

Original Research Article

Nutrient concentration of growth media affect relationship between root endophytic fungi and host plant

ABSTRACT

Aims: To clarify the effect of different nutrient concentration in growth media on the relationship between host plant, *Brassica campestris* and *Paraserianthes falcataria*, and endophytic fungi (EPF).

Study design: Inoculation of 33 EPF to 2 host plant and grew them on 2 media with different nutrient concentration.

Place and Duration of Study: Laboratory of Plant Nutrition and Soil Science, Faculty of Agriculture, Yamagata University, between 2016-2017.

Methodology: *Brassica campestris* and *Paraserianthes falcataria* were grown on 1/100 and 1/10 MS media. Thirty-three EPF isolated from forest soils in Indonesia were inoculated to both plants. *B. campestris* and *P. falcataria* were harvested, 28 and 37 days after transplant, respectively. Shoot dry weight and colonization rate were measured. Plant responsiveness to EPF inoculation were calculated based on shoot dry weight (SDW). Plant responsiveness (PR) was calculated by $PR = [SDW (inoculated) - SDW (control)] / SDW (inoculated)$.

Results: SDW of *B. campestris* grown on 1/100 MS inoculated with 2, 23 or 8 EPF were higher than, same as or lower than control plant, respectively. SDW of *B. campestris* grown on 1/10 MS inoculated with 24 or 9 EPF were same as or lower than control plant, respectively. SDW of *P. falcataria* grown on 1/100 MS inoculated with 1 or 32 EPF were higher than or same as control plant, respectively. SDW of *P. falcataria* grown on 1/10 MS inoculated with 11 or 22 EPF were same as or lower than control plant, respectively.

Conclusion: These results suggest that nutrient concentration in growth media affected relationship between plant and EPF.

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

1. INTRODUCTION

The term endophytic fungi (EPF) refer to fungi which colonize the plant tissue without causing any visible disease symptoms at any particular moment [1]. Practically, EPF was found to colonize in almost any part of the plant tissue, either it is leaf, stem and root [2]. Since their discovery, EPF have been studied in many types of plants including non-vascular plant like mosses [e.g. 3] and algae [e.g. 4] to vascular plant like shrubs [e.g. 3] and trees [e.g. 5]. Most of the isolated EPF belong to Ascomycota and Basidiomycota [1].

Root EPF are considered to have an important role in plant growth, like mycorrhizal fungi [6]. EPF is found to colonized 600 species of 114 families, representing its abundance in the nature. It was also found in temperate and tropical area [7].

Studies about EPF in tropical area are more limited than that in temperate area, particularly in tropical forest. There are increasing studies about EPF in tropical forest for example in American continent about leaf EPF by Arnold et al [8, 9] or root EPF by Rains et al [10]. However, study about root EPF in tropical forest remained scarce [11].

Evidences from studies in temperate and boreal area showed that effect of EPF colonization on plant growth is negative, neutral or positive [12, 13, 14]. From these evidence, mutualism-parasitism-paradigm has been considered to be applied on EPF [15], as in studies of mycorrhizal fungi [16].

Difference of plant growth response to EPF colonization is not only governed by the plant or EPF species but also environmental factors especially experimental condition [13]. Inside this experimental condition, nutrient status may be an important factor as it also affects relationship between plant and mycorrhizal fungi.

