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Effect of Arbuscular Mycorrhizal Fungi and Their Partner Bacteria on the Growth of Sesame Plants and the Concentration of Sesamin in the Seeds

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Abstract

Arbuscular mycorrhizal fungi (AMF) can stimulate the plant growth. *Pseudomonas* sp. (KCIGC01) NBRC109613 isolated from the spores of *Glomus clarum* IK97, an AMF, is reported to support the plant growth and development as partner bacteria (PB) for AMF [1]. In order to investigate the effect of *G. clarum* IK97 and *Pseudomonas* sp. (KCIGC01) NBRC109613 on the secondary metabolites, these microorganisms were inoculated to sesame plants. The inoculation of these microorganisms stimulated the growth of sesame. The rate of sesame root colonization in *G. clarum* IK97 + *Pseudomonas* sp. (KCIGC01) NBRC109613 inoculated plants (66.4% ± 4.4%) was higher than that in *G. clarum* IK97 alone inoculated plants (39.2% ± 5.8%). Furthermore, the content of sesamin in sesame seeds was increased by the inoculation of these microorganisms. In particular, the content of sesamin in the treatment inoculated with *G. clarum* IK97 and *Pseudomonas* sp. (KCIGC01) NBRC-109613 was 11.4 ± 1.5 mg/g seed. The results suggest that AMF and their partner bacteria can stimulate the growth and development of sesame plants and increase the content of sesamin in the seeds.

Keywords

Arbuscular Mycorrhizal Fungi, Partner Bacteria, Sesamin, Sesamolin

1. Introduction

Arbuscular mycorrhizal fungi (AMF) live as obligate symbionts on almost all of the terrestrial plant roots in-

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cluding many agriculturally and horticulturally important crop species [2] [3]. These AMF enhance the plant growth, plant water stress tolerance [4], plant health [5], nutrient cycling and soil quality [6]. Because of their beneficial effect on plant growth, AMF are important soil microorganisms for natural and managed ecosystems.

There are many publications about specific bacteria that promote interactions between AMF and plants. This may serve as a third partner in AMF symbiosis [7]-[9]. The cytoplasm of AMF spores contains some intracellular structures similar to bacteria called bacterium-like organisms, frequently located in the vacuoles [10] [11].

Pseudomonas sp. (KCIGC01) NBRC109613 was isolated from *G. clarum* IK97 spores [1]. This endobacterium in AMF spores was not only antagonistic microorganisms to the soil borne pathogens such as *Fusarium oxysporum* f. sp. *lactucae*, *Rosellinia necatrix* and *Rhizoctonia solani*, but also beneficial microorganisms on stimulation of phosphorus solubilization, ethylene production, nitrogen fixation, and hyphal growth of AMF [1]. Some kinds of endobacteria isolated from *Gigaspora margarita* spores have been known to act as partner bacteria (PB) of AMF as well as *Pseudomonas* sp. (KCIGC01) NBRC109613 [1].

Sesame (*Sesamum indicum* L.) seed is one of the world's important and oldest oilseed crops [12]. The chemical composition of sesame shows that the seed is an important source of oil (44% - 52.5%) and protein (18% - 23.5%) [13]. Sesame not only contains oil that has the monounsaturated fat, but also has various functional activities because of lignans in sesame seed [14]. Sesamin and sesamolin exist in relatively high contents as compared with other lignan compounds [15]. Sesamin and sesamolin were isolated and identified as insecticidal synergists in 1950's [16] [17]. On the other hand, their biosynthetic route and functional activity were recently elucidated [18] [19]. Sesame lignans have effects of antioxidants [20], antihypertensives [21] and immunomodulatory [22]. Furthermore, sesame lignans control metabolism of fatty acids [22], cholesterol [23], and alcohol [24] [25].

Harikumar [26] reported that indigenous AMF stimulated the plant biomass of sesame. But the quality of seeds by AMF inoculation was not investigated. The objective of this study is to investigate the effects of AMF and their PB on the growth of sesame plants and the concentration of lignans in sesame seeds.

