

Conceptual Framework On Fungal Diversity and Plant Growth of Endophytic Fungi

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Abstract

The diversity and plant growth-promoting capacity of endophytic fungi related with the five flower plant. The Sorenson's coefficient similitude records of the endophytic fungi from the five flower plant species went from 0.36 to 0.80. It was discovered that the likeness list between two developed flowers (0.8) or the closeness file between two wild flowers (0.71–0.76) was higher than the similitude list between one developed flower and one wild flower (0.36–0.48). The Shannon records (H) of the endophytic fungi from the five plant species went from 1.73 to 2.45, and the diversity files of the wild flowers were higher than those of the developed flowers.

Keywords: *Endophytic fungi, plant growth, fungal diversity.*

1. INTRODUCTION

The diversity and its biological parts of endophytic fungi of the developed flower just as the distinction of fungal diversity between the wild flower and the developed flower have never been accounted for as of not long ago. In the present examination, the diversity of endophytic fungi of three wild flowers and two developed flowers just as their plant growth promoting capacity was explored. The results would not just give knowledge into the fungal diversity of the cut-flower planted in the nursery yet in addition add to comprehend the potential functions of endophytic fungi related with the flower plant.

Endophytic fungi live in the tissues of different plants without creating any side effects of sickness in the host. This environmental specialty with the ceaseless metabolic associations between the endophytic microorganism and the host plant appear to serve an amazing solid developmental pressure improving the combination of optional metabolites of endophytes with novel properties. Previous examinations of these microorganisms demonstrated that they are magnificent makers of intensifies that can be exploited for agrochemical or therapeutic purposes because of their biological movement (e.g., antiviral, antimicrobial, anticancer, insecticidal, immunosuppressive and cancer prevention agent impacts).

2. MATERIAL AND METHODS

Study Site and Test Assortment

Rosa rugosa, Camellia japonica, and Delonix regia filling in wild, while Dianthus caryophyllus and Rosa half and half planted in the nursery were gathered from Yunnan, Southwest China in May 2012. Among them, R. rugosa, R. half breed, and D. caryophyllus were gathered from Dounan, Kunming (102°78' N, 24°89' E), C. japonica was gathered from Jingdian, Kunming (102°76' N, 25°08' E) and D. regia was gathered from Xinping area (101°99' N, 24°07' E). For each plant species, 15 sound people in any event 10 m separated from one another were picked, and three solid and separate branches from each plant were

gathered indiscriminately. All examples were brought to lab in sterile polythene sacks and handled inside 24 h.

Screening for Plant Growth-Promoting Endophytes

To get the sterile seedlings, the tobacco (*Nicotiana tabacum*) seeds were surface cleaned by plunging into 75% ethanol (2 min) and 10% sodium hypochlorite (2 min), at that point, washed with sterile water and planted onto the Murashige and Skoog medium enhanced with sucrose 15 g l⁻¹ and agar 8 g l⁻¹, and brooded at 25°C for developing. The seedlings becoming out were liberated from the fungal endophytes.

An aggregate of 102 sterile tobacco seedlings of comparative size were utilized to screen the plant growth-promoting endophytes. Furthermore, 24 isolates of 6 prevailing genera (*Alternaria*, *Cladosporium*, *Colletotrichum*, *Phomopsis*, *Phoma* and *Ascomycetes*, and 4 isolates from every taxon) were chosen aimlessly to test their plant growth-promoting capacity as follows: to acquire the endophyte-immunized seedlings, a few fungal plates (measurement 0.5 cm) were cut from a new culture (4–7 days) of each isolate and afterward vaccinated onto the roots of sterile seedlings. Control seedlings were vaccinated utilizing agar circles without the fungus. There were four seedlings were immunized with each isolate. All seedlings were hatched at 25°C under a 14-h photoperiod.

Prior to transplanting into plastic pots, 24 seedlings vaccinated with 24 diverse endophytes and 3 control seedlings were chosen aimlessly to test the accomplishment of immunization. Positive contaminated seedling was confirmed by isolating the strain, which was utilized to immunize the seedling previously, from the surface-cleaned root and additionally shoot of the tried seedling, while, a similar fungus was not isolated from the control seedlings.

There were three reproduces for every treatment. The stature of the seedlings was estimated and recorded like clockwork for 50 days. The isolates which could improve the growth of tobacco seedlings essentially were chosen to lead the accompanying pot experiment.