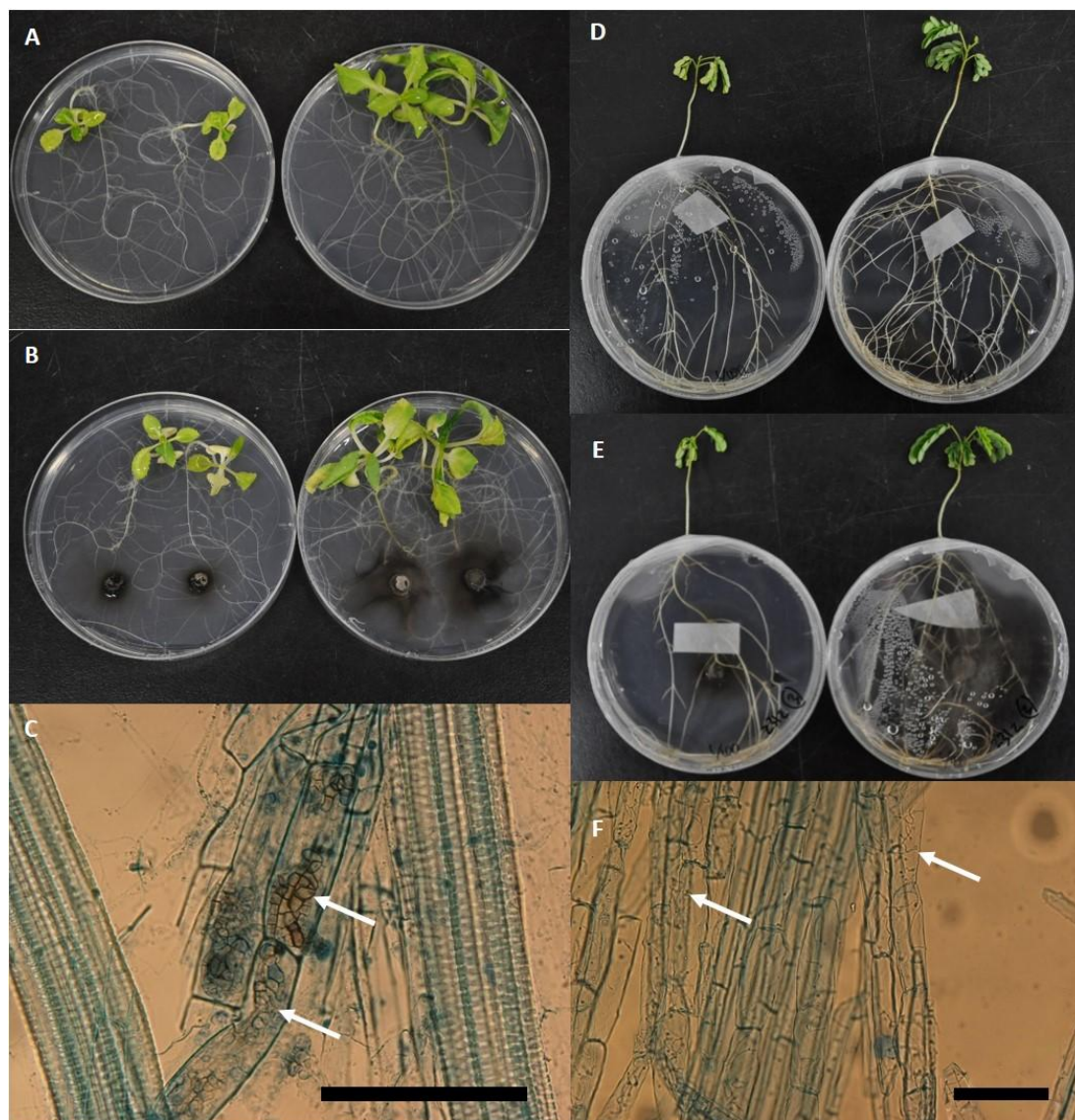
Murashige and Skoog (MS) media is being used to study the effect of EPF inoculation on plant growth. There are variation in nutrient concentration in MS media displayed as dilution strength of the MS media. Mandyam et al [12] used 1/10 strength MS media and observed positive response of plant growth to EPF inoculation. Lacercat-Didier et al [17] used full strength MS media and observed positive response of plant growth to EPF inoculation. Addition of more MS media in experiment of Mandyam and Jumpponen [15] did not show significant difference on plant response upon EPF inoculation. Although the plant and EPF species are different in these studies, there is same tendency showing that the effect of nutrient concentration of media to plant response upon EPF inoculation is not significant. However this conclusion is still premature until there are studies that confirm it by experiments involving different nutrient concentration in the same experimental condition including plant species and EPF strain. The objective of present study was to clarify the effect of two different nutrient concentration of growth media on relationship between host plants, *Brassica campestris* and *Paraserianthes falcataria*, and EPF.