2. Materials and Methods

2.1. Plants and Microorganisms

Seeds of sesame (*Sesamum indicum* L.) were bought from Takii Seed Co., Ltd. (Kyoto, Japan). Spores of *G. clarum* IK97, which had identified at Kazusa DNA research center [1], were collected from pot cultures of bahiagrass (*Paspalum notatum* Flügge.) by wet sieving methods. The inoculants of *G. clarum* IK97 were kept in refrigerator before use.

Pseudomonas sp. (KCIGC01) NBRC109613 was isolated from spores of *G. clarum* IK97 [1]. *Pseudomonas* sp. (KCIGC01) NBRC109613 was cultured with LB medium (1% polypeptone, 0.5% yeast extract, 0.5% NaCl) at 27˚C for 24 h. Then, the cells were washed by sterilized water one time and resuspended by sterilized water.

2.2. Experimental Design and Plant Growth

This experiment was carried out in greenhouse without a temperature control system. Seeds of sesame were sown in pots (24 cm in diameter) with a substrate mixture of vermiculite:zeolite (1:1, v/v). These soil materials contained no *G. clarum* IK97 and no *Pseudomonas* sp. (KCIGC01) NBRC109613. Two kinds of microorganisms were inoculated for each treatment at same time sowing. Mycorrhizal plants were inoculated by adding 5 g of an inoculum containing approximately 50 spores of *G. clarum* IK97. At bacterial treatment, *Pseudomonas* sp. (KCIGC01) NBRC109613 was inoculated into the soil at 3×10^9 cfu per pots. This bacterial inoculation was repeated monthly.

The experimental design included 4 treatments, each one consisting of 10 plants: CONT: uninoculated (control) plants; AMF: plants inoculated with *G. clarum* IK97 alone; AMF + PB: plants inoculated with *G. clarum* IK97 and *Pseudomonas* sp. (KCIGC01) NBRC109613; PB: plants inoculated with *Pseudomonas* sp. (KCIGC01) NBRC109613 alone.

One week after germination, the sesame plants were reduced to two plants per pots. All plants were fertilized weekly with 200 mL of Hoagland's solution containing macro-nutrients (525 mg of KNO_3, 1181 mg of $Ca(NO_3)_2 \cdot 4H_2O$, 490 mg of $MgSO_4 \cdot 7H_2O$, 136 mg of KH_2PO_4/1L tap water). Micro-nutrients (33.5 μg of $FeC_6H_5O_7 \cdot 5H_2O$, 2.23 μg of $MnSO_4 \cdot 4H_2O$, 0.29 μg of $ZnSO_4 \cdot 7H_2O$, 0.25 μg of $CuSO_4 \cdot 5H_2O$, 3.1 μg of H_3BO_3, 0.12 μg of $Na_2MoO_4 \cdot 2H_2O$, 5.8 μg of NaCl and 0.056 μg of $CoSO_4 \cdot 7H_2O$/1L tap water) was also applied to the pots once every two weeks.

2.3. Plants Harvest

The plants were harvested when the last seed was matured. The length of shoot was measured 3 times (1 month and 2 months after planting, and harvesting time). The number of flowers of each plant was counted. The period of harvesting (days) was evaluated from the date of first flowering to the date of last harvest. The dry weight of 30 seeds and all seeds of each plant were measured. In order to check the colonization of AMF, root samples were also taken, washed, and stained by the technique of Phillips and Hayman [27]. The percentage of AMF colonization in the roots was determined according to the method of Ishii and Kadoya [28].

2.4. The Analysis of Sesamin and Sesamolin Experimental Design and Plant Growth

Sesamin and sesamolin were extracted according to the modified methods by Yasumoto *et al*. [29]. One hundred mg of seeds were homogenized and extracted with 5 mL of chloroform-methanol mixture (2:1, v/v). The extracts were collected in centrifuge tubes and mixed ultrasonically for 30 min. After centrifugation (5000 rpm, 15 min), the supernatant was collected. The precipitate was dissolved in chloroform-methanol mixture and mixed ultrasonically. This extraction was repeated 3 times as mentioned above. The supernatants were combined and filtered with paper filters (Advantec No. 5C). The extracts were filtered through 0.22 μm filters and injected in a HPLC instrument (Hitachi, Tokyo, Japan).

