-1G significantly promoted the shoot growth, chlorophyll content and biomass of *Waito-C* rice seedlings as compared with CF of *Fusarium fujikuroi* and distilled water. CAC-1G was identified as strain of *Chaetomium globosum* LK4 by sequencing internal transcribed spacer regions and phylogenetic analysis of similar sequences. The CF analysis of *C. globosum* showed the presence of GAs (GA_1 0.67±0.13 ng/ml; GA_4 21.8±1.2 ng/ml; GA_9 0.51±0.11 ng/ml; GA_{12}13.4±0.41 ng/ml; GA_{20} 1.11±0.2 ng/ml) and IAA (16.71±1.42 µg/ml). The CF of *C. globosum* had higher GA_4, GA_{12} and GA_{20} than the CF of *F. fujikuroi*. The CF containing propagules of *C. globosum* was applied to the host-pepper plants. The results revealed significantly higher shoot growth, chlorophyll content, plant biomass and leaf area as compared to fungal-free medium and water applied plants. The present results of *C. globosum* can be reciprocated for improved plant growth and yield at field levels.

Introduction

Endophytic fungi live asymptomatically within plant tissues have been found in almost all plant species (Saikkonen *et al*., 1998; Schulz & Boyle, 2005). These poorly known fungi represent a trove of unexplored biodiversity, and a frequently overlooked component of forest (Reinhardt, 2007; Arnold, 2008) and crop ecology (Khan *et al*., 2011a). The endophyte-host interaction is mutualistic or neutral and may differ among hosts and on the basis of environmental conditions **(**Saikkonen *et al*,. 1998; Faeth & Fagan, 2002). Endophytic fungi draw three basic benefits from the host plants: nourishment, physical protection and adversities reproduction e.g. members of Clavicipitaceous and Dikarya (Hyde & Soytong, 2008). In return, the host plant is benefited by the endophyte through production of metabolites [(e.g. alkaloids, antibiotics, or toxins, growth regulators (Schulz & Boyle 2005, Khan *et al*., 2011a)], nutrient composition inside tissues, plant hormonal balance, chemical composition of root exudates, physical modification of soil, disease resistance and protection against external calamities (Waller *et al.,* 2005; Rahman & Saiga, 2005; Oses *et al.,* 2008).

These endophytes have been found as a novel source of various kinds of bioactive secondary metabolites (Schulz *et al*., 2002). However, there are few reports available about the endophytes secreting phytohormones like gibberellins (GAs), auxin etc. Previously, some endophytic fungal strains were reported to produce a variety of physiologically active and inactive GAs. This includes; *Fusarium fujikuroi, Sphaceloma manihoticola* (Bomke *et al*., 2008) *Phaeosphaeria* sp. L487 (Kawaide, 2006), *Phaeosphaeria* sp., *Neurospora crassa* (Rademacher 1994), *Sesamum indicum (Choi et al*., *2005), Cladosporium* sp. MH-6 (Hamayun *et al*., 2010), *Aspergillus fumigatus* (Khan *et al*., 2011a) *Penicillium funiculosum* (Khan *et al*., 2011b), *Exophiala* sp. LHL08 (Khan *et al*., 2011c), and *Curvularia protuberata* etc. These phytohormones producing endophytes have been also reported to play essential role in crop plant growth and metabolism. However, there is little information available on endophytes isolated from extreme environmental conditions.

Chilli pepper (*Capsicum annuum* L.) is an important vegetable as well as spice crop, used worldwide for domestic and commercial purposes. They are rich source of antioxidants, vitamin C, pro-vitamin A, E, and B (Bosland & Votava, 1999). Pepper is regarded as a sensitive to salinity and drought (Kanber *et al*., 1992). With expanding human population, food demands have been at sturdy rate and therefore, maintaining plant growth is crucial for crop yield. Symbiosis of such endophytic fungi offers advantages to host plants in transport and assimilation biochemicals necessary for plant growth and counteract biotic and abiotic stresses (Schulz & Boyle, 2005; Waller *et al.*, 2005; Reinhardt, 2007; Khan *et al.,* 2011abc; Davitt *et al.,* 2011). Previously, three different endophytic fungal strains (*Aspergillus favus*, *Coniothyrium* sp., and *Nigrospora* sp.) were isolated from pepper plant which improved plant growth and protected plants against pathogenic attack. However, we failed to find any report of phytohormones producing endophytic fungi from the isolated from pepper plants. GA-producing fungal endophytes might have potential to increase crop yields due to increasing concern about the excessive use of fertilizers in agricultural and the subsequent negative effect on the environment. In present work, we aimed to isolate phytohormones producing bioactive endophytic fungal strain from the roots of drought stressed pepper plants. We screened the

isolated strains through a dwaf GAs mutant rice line – *Waito- C*. The culture of the fungus was subjected to chromatographic techniques to isolate and detect phytohormones.