Fungal Isolation, Culture and Identification

For the isolation of endophytic fungi, 15 solid stems and 15 sound leaves were chosen from each plant species at arbitrary, washed in running faucet water, and handled as follows: the examples were cut into sections (around 5 × 5 mm) and surface cleaned by consecutively plunging into 0.5% sodium hypochlorite (2 min) and 70% ethanol. At that point, 100 leaf fragments and 100 stem portions from each plant species were put in a Petri dish containing potato dextrose agar (PDA) medium corrected with 0.5 g l⁻¹ streptomycin sulfate, hatched at 25°C, and checked each other day for 45 days. The fungi becoming out of the plant tissues were moved to new PDA plates. The adequacy of the surface sanitization was constrained by making engravings of purified fragments on PDA plates.

The fungal recognizable proof depended on the morphology of the state, the instrument of spore creation, and the spore characteristics shaped in PDA or autoclaved carnation leaves in water agar. Sterile isolates were arranged into various gatherings based on province surface, hyphal pigmentation, edge shapes, and growth rates. Twenty representative isolates from 10 morphological taxa (2 for every taxon) were additionally recognized dependent on their rDNA interior interpreted spacer (ITS) grouping analysis, taking note of that matches in GenBank didn't really give right names. The entirety of the isolates were saved in the Faculty of Life Sciences and Technology, Kunming University of Science and Technology

Pot Experiments

The isolates H25 (*Phomopsis*), B50 (*Cladosporium*), A38 (*Alternaria*), and A7 (*Alternaria*), which demonstrated better plant growth-promoting capacity in above experiments, were chosen to do pot experiments

An aggregate of 40 sterile tobacco seedlings of comparative size were chosen. For each isolate, eight sterile tobacco seedlings were immunized, and eight tobacco seedlings vaccinated with agar plates without the fungus were filled in as the control check (CK). All treatments were brooded at 25°C under a 14-h photoperiod for about a month. At that point, eight seedlings vaccinated with endophytes (two seedlings for each strain), and two control seedlings were chosen indiscriminately to test the achievement of immunization as the method portrayed before. At long last, the entirety of the endophyte-immunized seedlings and control seedlings were transplanted into the plastic pots (26 cm measurement × 21 cm stature, three seedlings in each pot) containing 6 kg waterway sand and perlite (v/v, 2:1). Prior to placing into the plastic pots, the waterway sand was gone through a 0.5 mm strainer and blended in with perlite completely and autoclaved at 121°C for 2 h for multiple times. There were six repeats for each strain. All experimental seedlings were refined in a sunlit nursery with regular light, a 16/8-h day/night cycle at 25/18°C and 60–80% relative moistness. Seedlings were consistently watered with deionized–refined water and each pot got 300 ml sterile Hoagland's supplement week by week.

Plant Harvest and Chemical Component Test

Following 2 months' growth, the plants were harvested and flushed with deionized water and blotched up water with sterile channel paper. At that point, the leaf territory was estimated and recorded (six repeats for every treatment), and 300 mg leaves were utilized to lead the chlorophyll content analysis as per the method of Well consume and Lichtenthaler. At last, the leftover seedlings were stove dried at 80°C to quantify dry weight, and afterward, the dissolvable sugar was preceded as the method of analyst.

3. DATA ANALYSIS

The colonization rate (CR) was determined as the all-out number of plant tissue pieces contaminated by at least one fungi partitioned by the complete number sections hatched. The relative recurrence (RF) was determined as the quantity of isolates of one animal types separated by the absolute number of isolates.

The endophytic fungal diversity was assessed utilizing the Shannon file, which has two principle segments, uniformity and the quantity of species. The Shannon list (H) was determined by the accompanying recipe:

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

where k is the all-out species number of one plot and P_i is the relative abundance of endophytic fungal species in a single plot. To assess the level of network closeness of the endophytic fungi between the two treatments, Sorenson's coefficient comparability file (Cs) was utilized and determined by the accompanying equation:

Where j is the quantity of endophytic fungal species coinciding in two treatments, an is the absolute number of endophytic fungal species in a single treatment and b is the complete number of endophytic fungal species in another treatment. The analysis of change was utilized for data analysis of factorial experiments. The distinctions of plant biomass between endophyte-vaccinated and uninoculated plants were controlled by the most un-huge contrast (LSD) test. What's more, the dismissal level was set at $p < 0.05$. The chi-squared test was utilized to analyze the distinction in the CR of the endophytes between the stem and leaf. All data were analyzed by SPSS 11.5.

4. RESULT AND DISCUSSION

Plant Growth-Promoting Ability of Endophytic Fungi

As indicated by the method referenced above, it was discovered that the entirety of the endophyte-immunized seedlings were positive contaminated, unexpectedly, the control seedlings were negative tainted.