Sesamin and sesamolin were separated by C30 packed column (Develosil C30-UG-5 (φ10 × 250 mm), Nomura Chemical Co., Ltd., Tokyo, Japan). The column was kept at 27˚C in column oven. Elution was carried out with an 70% methanol at 1.4 mL/min. The UV detector monitored the eluates at 280 nm. Purified sesamin and sesamolin (Nagara Science Co., Ltd., Tokyo, Japan) were used for generating a five-point calibration curve by comparing the peak area. The amounts of sesamin and sesamolin in each eluate were quantified by the calibration line. The concentration of sesamin and sesamolin was referred to the dry weight of grain of sesame.

3. Results

One month after planting, the shoot length was not affected by AMF or PB inoculation. However, the plant growth of sesame was stimulated by AMF inoculation at harvesting time. At harvesting time, the shoot length of AMF inoculated plants, AMF + PB inoculated plants, PB inoculated plants and no-inoculated plants was 102.8 cm, 96.1 cm, 93.4 cm and 86.3 cm, respectively. The AMF inoculated plants were higher than control plants significantly (**Figure 1**). The rate of sesame root colonization was higher in AMF + PB inoculated plants (66.4% ± 4.4%) than in AMF alone inoculated plants (39.2% ± 5.8%) ($t = -3.55$, $p = 0.002$). The inoculation of PB was stimulated the infection of AMF into sesame roots. Arbuscule and vesicle were observed in both treatments (**Figure 2**). No root colonization of the no-AMF (control) plants and PB alone inoculated plants was observed.

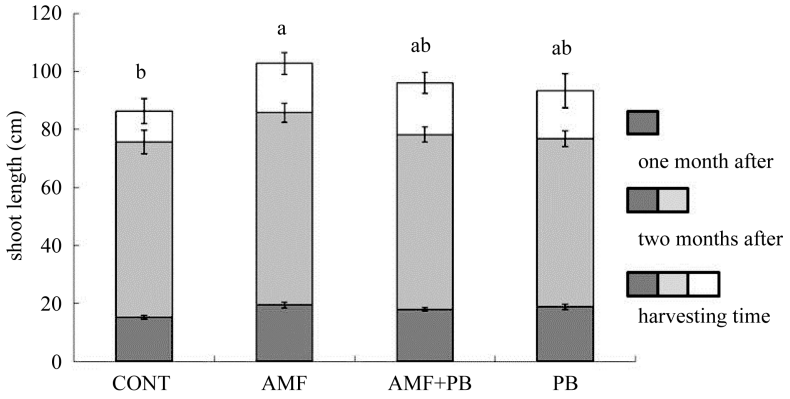

Figure 1. Effect of an arbuscular mycorrhizal fungus (AMF) and its partner bacterium (PB) on shoot length of sesame plants. *G. clarum* IK97 as AMF and *Pseudomonas* sp. (KCIGC01) NBRC109613 as PB were used. The vertical bars represent S.E. (n = 10). Bars with different small alphabets are significant among values mentioned in the graph, according to Duncan's multiple range test ($p \leq 0.05$).

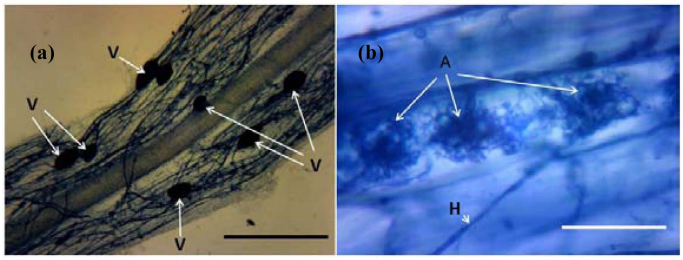

Figure 2. Photographs of sesame roots inoculated with an AMF and its PB. *G. clarum* IK97 as AMF and *Pseudomonas* sp. (KCIGC01) NBRC109613 as PB were used. (a) Sesame roots colonized with *G. clarum* IK97. Bar = 50 μm; (b) Arbuscule formation in an epidermal cell of a sesame root. Bar = 5 μm. The roots were stained with 0.05% trypan blue solution. A: arbuscule, H: hypha, V: vesicle.

