***Original Research Article***

# Nutrient concentration in growth medium affects relationship between root endophytic fungi and host plant

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## ABSTRACT

**Aims:** To clarify the effect of nutrient concentration in growth medium on the relationship between host plants, *Brassica campestris* and *Paraserianthes falcataria*, and endophytic fungi (EPF).
**Study design:** Inoculation of the two host plants growing in two media with different nutrient concentrations with 33 EPF.
**Place and duration of study:** Laboratory of Plant Nutrition and Soil Science, Faculty of Agriculture, Yamagata University, between 2016 and 2017.
**Methodology:** *B. campestris* and *P. falcataria* were grown on 1/100 and 1/10 strength Murashige and Skoog (MS) media. Both plants were inoculated with 33 EPF isolated from forest soils in Indonesia. *B. campestris* and *P. falcataria* were harvested 28 and 37 days after transplanting, respectively, and shoot dry weight (SDW) and colonization rate were measured. Plant response (PR) to EPF inoculation was calculated on the basis of SDW, as follows: PR = [SDW (inoculated) – SDW (control)] / SDW (inoculated).
**Results:** SDW of *B. campestris* grown on 1/100 strength MS medium inoculated with 2, 23 or 8 EPF was higher than, the same as, or lower than that of control plant, respectively. SDW of *B. campestris* grown on 1/10 strength MS medium inoculated with 24 or 9 EPF was the same as or lower than that of control plant, respectively. SDW of *P. falcataria* grown on 1/100 strength MS medium inoculated with 1 or 32 EPF was higher than or the same as that of control plant, respectively. SDW of *P. falcataria* grown on 1/10 strength MS medium inoculated with 11 or 22 EPF was the same as or lower than that of control plant, respectively.
**Conclusion:** These results suggest that nutrient concentration in growth medium affected the relationship between plant and EPF.



*Keywords: endophytic fungi, tropical forest, Brassica campestris, Paraserianthes falcataria, response*


## 1. INTRODUCTION

Endophytic fungi (EPF) refer to fungi that colonize plant tissue without causing any visible disease symptoms at any particular moment [1]. EPF colonize almost any plant tissue, be it leaf, stem or root [2]. Since their discovery, EPF have been studied in many types of plants, including non-vascular plants, such as mosses [3] and algae [4], and vascular plants, such as shrubs [3] and trees [5]. Most of the isolated EPF belong to Ascomycota and Basidiomycota [1].

Similar to mycorrhizal fungi, root EPF play an important role in plant growth [6]. EPF colonize 600 plant species from 114 families, indicating their abundance in nature. They are also found in temperate and tropical areas [7].

Studies of EPF in tropical areas are limited compared with those in temperate areas, particularly those in tropical forests. Nevertheless, studies of EPF in tropical forests are increasing in number, as exemplified by studies of leaf EPF in the American continent by Arnold et al. [8, 9] or root EPF by Rains et al. [10]. However, studies of root EPF in tropical forest have remained scarce [11].

Studies in temperate and boreal areas indicate that EPF colonization has a negative, neutral or positive effect on plant growth [12–14]. The application of the mutualism-parasitism paradigm to EPF has been considered [15], as in studies of mycorrhizal fungi [16]. The difference in plant growth response to EPF colonization is governed by not only plant or EPF species but also environmental factors, particularly experimental conditions [13]. Nutrient status may be an important factor as it also affects the relationship between plant and mycorrhizal fungi.

Murashige and Skoog (MS) medium is used to study the effect of EPF inoculation on plant growth. Nutrient concentration variation in MS medium is expressed as the dilution strength of MS medium. Mandyam et al. [12] used 1/10 strength MS medium and observed a positive response of plant growth to EPF inoculation. Lacercat-Didier et al. [17] used full-strength MS medium and observed a positive response of plant growth to EPF inoculation. The objective of the present study was to clarify the effect of two nutrient concentrations in growth medium on the relationship between host plants, *Brassica campestris* and *Paraserianthes falcataria*, and EPF.