Materials and Methods

Isolation of endophytic fungi: Roots were collected from pepper plants growing in drought stressed conditions. The soil characteristics during root sample collection were: 65% sand, 14.5% clay, 14.5% silt, 6.0% organic matter, pH 6.0-6.4, bulk density of 1.4gcm^3 and moisture content 41.82 hPa. Roots were thoroughly washed with tap water and consecutively treated with 1% Tween 80 solution, 1% perchloric acid and autoclaved double distilled water (DDW) for 5 minutes in shaking incubator (120rpm) (Khan *et al.,* 2011ab). The contaminants, rhizobacteria and mycorrhizal fungi were thus removed during surface sterilization. The endophytes were isolated as described by Waller *et al*., (2005) and Khan *et al*., (2011ab). Briefly, secondary root's pieces (0.5 cm) were selected and carefully placed on Hagem medium plates (0.5% glucose, 0.05% KH_2PO_4, 0.05% $MgSO_4.7H_2O$, 0.05% NH_4Cl, 0.1% $FeCl_3$, 80ppm streptomycin and 1.5% agar; pH 5.6±0.2) to isolate endophytic fungal spots. The newly emerged fungal spots were separated under sterilized conditions and grown on potatodextrose agar (PDA) medium plates for growth and storage (Khan *et al*., 2011a). Total eight different fungal strains were isolated and grown on PDA media. These strains were inoculated in Czapek broth (50 ml; 1% glucose, 1% peptone, 0.05% KCl, 0.05% $MgSO_4.7H_2O$, and 0.001% $FeSO_4.7H_2O$; pH 7.3±0.2) and grown for 7 days (shaking incubator -120 rpm; temperature 30ºC) to separate liquid culture medium and fungal mycelia (centrifugation 2500g at 4ºC for 15min). The culture medium (culture filtrate–CF, 50 ml) and mycelium (5.4gm) were immediately shifted to -70ºC freezer and then freeze-dried (Virtis Freeze Dryer, Gardiner, NY, USA) for 4-7 days. The lyophilized CF was diluted with one ml of autoclaved DDW, while the mycelia were used for genomic DNA extraction.

Screening for phytohormone production: To assess plant growth promoting or inhibiting and phytohormones producing fungal isolate, the CF of endophytes were screened to determine their effect on mutant *Waito-C* rice growth. *Waito–C* rice is a gibberellin biosynthesis mutant line. *Waito-C* rice seeds were surface sterilized with 2.5% sodium hypochlorite for 30 minutes, rinsed with autoclaved DDW, and then incubated for 24 h with 20-ppm uniconazol to obtained equally germinated seeds. Germinated rice seeds (moisture content 71%; germination 95%) were transplanted to autoclaved pots containing 0.8% water-agar medium and kept in growth chamber (day/night cycle: 14 hr- 28°C±0.2; 10 hr–25°C±0.2; relative humidity 70%; 18 seedlings per treatment) for further growth. After attaining two leaves stage, 10-µl of fungal CF was applied at seedling apex. One week after treatment, the shoot length, chlorophyll content and shoot fresh weight were recorded and compared with negative (autoclaved DDW) and positive controls (*Fusarium fujikuroi*). The wild-type strain of *F. fujikuroi* KCCM12329, provided by the Korean Culture Center of Microorganisms, was used as positive control. Upon screening results, bioactive fungal strain CAC-1G was selected for further experiments and identification.

DNA extraction and fungal isolate identification: Genomic DNA isolation and PCR was performed according to an established protocol (Khan *et al*., 2008). Fungal isolate was identified by sequencing the internal transcribed spacer (ITS) using 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′) (Taylor & Bruns 1999). 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 4 software (Tamura *et al.,* 2007), and the maximum parsimony tree was constructed using the same software. The bootstrap replications (1K) were used as a statistical support for the nodes in the phylogenetic tree.