As appeared in Table 4, it was discovered that distinctive isolate indicated diverse growth-promoting capacity. For instance, the leaf territory and the dry load of the seedlings vaccinated with A7 were altogether expanded when contrasted and the control ($p < 0.01$, LSD test). Nonetheless, the leaf territory of the seedlings vaccinated with B50 and A38 demonstrated no huge changes. For the dissolvable sugar substance, the entirety of the endophyte-vaccinated seedlings was fundamentally expanded in comparison with the control ($p < 0.01$, LSD test). Likewise, the chlorophyll substance of the seedlings vaccinated with the endophytes were essentially expanded ($p < 0.01$, LSD test), aside from that immunized with A7 ($p < 0.05$, LSD test).

TABLE 1: Number, CR, and Shannon list (H) of the endophytic fungi (EF) from the 5 flower plant species

Host plants	No. of segments plated (No. of segments colonized by EF)			No. of EF isolated			CR (%)			H
	Stem	Leaf	Total	Stem	Leaf	Total	Stem	Leaf	Total	Total
<i>C. japonica</i>	100 (85)	100 (74)	200 (159)	85	78	163	85.0 ^a	74.0 ^a	79.5	2.45
<i>R. rugosa</i>	100 (68)	100 (14)	200 (82)	70	14	84	68.0 ^a	14.0 ^b	41.0	2.36
<i>D. regia</i>	100 (63)	100 (1)	200 (68)	63	1	64	63.0 ^a	1.0 ^b	32.0	1.95
<i>D. caryophyllus</i>	100 (15)	100 (12)	200 (27)	15	12	27	15.0 ^a	12.0 ^a	13.5	1.77
<i>R. hybrid</i>	100 (10)	100 (9)	200 (19)	10	9	19	10.0 ^a	9.0 ^a	9.5	1.73
Total	500 (246)	500 (110)	1000 (356)	243	114	357	48.2 ^a	22.0 ^b	35.1	

TABLE 2: Numbers, taxa, and RFs of the endophytic fungi from the 5 flower plant species

Taxa	No. of strains isolated from the 5 flower plant species (RF%)					
	<i>C. japonica</i>	<i>R. rugosa</i>	<i>D. regia</i>	<i>D. caryophyllus</i>	<i>R. hybrid</i>	Total (RF%)
<i>Alternaria</i> sp. ^a	40 (24.54)	25 (29.76)	1 (1.56)	8 (29.63)	3 (15.79)	77 (21.57)
<i>Aspergillus</i> sp.1	–	2 (2.38)	–	1 (3.70)	2 (10.53)	5 (1.40)
<i>Aspergillus</i> sp.2	1 (0.61)	1 (1.19)	–	–	–	2 (0.56)
<i>Cladosporium</i> sp. ^a	4 (2.45)	6 (7.14)	–	5 (18.52)	3 (15.79)	18 (5.04)
<i>Colletotrichum</i> sp. ^a	16 (9.82)	–	2 (3.13)	–	–	18 (5.04)
<i>Geotrichum</i> sp.	3 (1.84)	–	–	–	–	3 (0.84)
<i>Gloeosporium</i> sp. ^a	4 (2.45)	4 (4.76)	8 (12.50)	–	–	16 (4.48)
<i>Melanconium</i> sp.	–	2 (2.38)	1 (1.56)	–	–	3 (0.84)
<i>Meria</i> sp.	–	–	–	1 (3.70)	1 (5.26)	2 (0.56)
<i>Ovulariopsis</i> sp.	2 (1.23)	–	4 (6.25)	–	–	6 (1.68)
<i>Penicillium</i> sp.	1 (0.61)	–	1 (1.56)	–	2 (10.53)	4 (1.12)
<i>Pestalotia</i> sp.	6 (3.68)	–	–	–	–	6 (1.68)
<i>Phoma</i> sp. ^a	2 (1.23)	1 (1.19)	7 (10.94)	2 (7.41)	1 (5.26)	13 (3.64)
<i>Phomopsis</i> sp.1 ^a	–	5 (5.95)	27 (42.19)	–	–	32 (8.96)
<i>Phomopsis</i> sp.2 ^a	4 (2.45)	2 (2.38)	7 (10.94)	–	–	13 (3.64)
<i>Phomopsis</i> sp.3 ^a	7 (4.29)	9 (10.71)	1 (1.56)	–	–	17 (4.76)
<i>Rhynchosporium</i> sp. ^a	1 (0.61)	10 (11.90)	–	–	–	11 (3.08)
<i>Stemphylium</i> sp.	3 (1.84)	–	–	1 (3.70)	–	4 (1.12)
Sterile mycelium I	6 (3.68)	3 (3.57)	2 (3.13)	2 (7.41)	–	13 (3.64)
Sterile mycelium II	4 (2.45)	5 (5.95)	1 (1.56)	7 (25.93)	7 (36.84)	24 (6.72)
Sterile mycelium III	6 (3.68)	1 (1.19)	1 (1.56)	–	–	8 (2.24)
Sterile mycelium IV	4 (2.45)	6 (7.14)	–	–	–	10 (2.80)
Sterile mycelium V	23 (14.11)	1 (1.19)	1 (1.56)	–	–	25 (7.00)
Ascomycetes (one species, unidentified) ^a	26 (15.95)	1 (1.19)	–	–	–	27 (7.56)
Total	163 (100)	84 (100)	64 (100)	27 (100)	19 (100)	357 (100)
Number of taxa	20	17	14	8	7	24