## 2. MATERIALS AND METHODS

### 2.1 Inoculation of host plants with EPF

*B. campestris* and *P. falcataria* were used in this experiment. *B. campestris* was reported to be responsive to EPF colonization [18–20]. *P. falcataria* is a target plant for reforestation in Indonesia [21, 22]. The seeds of *B. campestris* (cv. Harusakari, Watanabe seed, Japan) or *P. falcataria* were surface-sterilized by dipping into 5% NaClO for 3 or 10 min, respectively. Then, the seeds were rinsed three times with sterilized deionized water. The surface-sterilized seeds were sown on water agar (1% agar) and grown in a growth chamber (Biotron LPH-350S, NK system, Japan) at 27 $^{o}$C with a 16-hour photoperiod. Two or one 7-day-old seedling of *B. campestris* or *P. falcataria*, respectively, was transplanted onto 1/100 or 1/10 strength MS medium in an 85-mm-diameter plastic Petri dish (Fig. 1, modified from [23]) and grown in the growth chamber at 27 $^{o}$C with a 16-hour photoperiod. A piece of sterilized filter paper (± 1 cm x 2 cm) was placed on top of *P. falcataria* seedling to fix the roots to the medium (Fig. 1). One 5-mm-diameter mycelial plug of EPF isolate was inoculated at the distance of 5 mm from the most distant root of one seedling, 7 days after transplanting (DAT). The Petri dish was sealed with Parafilm$^{TM}$ and kept in the growth chamber until 28 DAT. The use of 1/10 strength MS medium was based on Mandyam et al. [12, 23]. However, in the present study, we included not only the basal salt but also sucrose in the medium composition. Thirty-three EPF isolates were used in this experiment. Some EPF isolates showed dark septate hyphae. These EPF isolates were isolated from the roots of *P. falcataria* and *Sorghum bicolor* that grow on forest soils in Indonesia. These forest soils were from three forests in Kalimantan Island (*Gmelina* sp., *Artocarpus champeden*, and Dipterocarp mixed forest, Dipterocarp primary forest, and *Macaranga* sp. secondary forest)

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and two forests in Java Island (*Tectona grandis* monoculture forest and *Swietenia macrophylla* monoculture forest).