The period from first flowering to last harvest tended to be shortened by AMF or PB inoculation (**Table 1**). The period was the longest in control plants. The dry weight of 30 seeds was significantly increased by AMF inoculation. The only AMF inoculation and the AMF + PB inoculation were able to induce a significant increase of the dry weight of 30 seeds. The greatest increase of the dry weight of 30 seeds was observed in the only AMF inoculated plants. The seed yield per plant of control plants was the least (1.8 ± 0.2 g). AMF or PB inoculation increased the seed yield. The seed yield per plant of AMF inoculation, AMF + PB inoculation and PB inoculation was 2.0 ± 0.2 g, 2.0 ± 0.1 g and 1.9 ± 0.1 g, respectively (**Table 1**).

The sesamin was detected prior to sesamolin by HPLC analysis (**Figure 3**). The separation of the peak was clear, and the concentration and area have liner relation. Any inoculation increased the amount of sesamin. AMF + PB inoculation was the most effective (11.4 ± 1.5 mg/g). The difference of the amount of sesamin between no-inoculated plants (control) and AMF + PB inoculated plants was significant (**Figure 4(a)**). On the other hand, the amount of sesamolin was not affected by inoculation of any microorganisms (**Figure 4(b)**).

4. Discussion

It was reported that satsuma mandarin trees that were inoculated with AMF had better fruit quality, such as Brix sugar content in the juice and good peel color as compared with no-AMF trees [30] [31]. In addition, inoculation with AMF was also shown to improve the quality of some crops such as tomato [32] and maize [33]. In this study, the inoculation of AMF and PB increased the content of sesamin in the seeds. Since the AMF and PB are known to contribute to the plant mineral nutrition and second metabolites, the content of sesamin would be increased.

In this study, the inoculation of PB promoted the mycorrhization. The colonization rate was increased from 39.2% ± 5.8% to 66.4% ± 4.4% by the inoculation of PB. Some *Pseudomonads* have the ability to attach to spore germination and hyphae of AMF as shown with *Gi. margarita in vitro* [34]. Meyer and Linderman [35] reported that *Glomus* and *Pseudomonas* dual inoculation enhanced root colonization. Thus, the interaction between *Pseudomonads* and AMF has many attentions [36] [37]. Further research is required to identify possible mechanisms mediating the reciprocal promotion of colonization by strains of AMF and *Pseudomonads*.

Furthermore, not only AMF but also many *Pseudomonas* species enhance plant growth and crop yield under greenhouse and field conditions [38] [39]. Some *Pseudomonas* species are generally recognized as plant growth promoting rhizobacteria (PGPR) because of suppress of disease, synthesis of plant growth promoting substance and production of antibiotics. In this study, the inoculation of *Pseudomonas* sp. (KCIGC01) NBRC109613 alone tended to increase the shoot length and the sesamin content of sesame but the difference was not significant. Because the *Pseudomonas* sp. (KCIGC01) NBRC109613 was isolated from *G. clarum* IK97, the strain could not adapt to live in soil environment. Gamalero *et al.* [40] reported that *Pseudomonas fluorescens* 92rk in tomato rhizosphere decreased from 2.34×10^8 down to 6.16×10^5 cfu/g root at 28 days after inoculation. In order to inoculate the *Pseudomonas* sp. (KCIGC01) NBRC109613 more efficiently, it needs to investigate the biological activity of this strain in the rhizosphere. Then the useful inoculation methods (e.g. amounts of cells and inoculation time) will be developed.

Table 1. Effect of an AMF and its PB on the growth of sesame plants.

	The period from first flowering to last harvest (days)		Dry weight of 30 seeds (g)		Seed yield per plant (g)	
CONT	48.5 ± 3.7	a	0.060 ± 0.003	a	1.8 ± 0.2	a
AMF	45.7 ± 1.2	a	0.067 ± 0.003	b	2.0 ± 0.2	a
AMF + PB	46.9 ± 1.7	a	0.066 ± 0.001	ab	2.0 ± 0.1	a
PB	45.7 ± 2.6	a	0.062 ± 0.002	ab	1.9 ± 0.2	a

AMF: *G. clarum* IK97, PB: *Pseudomonas* sp. (KCIGC01) NBRC109613. Mean ± standard error (n = 10). Values with different small alphabets are significant among values mentioned in the graph, according to Duncan's multiple range test ($p \leq 0.05$).