2. MATERIAL AND METHODS

2.1 Inoculation of EPF to host plants

B. campestris and *P. falcataria* were used in this experiment. *B. campestris* was reported to be responsive to EPF colonization [e.g. 18, 19, 20]. *P. falcataria* was a target plant for reforestation. *P. falcataria* was reported to be used for reforestation purpose [21, 22]. 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. Seeds were then rinsed 3 times with sterilized deionized water. Surface sterilized seeds were sown on water agar (1% agar) and grown in growth chamber (Biotron LPH-350S, NK system, Japan) at 27 °C with 16 hours photoperiod. Two or one 7-day-old seedling of *B. campestris* or *P. falcataria*, respectively, were transplanted on 1/100 or 1/10 strength Murashige and Skoog (MS) media in 85-mm-diameter plastic Petridish (Fig. 1) and grown in growth chamber days at 27 °C with 16 hours photoperiod. Sterilized filter paper (± 1 cm x 2 cm) was placed on top of *P. falcataria* to fix seedling root to the media (Fig. 1). One of 5-mm-diameter mycelial plug of EPF isolate were inoculated on 5-mm-distance from the most distant root of 1 seedling on 7 days after transplanting (DAT). Petridish were sealed by Parafilm™ and grown in growth chamber until 28 DAT. Utilization of 1/10 strength MS media was based on Mandyam et al [12, 23], however in the present study we included sucrose in the media composition, not only the basal salt. Thirty three EPF isolates were used in this experiment without differentiating whether it is dark septate endophyte or not. Thirty-three EPF isolates were

82 isolated from root of *P. falcataria* and *Sorghum bicolor* that grown on forest soils of
83 Indonesia. These forest soils were from three forests in Kalimantan Island (*Gmelina* sp.,
84 *Artocarpus champeden* and Dipterocarp mixed forest, Dipterocarp primary forest,
85 *Macaranga* sp. secondary forest) and two forests in Java Island (*Tectona grandis*
86 monoculture forest, *Swietenia macrophylla* monoculture forest).
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90 **Fig. 1. *Brassica campestris* inoculated without (A) or with isolate 2312(3) (B) grown on**
91 **1/100 (left) and 1/10 (right) MS media, 28 days after transplanting. Internal colonization**
92 **(arrow) by isolate 2312(3) in the root of *Brassica campestris* (C). *Paraserianthes***
93 ***falcataria* inoculated without (D) or with isolate 2312(3) (E) grown on 1/100 (left) and**
94 **1/10 (right) MS media, 37 days after transplanting. Internal colonization (arrow) by**
95 **isolate 2312(3) in the root of *Paraserianthes falcataria* (F). Black bar = 100 μm.**
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2.2 Growth parameters

Shoot of both plant species were cleaned under running tap water, rinse with deionized water. Shoot were oven dried at 70 °C for 72 hours and weighed for shoot dry weight (SDW). Plant response (PR) to EPF inoculation on 1/100 (PR_{1/100}) and 1/10 (PR_{1/10}) MS media was calculated using the formula of mycorrhizal dependency of Plenchette et al [24]: $PR = [SDW (inoculated) - SDW (control)] / SDW(inoculated)$.