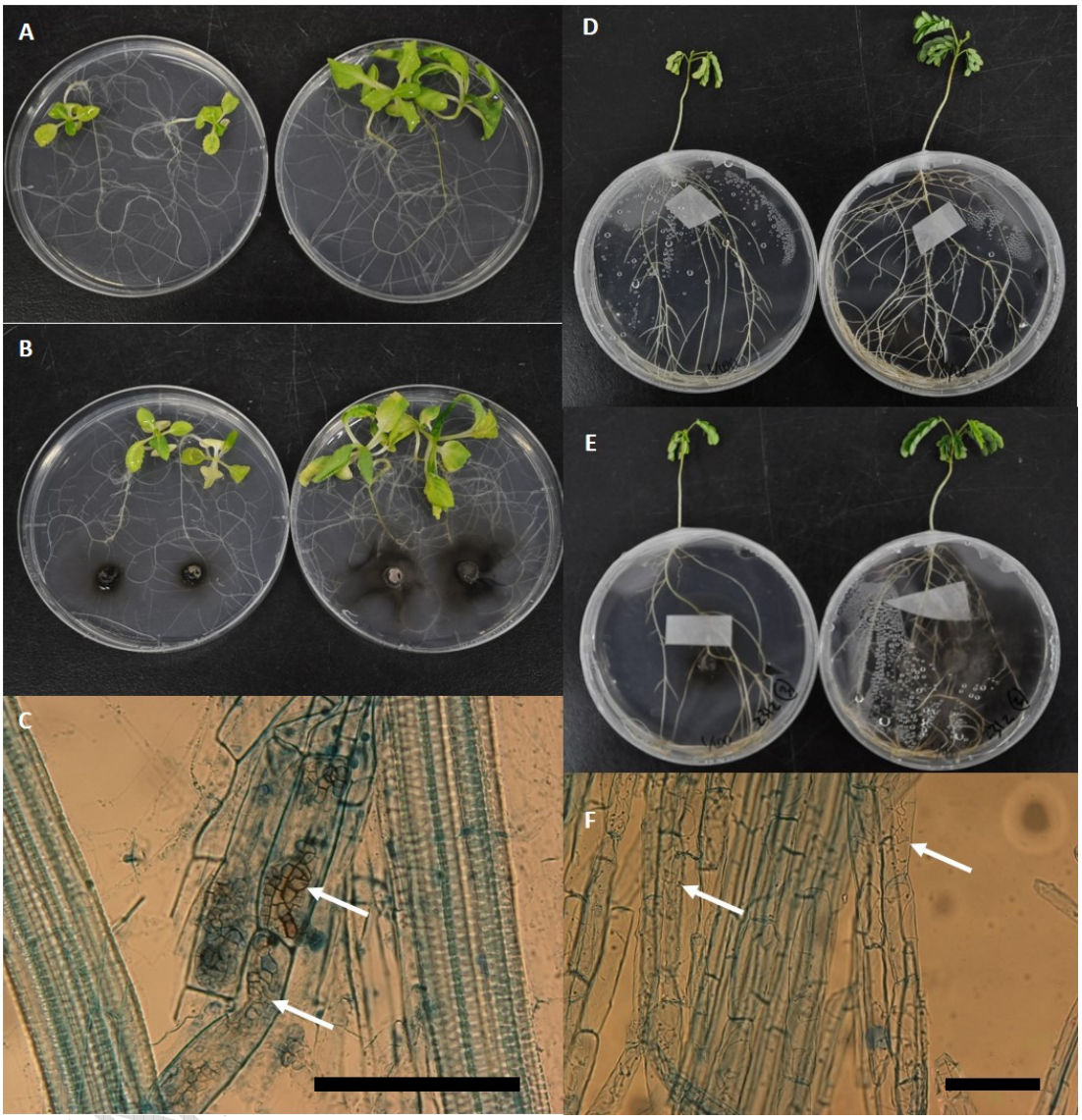


**Fig. 1. *Brassica campestris* not inoculated (A) or inoculated (B) with isolate 2312(3) and grown on 1/100 (left) and 1/10 (right) MS media, 28 DAT. Internal colonization (arrow) of *B. campestris* roots by isolate 2312(3) (C). *Paraserianthes falcataria* not inoculated (D) or inoculated (E) with isolate 2312(3) and grown on 1/100 (left) and 1/10 (right) MS media, 37 DAT. Internal colonization (arrow) of *P. falcataria* roots by isolate 2312(3) (F). Black bar = 100 µm.**

## 2.2 Growth parameters

The shoots of both plant species were cleaned under running tap water and rinsed with deionized water. The shoots were oven-dried at 70 $^{o}$C for 72 hours and weighed to

determine SDW. Plant response (PR) to EPF inoculation onto 1/100 and 1/10 strength MS media was calculated using the equation for mycorrhizal dependency formulated by Plenchette et al. [24]: PR = [SDW (inoculated) – SDW (control)] / SDW (inoculated).

## 2.3 Colonization by endophytic fungi

The roots were stained with aniline blue dye as described by Tawaraya et al. [25]. The roots of *B. campestris* or *P. falcataria* were cleaned by dipping into 10% (w/v) KOH solution and heated in a water bath at 80 $^{o}$C for 5 min or 15 min, respectively. Then, the roots were rinsed with tap water, acidified with 1% (w/v) HCl, and rinsed again with tap water. The roots were dipped into 0.05% aniline blue solution (Aniline blue, Wako, Japan) and heated again at 90 $^{o}$C for 5 min. After rinsing with tap water, the roots were transferred to a Petri dish and lactic acid-glycerol solution was added. The roots were mounted on glass slides and covered with cover glass. Colonization was observed under a microscope (Eclipse 80i, Nikon, Japan) at 200x magnification. The presence of fungal structures inside plant root indicated internal colonization (Tables 1 and 2). The presence of fungal structures on the surface of plant root indicated external colonization. Percentage colonization was estimated by the gridline intersect method on 100 intersections [26].

## 2.4 Statistical analysis

The significant difference in PR between 1/100 and 1/10 strength MS media and the significant difference in SDW between inoculated plant and respective control were determined by the Student's t-test using Kaleida Graph 4.1 software (Synergy software 2012, USA). Two of the 33 isolates used to inoculate *B. campestris* were excluded from statistical analysis because less than three replication plants survived until harvest. Those isolates were 2613(5)-1 and 2655(2). Thus, only 31 isolates were included in the statistical analysis.

# 3. RESULTS AND DISCUSSION

## 3.1 Results

### 3.1.1 Growth response of *B. campestris* to EPF inoculation

Two isolates increased, eight isolates decreased, and 21 isolates did not affect SDW of *B. campestris* grown on 1/100 strength MS medium (Table 1). Two isolates increased, three isolates decreased, and 26 isolates did not affect SDW of *B. campestris* grown on 1/10 strength MS medium. The number of isolates that increased SDW of *B. campestris* grown on 1/100 strength MS medium was the same as that grown on 1/10 strength MS medium. The number of isolates that decreased SDW of *B. campestris* grown on 1/100 strength MS medium was larger than that grown on 1/10 strength MS medium.

SDW of *B. campestris* grown on 1/100 strength MS medium inoculated with 2312(3) or 2334(2) was 1.6- or 1.8-fold significantly higher than control, respectively. SDW of *B. campestris* grown on 1/10 strength MS medium inoculated with 2334(2) was 1.4-fold significantly higher than control. SDW of *B. campestris* grown on 1/10 strength MS medium inoculated with 2312(3) and that grown on 1/100 and 1/10 strength MS media inoculated with 2331(2) or 2332(5) were not significantly different from control. Even so, SDW of *B. campestris* grown on 1/100 strength MS medium inoculated with 2331(2) or 2332(5) was 1.3- or 1.4-fold higher than control. SDW of *B. campestris* grown on 1/10 strength MS

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medium inoculated with 2312(3), 2331(2) or 2332(5) was 1.1-, 1.3- or 1.2-fold higher than control, respectively.

*B. campestris* inoculated with three isolates showed higher PR in the medium with low nutrient concentration than the one with high nutrient concentration (Fig. 2). *B. campestris* inoculated with 26 isolates showed the same PR in both nutrient concentrations. *B. campestris* inoculated with four isolates exhibited lower PR in the medium with low nutrient concentration than the one with high nutrient concentration.