Phytohormones extraction, isolation and detection from bioactive CF: To extract, isolate and characterize GAs secreted in the pure fungal culture of bioactive endophyte, it was inoculated in Czapek broth (120 ml) for 7 days at 30°C (shaking incubator-120 rpm). The culture medium of *F. fujikuroi* and bioactive endophyte (CF; 50 ml) were used to extract and purify GAs as described by Hamayun *et al*., (2010). Briefly, the pH of the CF was adjusted to 2.5 using 6 N HCl and was partitioned with ethyl acetate (EtOAc). Before partitioning, deuterated GAs internal standards (20 ng; [17, 17-$^2H_2$] GA_1, GA_3, GA_4, and GA_{12}) were added in the CF. Tritiated GAs i.e. [1, 2-$^3H_2$] GA_9 and [1,2-$^3H_2$] GA_{20} were also added (obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia). The organic layer was vacuum dried and added with 60% methanol (MeOH) while the pH was adjusted to 8.0±0.3 using 2 N NH_4OH. Similarly, endogenous GAs from cucumber plants treated with and without endophytic fungus and salinity stress were extracted from 0.5 g of freeze-dried plant samples according to the method of Lee *et al*., [31]. The CF extract was subjected to chromatographic and mass spectroscopy techniques for identification and quantification of GAs. The dried samples were subjected to high performance liquid chromatography (HPLC) using a 3.9×300 m Bondapak C18 column (Waters Corp., Milford, MA, USA) and eluted at 1.0ml/min with the following gradient: 0 to 5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5 to 35 min, linear gradient from 28% to 86% MeOH; 35 to 36 min, 86% to 100% MeOH; 36 to 40 min, isocratic 100% MeOH. Forty-eight fractions of 1.0 ml each were collected. 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 SIM. 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 whereas the same was done for endogenous GAs of cucumber plants. The fungal CF GAs were calculated from the peak area ratios of sample GAs to corresponding internal standards. The retention time was determined using hydrocarbon standards to calculate the KRI (Kovats retention index) value. The limit of detection was determined for all GAs. GC/MS SIM limit of detection was 40pg/ml for fungal CF. The data was calculated in nano-grams per milliliter (for fungal CF) and repeated three times.

The bioactive CF was assess for the presence of IAA with the help of High Performance Liquid Chromatography (HPLC) system, equipped with a differential ultraviolet (UV) detector absorbing at 280nm and a C18 (5µm; 25 x 0.46 cm) column. Mobile phase was methanol and water (80:20 [v/v]) at a flow rate of 1.5 ml/min. The sample injection volume was 10µl. Retention times for the analyte peaks were compared to those of authentic internal standards added to the medium and extracted by the same procedures used with fungal cultures. Quantification was done by comparison of peak area (Shahab *et al*., 2009).

Host-plant growth: The pepper seeds were surface sterilized as described earlier and the germinated seeds (28°C and relative humidity of 60%) were sown in autoclaved soil (200 g/pot of soil at 121°C for 90 min). The soil characteristics were: peat moss (13-18%), perlite (7-11%), coco-peat (63-68%) and zeolite (6-8%), with macro-nutrients present as: NH_4- ~90 mg Kg^{-1}; NO_3- ~205 mg Kg^{-1}; P_2O5 ~350 mg Kg^{-1} and K_2O ~100 mg Kg^{-1}. The culture medium (20 ml/pot) along with the propagules (10) of bioactive endophyte was applied to the one week grown pepper (*Capsicum annuum* L.) plants. For comparison, two kinds of control plants received (i) 20 ml/pot of endophyte-free medium (containing 1% glucose, 1% peptone, 0.05% KCl, 0.05% $MgSO_4.7H_2O$, and 0.001% $FeSO_4.7H_2O$; pH 7.3±0.2; shaking for 10 days at 30°C) and (ii) distilled water. The endophytic fungi and pepper plants were grown together for two weeks in growth chamber (day/night cycle: 14 hr- 28°C ±0.3; 10 hr –25°C ±0.3; relative humidity 60–65%; 18 plants per treatment) and irrigated with distilled water. The growth parameters i.e. shoot length and shoot fresh weights were measured for harvested cucumber plants, while chlorophyll content of fully expanded leaves were analyzed with the help of chlorophyll meter (SPAD-502 Minolta, Japan). Dry weights were measured after drying the plants at 70ºC for 72h in oven. Total leaf area was measured with Laser Leaf Area meter (CI-203 model, CID Inc., USA). For each measurement, readings were recorded in triplicates.