2.3 Observation of EPF colonization

Roots were stained by aniline blue dye as described by Tawaraya et al [25]. Roots of *B. campestris* or *P. falcata* were cleaned by dipping into 10% (w/v) KOH solution and heated in water bath at 80 °C for 5 min or 15 min, respectively. Roots were rinsed by tap water, acidified by 1% (w/v) HCl and rinsed again by tap water. Then roots were dipped into 0.05% aniline blue solution (Aniline blue, Wako, Japan) and heated again at 90 °C for 5 min. After rinsed by tap water, roots were transferred to Petridish and lactic acid-glycerol solution was added, subsequently. Roots were put on glass slide and covered with glass cover. Presence of colonization were observed under microscope (Eclipse 80i, Nikon, Japan) at 200x magnification. Presence of fungal structure inside the plant root was considered as internal colonization (Tables 1 and 2). Presence of fungal structure on the surface of plant root was considered as external colonization. Percentage of colonization was estimated by the gridline intersect method on 100 intersections [26].

2.4 Statistical analysis

Significant difference of plant responsiveness between 1/100 and 1/10 MS media and significant difference of SDW between inoculated plant and respective control were determined by Student's T-test using Kaleida Graph 4.1 software (Synergy software 2012, USA). Two from 33 isolates that inoculated to *B. campestris* were excluded from statistical analysis because less than three replication plant 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, 8 isolates decreased and 21 isolates did not affect SDW of *B. campestris* grown on 1/100 MS media (Table 1). Two isolates increased, 3 isolates decreased and 26 isolates did not affect SDW of *B. campestris* grown on 1/10 MS media. Number of isolates that increased SDW of *B. campestris* grown on 1/100 MS media were the same as that grown on 1/10 MS media. Number of isolates that decreased SDW of *B. campestris* grown on 1/100 MS media were higher than that grown on 1/10 MS media.

SDW of *B. campestris* inoculated with 2312(3) or 2334(2) grown on 1/100 MS media were 1.6 or 1.8 fold significantly higher than control, respectively. SDW of *B. campestris* inoculated with 2334(2) grown on 1/10 MS media were 1.4 fold higher than control. SDW of *B. campestris* inoculated with 2312(3) grown on 1/10 MS media and inoculated with 2331(2) or 2332(5) grown on 1/100 and 1/10 MS media were not significantly higher than control. SDW of *B. campestris* inoculated with 2331(2) or 2332(5) grown on 1/100 MS media were

1.3 or 1.4 fold higher than control. SDW of *B. campestris* inoculated with 2312(3), 2331(2) or 2332(5) that grown on 1/10 MS media were 1.1, 1.3 or 1.2 higher than control, respectively.

B.campestris inoculated with 3 isolates had higher PR in low than high nutrient concentration (Fig. 2). *B.campestris* inoculated with 26 isolates had same PR in both nutrient concentration. *B.campestris* inoculated with 4 isolates had lower PR in low than high nutrient concentration.

3.1.2 EPF Colonization in root of *B. campestris*

Internal colonization was not always observed in root of inoculated *B. campestris* (Table 1). Number of intersection for determination of colonization rate were between 11-100 intersections depending on the availability of root. Internal and external colonization was observed in *B. campestris* inoculated with 2312(3) (Fig. 1C) or 2334(2) which showed significantly higher SDW than control. Even so, external colonization was observed on the surface of root of this inoculated *B. campestris*. Internal and external colonization was also observed in inoculated *B. campestris* which showed significantly lower SDW than control for example in *B. campestris* inoculated with 2655(2) grown on 1/10 MS media.

Table 1. Shoot dry weight, internal and or 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 MS		1/10 MS		1/100 MS	1/10 MS	1/100 MS	1/10 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
2632(1)	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.

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 and respective control was determined by Student's T-test (** $P < .0001$, ** $P < .01$, * $P < .05$, ns not significant; $n = 5$). †Replication plant that survived until the time of harvest was less than 3, thus excluded from statistical analysis. Colonization rate could not be determined in some plants, indicated by n.d (not determined), due to not enough amount of roots for observation.