### 3.1.2 EPF colonization of *B. campestris* root

Internal colonization was not always observed in the roots of inoculated *B. campestris* (Table 1). The number of intersections for colonization rate determination was between 11 and 100 depending on root availability. *B. campestris* inoculated with 2312(3) (Fig. 1C) or 2334(2) exhibited internal and external colonization, and both showed significantly higher SDW than control. Internal and external colonization was also observed in inoculated *B. campestris* that showed significantly lower SDW than control, for example, in *B. campestris* grown on 1/10 strength MS medium inoculated with 2655(2).

**Table 1. Shoot dry weight and internal/external colonization of *Brassica campestris* inoculated with or without endophytic fungi, 28 days after transplanting**

| EPF isolate | SDW (mg/plant) | | | | Internal colonization (%) | | External colonization (%) | |
|---|---|---|---|---|---|---|---|---|
| | 1/100 strength MS | | 1/10 strength MS | | 1/100 strength MS | 1/10 strength MS | 1/100 strength MS | 1/10 strength MS |
| Control | 5.4 | | 19.0 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2312(3) | 8.7 | ** | 20.6 | ns | 14.1 | 23.9 | 27.9 | 44.3 |
| 2331(2) | 7.2 | ns | 24.1 | ns | 90.8 | 78.6 | 0.0 | 5.2 |
| 2332(5) | 7.5 | ns | 21.8 | ns | 0.0 | 1.7 | 25.9 | 43.9 |
| 2334(2) | 9.9 | ** | 28.2 | * | 57.0 | 57.8 | 15.4 | 17.6 |
| Control | 10.3 | | 28.2 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2313(1) | 4.7 | * | 0.0 | | n.d. | n.d. | n.d. | n.d. |
| 2331(1)-1 | 6.8 | ns | 18.1 | ns | 0.0 | 0.0 | 74.0 | 90.0 |
| 2331(1)-2 | 8.7 | ns | 18.7 | ns | 23.3 | 52.9 | 60.6 | 46.4 |
| 2332(2) | 4.5 | * | 14.9 | ns | n.d. | n.d. | n.d. | n.d. |
| 2352(5) | 4.6 | * | 17.6 | ns | n.d. | n.d. | n.d. | n.d. |
| 2354(1)-1 | 10.0 | ns | 18.7 | ns | 2.4 | 10.7 | 20.4 | 40.6 |
| 2624(5) | 5.3 | * | 16.1 | ns | n.d. | n.d. | n.d. | n.d. |
| 2633(5)-1 | 4.5 | * | 17.2 | ns | n.d. | n.d. | n.d. | n.d. |
| 2633(5)-2 | 9.0 | ns | 29.4 | ns | 0.0 | 31.9 | 11.3 | 19.7 |
| Control | 8.0 | | 22.3 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 26321) | 7.7 | ns | 27.6 | ns | 0.5 | 2.0 | 39.0 | 6.6 |
| 2633(1) | 9.1 | ns | 29.2 | * | 3.3 | 10.5 | 47.6 | 34.3 |
| 2651(3) | 5.6 | * | 19.6 | ns | n.d. | n.d. | n.d. | n.d. |
| 2653(3)-1 | 6.8 | ns | 24.8 | ns | n.d. | n.d. | n.d. | n.d. |

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|---|---|---|---|---|---|---|---|---|
| Control | 6.1 | | 15.0 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2612(4) | 6.5 | ns | 15.9 | ns | 0.0 | 0.0 | 0.0 | 0.0 |
| Control | 4.5 | | 16.4 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2613(5)-1 | 2.53† | | 1.59† | | n.d. | n.d. | n.d. | n.d. |
| 2613(5)-2 | 4.4 | ns | 15.2 | ns | 0.0 | 0.0 | 1.6 | 1.0 |
| 2624(1) | 4.3 | ns | 15.6 | ns | 0.4 | 0.0 | 1 | 0.0 |
| 2632(5) | 4.6 | ns | 14.5 | ns | 0.0 | 0.0 | 4.9 | 0.0 |
| 2633(2) | 5.7 | ns | 17.3 | ns | 0.0 | 0.0 | 31.4 | 22.4 |
| 2635(4) | 4.5 | ns | 13.1 | ns | 0.0 | 0.0 | 27.5 | 7.5 |
| 2655(2) | 0† | | 2† | | n.d. | n.d. | n.d. | n.d. |
| Control | 12.1 | | 31.0 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2331(5) | 10.5 | n | 27.3 | ns | 0.0 | 4.0 | 0.8 | 13.4 |
| 2354(1)-2 | 10.4 | ns | 21.4 | * | 0.0 | 0.0 | 10.6 | 4.6 |
| 2612(2) | 9.9 | ** | 21.2 | * | 0.0 | 0.0 | 0.0 | 3.8 |
| 2612(3)-1 | 10.6 | n | 23.6 | ns | 0.0 | 0.0 | 0.2 | 7.6 |
| 2652(4) | 8.2 | * | 21.6 | * | 0.0 | 0.0 | 65.6 | 32.6 |
| Control | 4.7 | | 17.1 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2651(4) | 5.8 | ns | 19.2 | ns | 0.2 | 0.0 | 1.2 | 1.6 |
| 2652(1) | 5.0 | ns | 19.4 | ns | 0.0 | 0.0 | 0.0 | 0.0 |
| 2652(3) | 5.5 | ns | 18.2 | ns | 0.0 | 0.0 | 0.0 | 0.0 |