TABLE 3: The Sorenson’s coefficient similarities indices (Cs) of the fungal endophytes from the 5 flower plant species

Plant	<i>R. rugosa</i>	<i>R. hybrid</i>	<i>D. caryophyllus</i>	<i>C. japonica</i>
<i>R. hybrid</i>	0.42			
<i>D. caryophyllus</i>	0.48	0.80		
<i>C. japonica</i>	0.76	0.37	0.43	
<i>D. regia</i>	0.71	0.38	0.36	0.71

The Sorenson's coefficient comparability file between two developed flowers or the likeness record between two wild flowers was higher than that between one developed flower and one wild flower. The result recommended that the endophytic fungal network was primarily impacted by the natural factors as opposed to the host plant species. The comparable result was accounted for by different analysts.

TABLE 4: The biomass of seedlings inoculated or inoculated with the endophytic fungi

Treatments	Leaf area (cm ²)	Plant dry weight (g)	Chlorophyll (mg/g FM)	Soluble sugar (mg/g FM)
CK	35.51 ± 20.14	0.32 ± 0.09	104.62 ± 0.29	27.26 ± 1.59
H25	73.25 ^{**} ± 13.37	0.46 [*] ± 0.07	142.67 ^{**} ± 1.34	54.78 ^{**} ± 1.24
B50	38.19 ± 6.89	0.50 [*] ± 0.09	142.69 ^{**} ± 0.14	46.14 ^{**} ± 4.68
A38	40.39 ± 13.11	0.54 ^{**} ± 0.08	145.64 ^{**} ± 0.57	38.26 ^{**} ± 3.98
A7	70.55 ^{**} ± 22.85	0.52 ^{**} ± 0.05	121.73 [*] ± 0.57	52.32 ^{**} ± 7.54

Note: Values with ‘*’ or ‘**’ in the same column refer to significant difference at the 0.05 and 0.01 levels according to LSD test, respectively. FM means the fresh weight

It has been accounted for that distinctive endophytes could improve plant's growth through various instruments. For example, researcher found that the endophytic fungi *Phomopsis liquidambari* can create enzyme to debase the phenolic corrosive allelochemicals which impacts affect the growth of plants to lighten the impacts of the natural pressure; researcher revealed that *Phoma glomerata* and *Penicillium* sp. could emit gibberellins (GAs) and indoleacetic corrosive to altogether promote the growth of GAs-insufficient rice, and researcher announced that the nitrogen-fixing endophytes of sugarcane contribute abundant nitrogen to the plant and improve plant growth. In the present investigation, it was discovered that various strains demonstrated diverse plant growth-promoting capacity (Table 4). It was assumed that these isolates improve tobacco seedlings' growth through various ways. Notwithstanding, to comprehend the instruments of the endophytic fungi in plant growth promoting, it actually needs more works.

Solvent sugars assume a clearly focal function in the plant structure and digestion at the cell and whole organism levels. They are engaged with the reactions to various anxieties, and they go about as supplement and metabolite flagging molecules that actuate hormone transduction pathways.

5. CONCLUSION

Chlorophyll is a key part of photosynthesis needed for the assimilation of daylight and hindrance of chlorophyll biosynthesis can prompt a few plants growth restraint and cell demise. In the present investigation, the substance of dissolvable sugar and chlorophyll of tobacco seedlings vaccinated with the endophytes were fundamentally expanded in comparison with the control ($p < 0.01$, LSD test; Table 4), which demonstrated that these endophytes are advantages to the hosts' growth. Thusly, these endophytes may have a potential use in horticulture to lighten ecological pressure and diminish farming expense later on.

6. REFERENCES

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