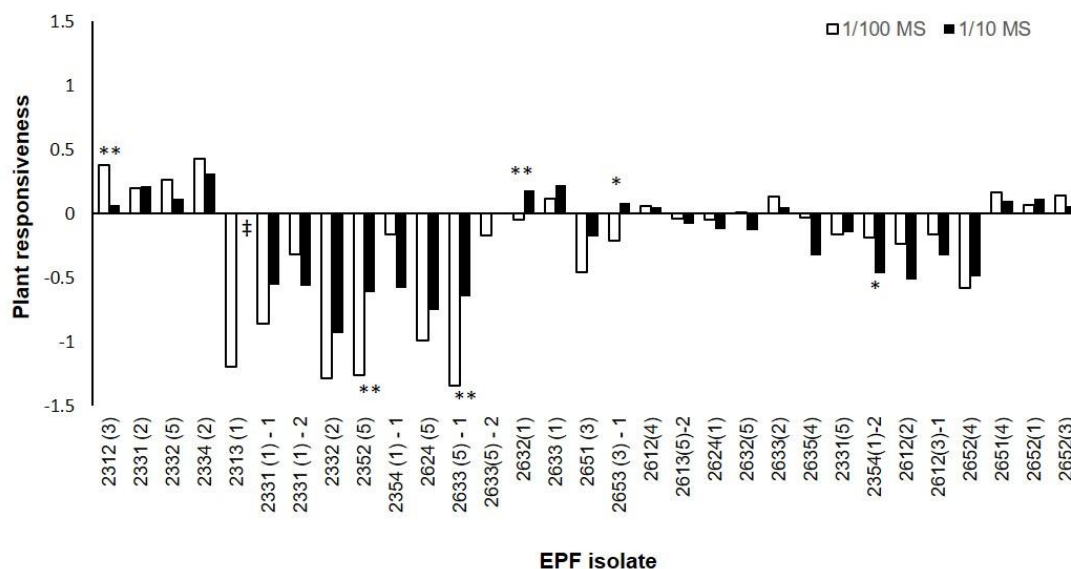


Fig. 2. Responsiveness of *Brassica campestris* to inoculation with 31 isolates of endophytic fungi grown on 1/100 strength MS media (open bar) or 1/10 strength MS media (closed bar). Values higher than 0 on x-axis indicate positive response, while values lower indicate negative. Significant difference between plant responsiveness grown on 1/100 MS and 1/10 MS media was determined by Student's T-test (** $P < .01$, * $P < .05$; n= 5). ‡ Denominator was zero.

3.1.3 Growth response of *P. falcatoria* to EPF inoculation

One isolate increased, no isolate decreased and 32 isolates did not affect SDW of *P. falcatoria* grown on 1/100 MS media (Table 2). No isolate increased, 11 isolates decreased and 22 isolates did not affect SDW of *P. falcatoria* grown on 1/10 MS media. Number of isolates that decreased SDW of *P. falcatoria* grown on 1/100 MS media were lower than that grown on 1/10 MS media.

P. falcatoria inoculated with 8 isolates had higher PR in low than high nutrient concentration (Fig. 3). *P. falcatoria* inoculated with 24 isolates had same PR in both nutrient concentrations. *P. falcatoria* inoculated with 1 isolate had lower PR in low than high nutrient concentration.

3.1.4 EPF Colonization in root of *P. falcatoria*

Internal colonization was not always observed in root of inoculated *P. falcatoria* (Table 2). Number of intersection for determination of colonization rate were between 30-100 intersections depending on the availability of root. Internal and external colonization was observed in *P. falcatoria* inoculated with 2312(3) (Fig. 1F) although there were no significant difference between SDW of inoculated plant and control. Internal colonization was not observed in *P. falcatoria* inoculated with 2651(4) which showed significantly higher SDW of inoculated plant than control. Internal and external colonization were observed in inoculated *P. falcatoria* which showed significantly lower SDW than control for example in *P. falcatoria* inoculated with 2352(5) grown on 1/10 MS media.