*Significant difference between inoculated plant and respective control was determined by the Student's t-test (*** P < .0001; ** P < .01; * P < .05; ns, not significant; n= 5). †The number of replication plants that survived until time of harvest was <3 and thus excluded from statistical analysis. Colonization rate could not be determined in some plants due to insufficient number of roots for observation, and is indicated by n.d. (not determined).*

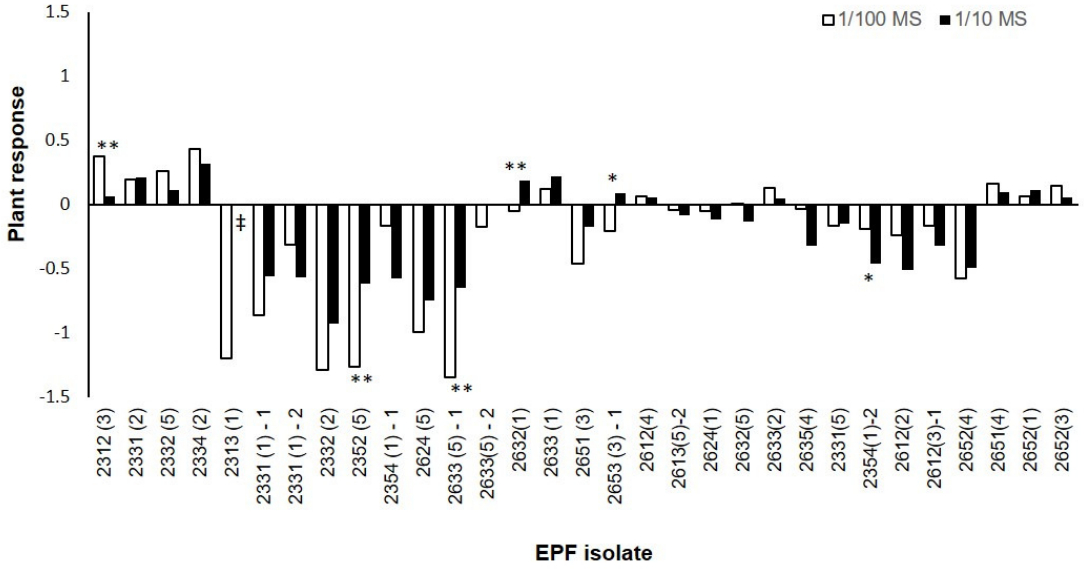


**Fig. 2. Response of *Brassica campestris* to inoculation with 31 EPF isolates and growth on 1/100 strength MS medium (open bar) or 1/10 strength MS medium (closed**

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**bar). Values higher (lower) than 0 on the x-axis indicate positive (negative) response. Significant difference in PR between plant grown on 1/100 strength MS medium and that grown on 1/10 strength MS medium was determined by the Student's t-test (** *P* < .01, * *P* < .05; n= 5). ‡ The denominator was zero.**

### 3.1.3 Growth response of *P. falcataria* to EPF inoculation

One isolate increased, no isolate decreased, and 32 isolates did not affect SDW of *P. falcataria* grown on 1/100 strength MS medium (Table 2). No isolate increased, 11 isolates decreased, and 22 isolates did not affect SDW of *P. falcataria* grown on 1/10 strength MS medium. The number of isolates that decreased SDW of *P. falcataria* grown on 1/100 strength MS medium was smaller than that grown on 1/10 strength MS medium.

*P. falcataria* inoculated with eight isolates showed higher PR in the medium with low nutrient concentration than in that with high nutrient concentration (Fig. 3). *P. falcataria* inoculated with 24 isolates exhibited the same PR in both nutrient concentrations. *P. falcataria* inoculated with one isolate showed lower PR in the medium with low nutrient concentration than in that with high nutrient concentration.