Results

Screening of plant hormone secreting bioactive endophyte: We isolated 13 endophytic fungi from 31 different secondary roots of three pepper plants. These endophytic fungi were grown on Hegam minimal media for seven days. The frequency of endophyte isolation was 2.34. However, upon morphological trait analysis of 13, only eight were different on the basis of colony shape, height and color of aerial hyphae, base color, growth rate, margin characteristics, surface texture and depth of growth into medium (Arnold *et al*., 2007). The CF of these endophytes were screened on dwarf *Waito-C* rice seedlings. The results showed that four fungi exhibited growth stimulatory effects and 3 strains exhibited inhibitory effects as compared to both DDW and *F. fujikuroi* treated rice seedlings (Table 1). In growth promotive strains, endophyte CAC-1G significantly increased the shoot length, shoot fresh weight, chlorophyll content and leaf area of pepper plants as compared to other endophytic CF and controls. In inhibitory strains, the CF of CAC-1A significantly suppressed the growth attributes of Waito-C rice seedlings as compared to control (Table 1).

Table 1. Effect of CF of endophytic fungal strains isolated from the roots of field grown cucumber plants on the growth of *Waito-C* rice seedlings.

Strains	TL (cm)	SL (cm)	CC (SPAD)	FW (g)
Control (Ff)	15.5 ± 0.29c	9.4 ± 0.32b	34.3 ± 1.4c	0.63 ± 0.19b
Control (DW)	13.25 ± 0.25d	7.2 ± 0.31d	32.87 ± 1.7d	0.61 ± 0.13b
CAC-1C	12.73 ± 0.37d	7.4 ± 0.21d	30 ± 0.58e	0.47 ± 0.11d
CAC-1G	18.26 ± 0.15a	11.5 ± 0.35a	40.8 ± 1.7a	0.71 ± 0.10a
CAC-1J	15.8 ± 0.32b	9.46 ± 0.29b	38.6 ± 1.1b	0.61 ± 0.12b
CAC-1F1	15.43 ± 0.44c	8.7 ± 0.31c	32.6 ± 1.36d	0.56 ± 0.18c
CAC-1E	14.2 ± 0.56d	8.46 ± 0.24c	35.13 ± 1.8c	0.59 ± 0.14c
CAC-1A	14.6 ± 0.42c	8.16 ± 0.55c	33 ± 1.11d	0.57 ± 0.22c
CAC-1H	14.46 ± 0.74c	8.33 ± 0.74c	35.8 ± 1.2c	0.55 ± 0.12c
CAC-IK	16.27 ± 0.67b	9.93 ± 0.33b	38.8 ± 1.6b	0.66 ± 0.14b

Control (Ff) = rice seedlings treated with the CF of a Wild-type strain of *F. fujikuroi* KCCM12329; Control (DW) = rice seedlings treated with autoclaved distilled water. SPAD = Soil plant analysis development. TL = total length; SL = shoot length; CC = chlorophyll content; FW= fresh weight. In each column, treatment means having different letter (s) are significantly (p<0.05) different as evaluated by DMRT. Values are mean ± SD (n=18).

CAC-1G identification and phylogenetic analysis: Phylogenetic analysis of the ITS sequence of CAC-1G was carried out through Mega 4.0 using maximum parsimony (MP) method to make a consensus tree from 15 (14 references and 1 clone) aligned ITS1 and ITS4 with 1,000 bootstrap replications. Results of BLASTn search revealed that sequence of fungal strain CAC-1G has 100% sequence similarity with *Chaetomium globosum*. In MP dendrogram, CAC-1G formed 63% bootstrap support with *C. globosum* (Fig. 1). On the basis of sequence similarity and phylogenetic analysis, fungal isolate CAC-1G was identified as *C. globosum* LK4. The 18S rDNA sequence was submitted to NCBI GenBank and was given accession no. JQ288106.