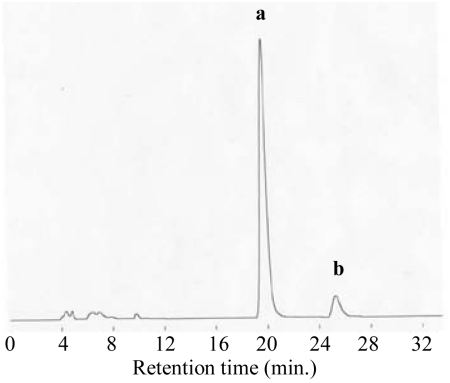

Figure 3. The chromatogram of sesamin (a) and sesamolin (b) on HPLC.

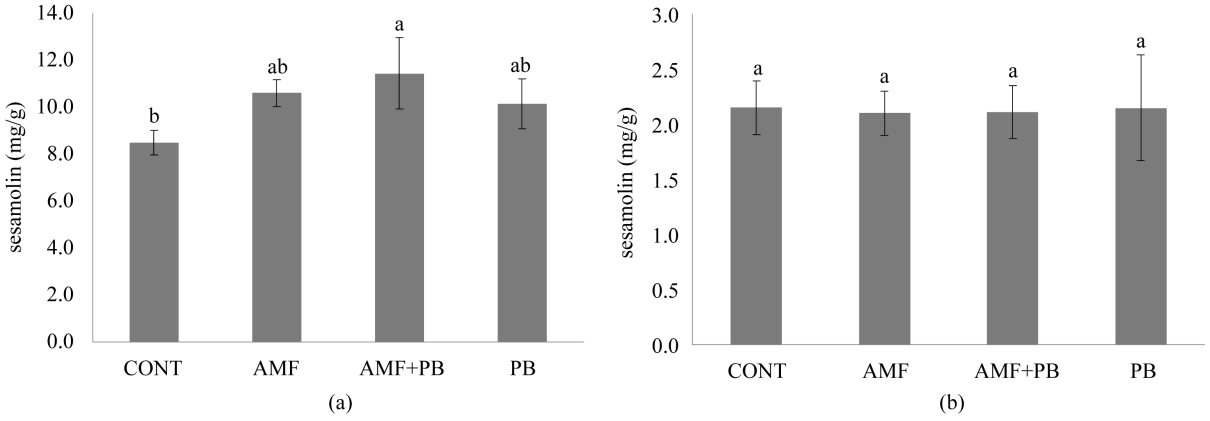

Figure 4. Effect of an AMF and its PB on the contents of sesamin and sesamolin in sesame seeds. *G. clarum* IK97 as AMF and *Pseudomonas* sp. (KCIGC01) NBRC109613 as PB were used. (a) Sesamin content in sesame seeds; (b) Sesamolin content in sesame seeds. The vertical bars represent S.E. (n = 10). Bars with different small alphabets are significant among values mentioned in the graph, according to Duncan's multiple range test ($p \leq 0.05$).

In this study, the AMF colonization rate in treatment of AMF + PB was significantly higher than that in treatment of AMF alone. However, there was no significant difference in the shoot length, the yield per plant, sesamin content and sesamolin content between these two treatments. Ishii *et al.* [41] reported that the growth of trifoliate orange seedlings was not affected by the difference of the percentage of AMF colonization, too. The AMF colonization rate in treatment of cadaverine (76.7%) was higher than that in control (26.6%). However, there was no significant difference in growth of trifoliate orange seedlings. When the AMF occupy some parts of roots, they could transfer the nutrient to plants and get the photosynthetic products from plants. They do not need to infect a wide part of roots for the exchange of materials between host plants.

Increased quality, in terms of taste and nutria value, can become an additional target in agriculture. Consum-

ers have paid their attention on the aspects regarding the quality of foods and agricultural products in relation to health and environmental concerns. Further investigations are needed to understand the mechanism by which AMF and their partner bacteria control plant growth and food quality.

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