### 3.1.4 EPF colonization of *P. falcataria* root

Internal colonization was not always observed in the roots of inoculated *P. falcataria* (Table 2). The number of intersections for colonization rate determination was between 30 and 100 depending on root availability. *P. falcataria* inoculated with 2312(3) exhibited internal and external colonization (Fig. 1F) although there was no significant difference in SDW between the inoculated plant and control. Internal colonization was not observed in *P. falcataria* inoculated with 2651(4), which showed significantly higher SDW than control. Internal and external colonization was observed in inoculated *P. falcataria* that showed significantly lower SDW than control, for example, in *P. falcataria* grown on 1/10 strength MS medium inoculated with 2352(5).

**Table 2. Shoot dry weight and internal/external colonization of *Paraserianthes falcataria* inoculated with or without endophytic fungi, 37 days after transplanting**

| EPF isolate | SDW (mg/plant) | | | | Internal colonization (%) | | External colonization (%) | |
|---|---|---|---|---|---|---|---|---|
| | 1/100 strength MS | | 1/10 strength MS | | 1/100 strength MS | 1/10 strength MS | 1/100 strength MS | 1/10 strength MS |
| Control | 23.0 | | 51.0 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2313(1) | 21.7 | ns | 53.1 | ns | 0.0 | 0.0 | 0.3 | 14.3 |
| 2612(2) | 25.5 | ns | 54.8 | ns | 0.0 | 0.0 | 0.0 | 2.8 |
| 2612(3)-1 | 22.1 | ns | 56.4 | ns | 0.0 | 0.0 | 0.0 | 0.5 |
| 2613(5)-1 | 22.7 | ns | 40.1 | * | 0.0 | 0.0 | 0.0 | 8.5 |
| 2633(2) | 26.6 | ns | 46.7 | ns | 0.0 | 0.0 | 0.0 | 5.5 |
| 2633(5)-1 | 22.3 | ns | 50.7 | ns | 0.0 | 0.0 | 0.5 | 1.5 |
| 2652(1) | 23.2 | ns | 37.7 | * | 0.0 | 0.0 | 0.0 | 2.3 |
| Control | 23.7 | | 54.3 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2331(1)-1 | 19.8 | ns | 50.6 | ns | 0.0 | 0.0 | 0.3 | 7.5 |

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|---|---|---|---|---|---|---|---|---|
| 2331(1)-2 | 21.5 | ns | 48.9 | ns | 0.0 | 0.0 | 0.3 | 10.5 |
| 2354(1)-2 | 23.6 | ns | 58.6 | ns | 0.0 | 0.0 | 0.0 | 1.0 |
| 2613(5)-2 | 20.6 | ns | 58.1 | ns | 0.0 | 0.0 | 0.0 | 0.5 |
| 2624(5) | 23.0 | ns | 52.3 | ns | 0.0 | 0.0 | 1.3 | 5.8 |
| 2632(1) | 22.5 | ns | 51.2 | ns | 0.0 | 0.0 | 1.3 | 3.3 |
| 2633(5)-2 | 18.4 | ns | 55.7 | ns | 0.0 | 0.0 | 0.3 | 1.8 |
| Control | 21.9 | | 58.8 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2354(1)-1 | 25.7 | ns | 51.8 | ns | 0.0 | 0.0 | 1.8 | 0.5 |
| 2612(4) | 24.0 | ns | 42.8 | ns | 0.0 | 0.0 | 0.0 | 0.3 |
| 2624(1) | 18.4 | ns | 49.8 | * | 0.0 | 0.0 | 0.0 | 0.0 |
| 2633(1) | 21.4 | ns | 59.0 | ns | 3.5 | 0.5 | 4.0 | 1.0 |
| 2651(4) | 26.9 | * | 53.1 | ns | 0.0 | 0.0 | 0.3 | 0.3 |
| 2652(3) | 27.7 | ns | 54.6 | ns | 0.0 | 0.0 | 0.0 | 0.0 |
| 2653(3)-1 | 22.1 | ns | 52.4 | ns | 5.5 | 7.3 | 10.8 | 5.5 |
| 2655(2) | 23.2 | ns | 44.5 | * | 23.5 | 0.5 | 0.0 | 1.0 |
| Control | 20.5 | | 52.3 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2331(5) | 18.3 | ns | 50.3 | ns | 5.5 | 41.3 | 1.5 | 18.8 |
| 2332(2) | 21.6 | ns | 28.0 | *** | 0.0 | 0.0 | 0.0 | 0.0 |
| 2352(5) | 16.5 | ns | 15.4 | ** | 3.3 | 6.3 | 5.6 | 25.0 |
| 2632(5) | 17.7 | ns | 39.1 | * | 4.3 | 3.8 | 3.0 | 5.3 |
| 2635(4) | 21.2 | ns | 38.1 | * | 0.0 | 0.5 | 4.5 | 16.8 |
| 2651(3) | 17.7 | ns | 23.5 | ** | 6.6 | 0.9 | 10.8 | 13.4 |
| 2652(4) | 20.3 | ns | 37.3 | * | 0.0 | 0.0 | 11.0 | 24.3 |
| Control | 23.1 | | 56.0 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2312(3) | 24.2 | ns | 58.8 | ns | 5.8 | 21.5 | 6.8 | 14.3 |
| 2332(5) | 23.2 | ns | 63.3 | ns | 3.3 | 7.5 | 5.3 | 14.3 |
| 2334(2) | 23.3 | ns | 52.1 | ns | 2.5 | 0.5 | 5.8 | 1.0 |
| Control | 25.3 | | 62.3 | | 0.0 | 0.0 | 0.0 | 0.0 |
| 2331(2) | 21.1 | ns | 57.1 | * | 0.3 | 0.5 | 0.0 | 0.5 |