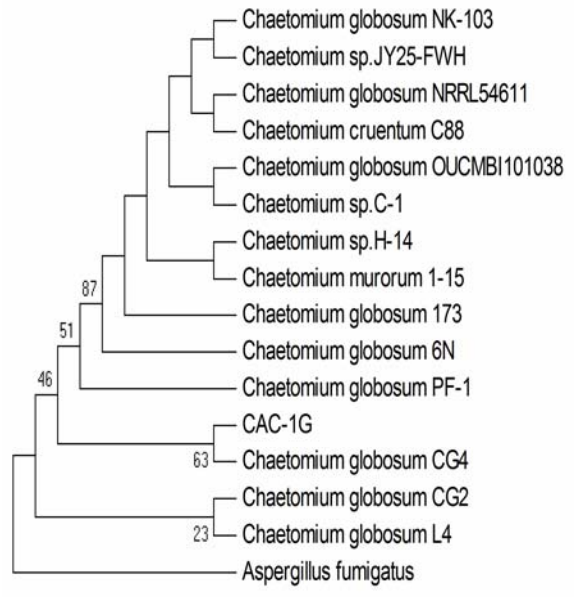

Fig. 1. Phylogenetic tree formed after maximum parsimony method using ITS sequence of *Chaetomium globosum* and related fungi. CAC-1G formed a clad (63% bootstrap support) with *C. globosum*. *Aspergillus fumigatus* was taken as an out group.

GAs and IAA secretion by *C. globosum* LK4: The pure culture filtrate of *C. globosum* LK4 was analyzed for the presence of GAs and IAA. In GAs extraction, isolation and detection, we found that the CF of the *C. globosum* had five different physiologically active and non-active gibberellins (Fig. 2). The GAs was detected through GC/MS selected ion monitor. Among biologically active GAs, GA_1 (0.67±0.13 ng/ml) and GA_4 (21.8±1.2 ng/ml) were found in the HPLC fractions. In physiologically inactive GAs, GA_9 (0.51±0.11 ng/ml), GA_{12} (13.4±0.41 ng/ml), GA_{20} (1.11±0.2 ng/ml) and were present in the CF. The quantities of bioactive GA_4 and GA_{12} were significantly higher as compared to other GAs (Fig. 2). In the culture of *F. fujikuroi*, we found bioactive GA_3 (12.4±1.03 ng/ml) and GA_4 (3.1±0.4 ng/ml), GA_9 (0.8±0.21 ng/ml), GA_{12} (13.1±1.11 ng/ml), GA_{20} (0.95±0.31 ng/ml). Besides GAs, we also found IAA in the growing culture medium of *C. globosum*. The quantity of IAA was 16.71±1.42 µg/ml.

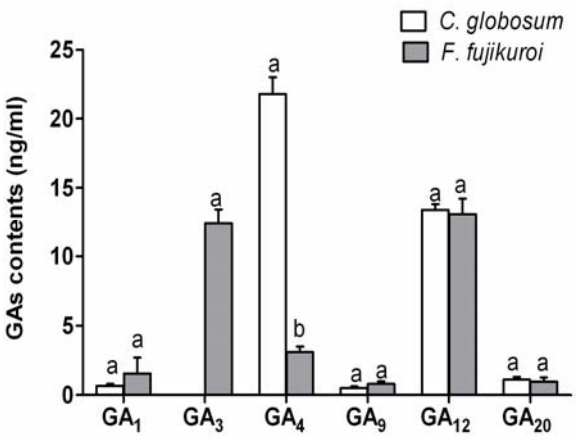

Fig. 2. Gibberellins detection in the CF of *C. globosum* and *F. fujikuroi*. For each set of treatment, the different letter(s) indicates significant differences between the CF of *C. globosum* and *F. fujikuroi* treatments at $p<0.05$ level by DMRT.

Effect of *C. globosum* on host-plant growth: The *C. globosum* inoculation to the host pepper plants significantly increased the shoot length as compared with the endophyte-free medium and water applied control plants. Similarly, the chlorophyll content, leaf area and shoot biomass were also significantly higher in plants inoculated with *C. globosum* as compared to control plants (Fig. 3).