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

EPF isolate	Shoot DW (mg/plant)		Internal colonization (%)		External colonization (%)	
	1/100 MS	1/10 MS	1/100 MS	1/10 MS	1/100 MS	1/10 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
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 Student's T-test
 (***) $P < .0001$, ** $P < .01$, * $P < .05$, ns not significant; n= 5)

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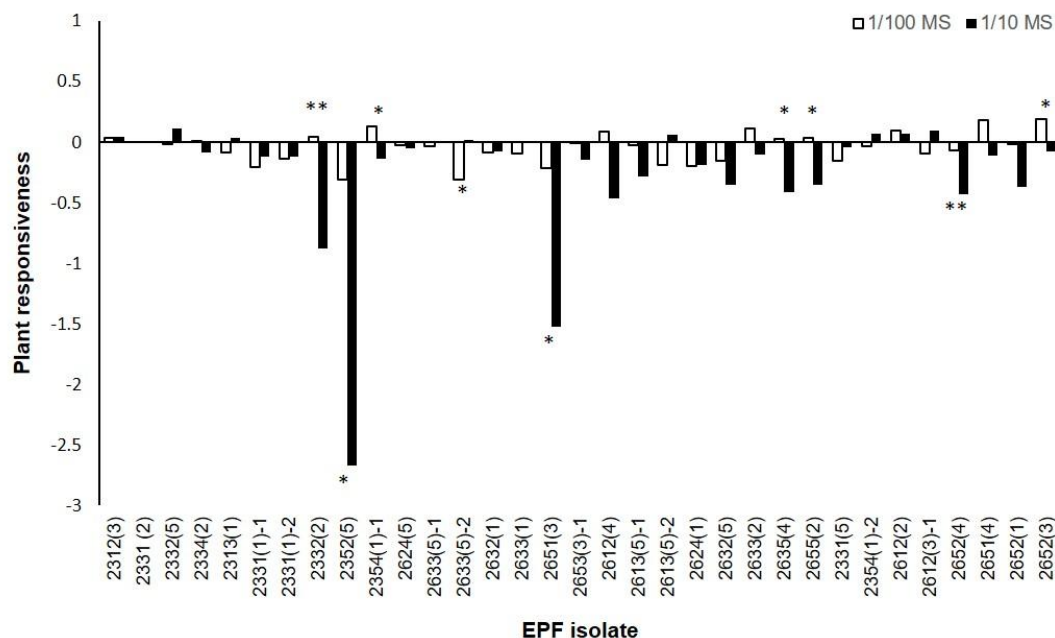


Fig. 3. Responsiveness of *Paraserianthes falcataria* to inoculation with 33 isolates of endophytic fungi grown on 1/100 strength MS media (open bar) or 1/10 strength MS media (closed bar). Values higher than 0 on x-axis indicate positive response, while values lower indicate negative. Significant difference between plant responsiveness grown on 1/100 MS and 1/10 MS media was determined by Student's T-test ($P < .01$, * $P < .05$; $n = 5$).**

3.2 Discussion

3.2.1 Nutrient concentration of growth media affected the relationship between host plant and EPF

Study about EPF in tropics is increasing, especially leaf EPF, for example in Panama [8, 9] and India [27]. Study about root EPF is scarcer. These studies were concerned only about the diversity of EPF without research about its effect on plant growth. Studies in temperate by Mandyam et al [12, 23] showed that EPF inoculation resulted in plant response ranging from parasitism to mutualism. Present study was intended to know the effect of inoculation of tropical EPF on plant growth, with concern of nutrient concentration in media as a possible factor determining the plant response to EPF inoculation. In addition, due to the lack of studies in tropics about effect of EPF on plant growth, evidences from temperate was used to explain the results of present study.