*Significant difference between inoculated and respective control was determined by the Student's t-test (*** P < .0001; ** P < .01; * P < .05; ns, not significant; n= 5).*

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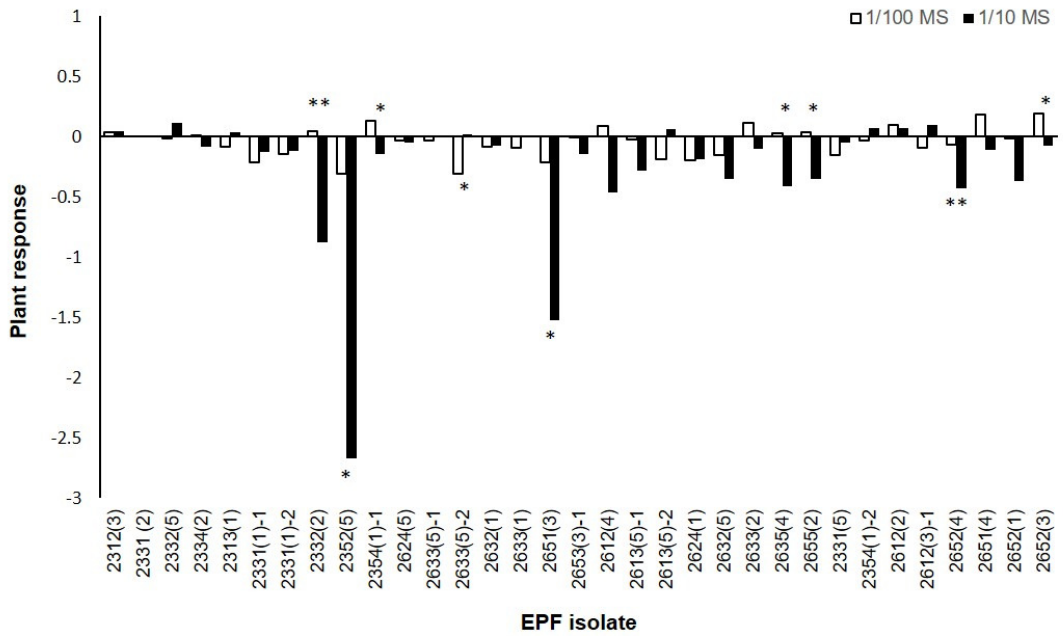


**Fig. 3. Response of *Paraserianthes falcataria* to inoculation with 33 EPF isolates and growth on 1/100 strength MS medium (open bar) or 1/10 strength MS medium (closed bar). Values higher (lower) than 0 on the x-axis indicate positive (negative) response. Significant difference in PR between plant grown on 1/100 strength MS medium and that grown on 1/10 strength MS medium was determined by the Student's t-test (** *P* < .01, * *P* < .05; n= 5).**

## 3.2 Discussion

### 3.2.1 Nutrient concentration in growth medium affects the relationship between host plant and EPF

Studies of EPF, particularly leaf EPF, in the tropics are increasing, as exemplified by studies conducted in Panama [8, 9] and India [27], whereas studies of root EPF are scarce. Studies of root EPF in a temperate country by Mandyam et al. [12, 23] showed that EPF inoculation resulted in such PRs as parasitism and mutualism. In addition, Mandyam et al. [12] and Lacercat-Didier et al. [17] recorded positive PR upon EPF inoculation despite using MS media with different nutrient concentrations. Based on those two studies, it seems that nutrient concentration in medium does not have any effect on the relationship between EPF and plant. However, further work involving different nutrient concentrations under the same experimental conditions including plant species and EPF strain is needed to come to a definite conclusion. In the present study, our intent was to clarify the effect of inoculation of tropical EPF on plant growth, with nutrient concentration in medium as a possible factor determining PR to EPF inoculation. In addition, due to lack of studies of the effect of EPF on plant growth in the tropics, evidence obtained from studies in temperate countries was used to explain the results of the present study.