Discussion

Increase in human population has exerted tremendous pressure on agriculture land to obtain higher crop productivity and satisfy the food demand. To avoid continues depletion of natural resources and achieve goals of sustainable agriculture development, various eco-friendly alternatives are available. Among them, symbiotic fungal associations with crops show considerable promise because of their effectiveness, habit-specific mode of action and ability to provide multiple benefits (Schulz & Boyle, 2005; Diene and Narisawa, 2009; Lamit and Gehring, 2012; Rodriguez *et al*., 2012). Endophytes have been presumed as one of the future resources for increasing crop productivity and alternative to synthetic chemicals (Diene & Narisawa, 2009). A plant endophytic fungus produces many natural bioactive compounds with dominant importance in agriculture, medicine and food industry (Schulz & Boyle, 2005; Zhao *et al*., 2010; Khan *et al*., 2011abc). In the present study, we isolated an endophytic fungus from the roots of drought stressed pepper plants. It was identified as a strain of *C. globosum* LK4 by sequencing the 18S rDNA regions. *Chaetomium* is a large genus of the fungal family *Chaetomiaceae* (Ascomycota) with over an hundred marine- and terrestrial-derived species. To date, more than 200 compounds have been reported from this genus (Li *et* al., 2011). Previously, endophyte *C. globosum* has been isolated from various economically important plants like *Ginkgo biloba* (Li *et al.,* 2011). From *C. globosum* a verity of bioactive secondary metabolites have been isolated and identified as reported by Qin *et al*., (2009) and Li *et al*., (2011). However, in present study we unveiled the phytohormone production capacity of this endophyte.

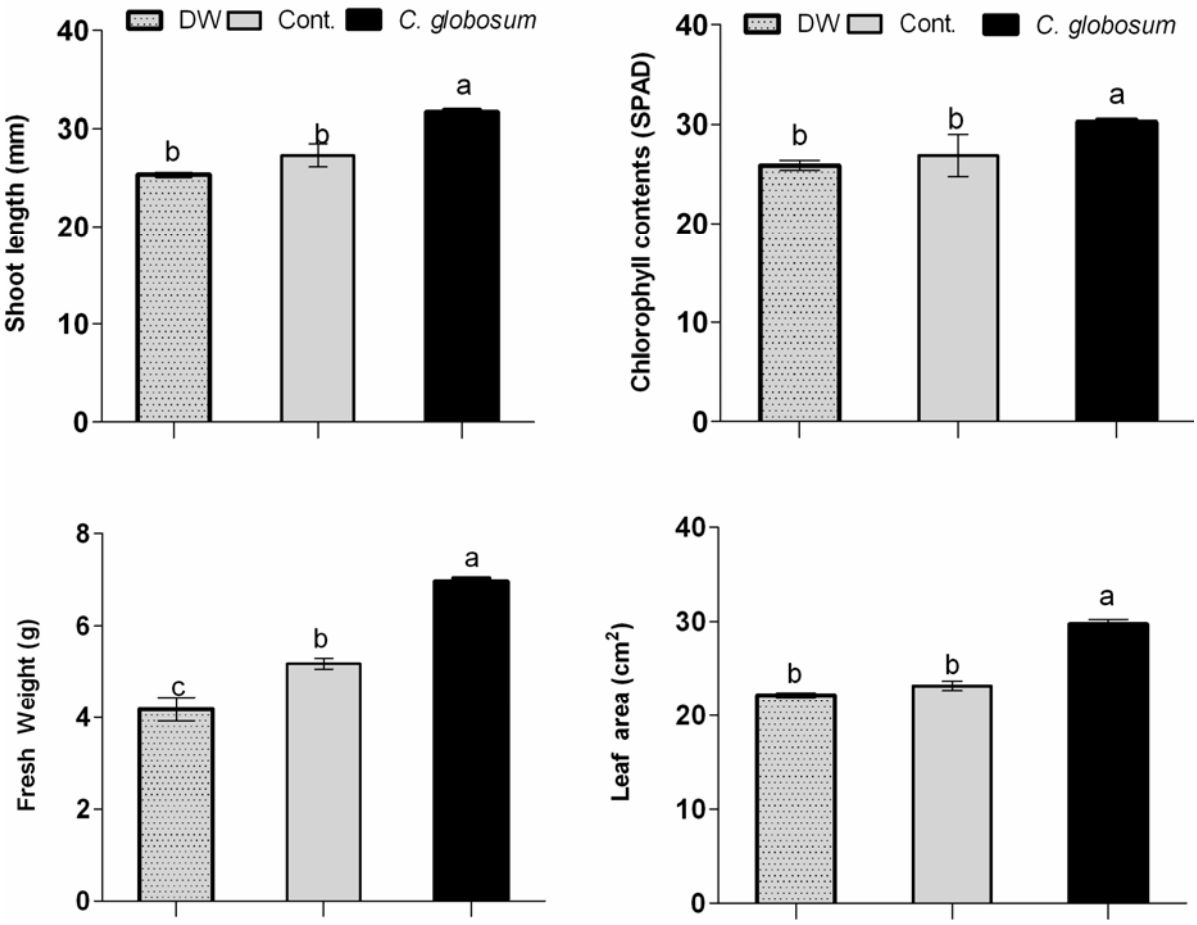

Fig. 3. Effect of endophyte-host association on the growth attributes of pepper plants. Each value is the mean ± SE of 6 plants per replicate per treatments (three replicates). For each set of treatment, the different letter(s) indicates significant differences between endophyte-host and without endophyte-host treatments at $p<0.05$ level by DMRT.