SDW of two inoculated *B. campestris* and one inoculated *P. falcataria* were increased if grown on 1/100 MS but not on 1/10 MS media. Number of inoculated *B. campestris* and *P. falcataria* with more positive PR were also observed higher when grown on 1/100 MS than that grown on 1/10 MS. The difference between 1/100 MS and 1/10 MS media were the concentration of all nutrient contained in the media. Mutualism between *B. campestris* or *P. falcataria* and EPF were achieved when the nutrient concentration was low. However it is not clear which nutrient was the driver of the mutualism in this association. In case of mycorrhizal association, particularly arbuscular mycorrhizal association, phosphorus

concentration in the media is generally thought to be one of important driver of mutualism [28]. There are some studies that documented the importance of phosphorus in association between EPF and plant. One of those studies were conducted by Hiruma et al [29] whom inoculated *Colletotrichum tofieldiae* to *Arabidopsis thaliana* and grown it on half strength MS media without sucrose with two concentration of P: 0.68 mg 100 g⁻¹ (low P) and 8.51 mg 100 g⁻¹ (high P). In the result, *C. tofieldiae* increased shoot fresh weight (SFW) of *A. thaliana* when grown on MS media with low P but not on high P. Beside phosphorus, nitrogen is also an important macronutrient which possibly has effect on relationship between EPF and plant. Usuki and Narisawa [18] inoculated *Heteroconium chaetospora* to *B. campestris* and grew the on basal agar media with different nitrogen forms (NO₃, NH₄, glutamine, leucine, phenylalanine and valine). Dry weight of *B. campestris* was increased by inoculation of *H. chaetospora* when the media was supplied with organic nitrogen form but not with inorganic nitrogen. Present study only used inorganic nitrogen in the media. Inorganic nitrogen in the media may not affected the relationship between EPF and plant. The concentration of phosphorus in the growth media may had effect in relationship between EPF and host plant in the present study.

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

Number of inoculated *B. campestris* with decreased SDW when grown on 1/10 MS were higher than that grown on 1/100 MS media. The nutrient concentration in 1/10 MS were higher than 1/100 MS. Beside higher phosphorus in 1/10 MS media, carbon from sucrose was one possible nutrient affected association between EPF and host plant. EPF can survive by being biotroph or saprotroph. Thus acquiring carbon from the growth media without forming symbiosis with plant is possible for EPF. If the EPF acquire the carbon and other nutrients from the growth media, there is possibility that it will indirectly limit nutrient availability for plant growth. This prediction may be prevail for EPF which is not parasitic fungi to the plant. If the EPF is parasitic to the plant, when carbon and other nutrient is sufficient for the EPF, it is likely to colonize and limit plant growth directly. However, further studies is needed to confirm this predictions.

3.2.2 Effect of EPF inoculation to different plant species

SDW of *B. campestris* and *P. falcata* inoculated with same EPF isolates were increased, not affected or decreased compared to control plant. EPF that increased SDW of *B. campestris* did not always increase SDW of *P. falcata*. Different plant response to inoculation of same EPF were also observed in the study of Mandyam et al [23]. Mandyam et al [23] inoculated *Microdochium* sp. and *Periconia macrospinos* to leek (*Allium porrum* L.) and C₄ grass *Andropogon gerrardii* Vitman. The colonization inside the root of *A. porrum* was observed but the total biomass were not affected or decreased by inoculation of EPF. The colonization inside the root of *A. gerrardii* was observed, but in contrast to *A. porrum*, total biomass of *A. gerrardii* was increased or not affected by inoculation of EPF. In different study, Mandyam et al [12] inoculated 4 strains of *Microdochium* sp. and 34 strains of *Periconia* sp. to three genotypes of *Arabidopsis thaliana* (Col-0, Cvi-0, Kin-1). Inoculation of same EPF resulted in different plant response among the *A. thaliana* genotypes. This evidences emphasize that plant response to EPF inoculation were different not only depending on the plant species but also plant genotypes.

305 4. CONCLUSION

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307 Nutrient concentration of growth media affected the plant responsiveness to inoculation with
308 EPF. Positive response in term of plant growth was observed in lower nutrient concentration
309 of MS media. Further research is needed to determine the element that was the main factor
310 affecting relationship between host plant and EPF.

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314 COMPETING INTERESTS

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316 Authors have declared that no competing interests exist.

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