Two inoculated *B. campestris* and one inoculated *P. falcataria* exhibited increased SDW when grown on 1/100 strength MS medium but not on 1/10 strength MS medium. The number of inoculated *B. campestris* and *P. falcataria* with more positive PR was larger when

grown on 1/100 strength MS medium than 1/10 strength MS medium. The difference between 1/100 and 1/10 strength MS media was the concentrations of all the nutrients contained in the media. Mutualism between *B. campestris* or *P. falcataria* and EPF was achieved when nutrient concentration was low. However, we could not clarify which nutrient was the driver of mutualism in this association. In the case of mycorrhizal association, particularly arbuscular mycorrhizal association, phosphorus (P) concentration in the media is generally thought to be one of the important drivers of mutualism [28]. Some studies have documented the importance of P in the association between EPF and Brassicaceae species. Hiruma et al. [29] inoculated *Arabidopsis thaliana* with *Colletotrichum tofieldiae* and grew it on half-strength MS media without sucrose and with two concentrations of P: 0.68 mg 100 $g^{-1}$ (low P) and 8.51 mg 100 $g^{-1}$ (high P). *C. tofieldiae* increased SFW of *A. thaliana* grown on MS medium with low P. Almario et al. [30] inoculated *Arabis alpina* with Helotiales species and grew it on MS agar with two concentrations of P: 100 µM (low P) and 1000 µM (high P). The results corresponded to the present study and Hiruma et al.'s study [29], namely, growth of *A. alpina* was promoted when conducted on medium with low P.

Nitrogen is also an important macronutrient that possibly exerts an effect on the relationship between EPF and plant. Usuki and Narisawa [18] inoculated *B. campestris* with *Heteroconium chaetospira* and grew it on basal agar medium with different forms of nitrogen ($NO_3$, $NH_4$, glutamine, leucine, phenylalanine, and valine). *B. campestris* dry weight was increased by inoculating *H. chaetospira* when the medium contained organic nitrogen and not inorganic nitrogen. In the present study, we used only inorganic nitrogen in the medium and found that it may not affect the relationship between EPF and plant. P concentration in the growth medium may have an effect on the relationship between EPF and host plant.

Some inoculated *B. campestris* and *P. falcataria* showed no difference in SDW even if those plants were grown on 1/100 strength MS medium. Hiruma et al. [29] also inoculated *A. thaliana* with *C. incanum*. However, SFW of inoculated *A. thaliana* was the same as control. The result was different when *A. thaliana* was inoculated with *C. tofieldiae*. These findings indicate that EPF show functional diversity in promoting plant growth.

The number of inoculated *B. campestris* with decreased SDW when grown on 1/10 strength MS medium was larger than that grown on 1/100 strength MS medium. Nutrient concentration in the 1/10 strength MS medium was higher than that in the 1/100 strength MS medium. Besides the high P in the 1/10 strength MS medium, carbon from sucrose is a possible nutrient affecting the association between EPF and host plant. EPF can survive by being a biotroph or a saprotroph. In this regard, acquiring carbon from the growth medium without forming symbiosis with plant is possible for EPF. By acquiring carbon and other nutrients from the growth medium, EPF may indirectly limit nutrient availability for plant growth. This hypothesis may apply to EPF that are not parasitic. If the EPF are parasitic, when carbon and other nutrients are sufficient for the EPF, the EPF are likely to colonize and limit plant growth directly. However, further studies are needed to confirm this hypothesis.

### 3.2.2 Effect of EPF inoculation on plant species

SDWs of *B. campestris* and *P. falcataria* inoculated with same EPF isolates were increased, not affected, or decreased relative to control plant. EPF that increased SDW of *B. campestris* did not always increase SDW of *P. falcataria*. Different PRs to the inoculation of the same EPF were also observed by Mandyam et al. [23]. Mandyam et al. [23] inoculated leek (*Allium porrum* L.) and $C_4$ grass (*Andropogon gerardii* Vitman) with *Microdochium* sp. and *Periconia macrospinosa*. Internal colonization of *A. porrum* root was observed but the total biomass was not affected by the EPF inoculation. Internal colonization of *A. gerardii* root was observed, but in contrast to *A. porrum*, the total biomass of *A. gerardii* was

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increased or not affected by the EPF inoculation. Mandyam et al. [12] inoculated three genotypes of *Arabidopsis thaliana* (Col-0, Cvi-0, Kin-1) with four strains of *Microdochium* sp. and 34 strains of *Periconia* sp. Inoculation of the same EPF resulted in different PRs among the *A. thaliana* genotypes, underscoring the fact that PR to EPF inoculation differs with not only plant species but also plant genotype.

## 4. CONCLUSION

Nutrient concentration of growth medium affected PR to inoculation with EPF. Positive response in terms of plant growth was observed in MS medium with low nutrient concentration. Further research is needed to determine the element affecting the relationship between host plant and EPF.

## COMPETING INTERESTS

The authors declare no conflicts of interest.

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