Previously, reports are available which elaborate the GAs and auxins secretion by endophyte (Hamayun *et al*., 2010; Khan *et al*., 2011abc). Gibberellins are the most important phytohormones and play an essential role in plant growth and development (Bomke & Tudzynski, 2009). In present study, we found that endophyte *C. globosum* produces various physiologically active and in-active GAs in its culture medium. It was observed that the CF of *C. globosum* significantly promotes the growth attributes of mutant rice *Waito*-C. Dwarf rice (*Waito*-C) is GAs biosynthesis mutant line having passive *dy* gene which synthesize bioactive GAs through C13 hydroxylation pathway (Ikada *et al*., 2001). The *Waito*-C rice seeds were treated with uniconazol to further suppress the GAs biosynthesis pathway (Ikada *et al*., 2001). The use of *Waito*-C rice can help in detection of a very small amount of GAs if present in the sample (Hamayun *et al*., 2010; Khan *et al*., 2011a). Agar and water, on the other hand, was used as growing medium for rice. During the bioassay, the rice seeds were devoid of any nutrients to accurately measure the sole effect of fungal CF (Soon-Ok *et al*., 2007). *Fusarium fujikuroi* corresponds to the mating group C of *Fusarium fujikuroi* and has the ability to produce GAs on industrial level (Takahashi *et al*., 1991). We compared the effect of CF of Wild type *F. fujikuroi* with that of *C. globosum* and found a similar growth promoting behaviour. The further comply the findings, CF of both *C. globosum* and *F. fujikuroi* were analyzed for GAs production through GC MS/SIM which is an established method to identify targeted novel secondary metabolites (Higgs *et al*., 2001). The repetition of our experiment and correlation with GAs detection in corresponding to deuterated GAs standards further helped to confirm our findings of GAs production. Several researchers reported the plant growth promoting characteristics and secretion of secondary metabolites including phytohormones of endophytic fungi mostly associated with roots (Rademacher, 1994; Kawaide, 2006; Khan *et al*., 2011abc). In the CF, we found GA_4 though the quantity of GA_1 was very low. In fungus, GA_{12}-aldehyde is 3b-hydroxylated to GA_{14}-aldehyde and then oxidized to form GA_{14}. The subsequent conversion of GA_{14} to GA_4 is comparable to the production of GA_9 and GA_{20} in plants. Desaturation of GA_4 results in the formation of GA_7 and then GA_3, which is the main product reported from *F. fujikuroi* (Bomke & Tudzynski, 2009; Khan et al., 2012). The endophyte also produced IAA in its culture medium. The presence of IAA in *C. globosum*

clearly suggests the existence of IAA biosynthesis pathway as reported for some other classes of fungi by Tuomi *et al*., (1993).

Endophytic mutualism can extend beneficial growth regulatory effects on host-plant under normal as well as extreme environmental conditions. In current study, the *C. globosum* has significantly increased the shoot growth and allied growth characteristics of the host pepper plants. The plant had higher chlorophyll content, shoot biomass and leaf area compared to both the controls, indicating growth ameliorative impacts on plants. In endophyte-host symbioses, secondary metabolites may be a contribution of the endophytic partner for such mutualistic relationship. Plants treated with endophytes are often healthier than those lacking such interaction (Schulz & Boyle, 2005), which may be attributed to the endophyte secretion of phytohormones such as IAA (Khan *et al*., 2011b) and GAs (Rademacher, 1994; Bomke *et al*., 2008; Kawaide, 2006; Hamayun *et al*., 2010). As such the practical applications of endophytes as potential sources of bioorganic nutrients and as biocontrol agents can significantly improve yields in eco-friendly method (Diene & Narisawa, 2009; Khan *et al*., 2012; Naz and Bano, 2012). Understanding such endophytic interactions can therefore help to improve the quality and productivity of agricultural crops.

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