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Original Article

IAA-producing bacteria as a biostimulant: Effects on seed quality of vegetable soybean (*Glycine max* (L.) Merrill) through soaking and coating applications

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Abstract

Vegetable soybean is a nutrient-dense crop crucial to human nutrition, but its high fat content accelerates seed deterioration, presenting significant challenges for maintaining seed quality during storage. This study investigates the potential of plant growth-promoting rhizobacteria (PGPR), specifically indole-3-acetic acid (IAA)-producing bacteria, to enhance seed quality. The research was conducted using a completely randomized design (CRD) with four replications. From 10 bacterial isolates obtained from soil, three were identified as high IAA producers*: Enterobacter kobei* (isolate 1), *Agrobacterium radiobacter* (isolate 2), and *Agrobacterium radiobacter* (isolate 3). The results demonstrated that seed coating with *E. kobei* (isolate 1) significantly improved the speed of germination and mean germination time compared to untreated seeds when tested under sand conditions. Additionally, seed soaking with *E. kobei* (isolate 1) enhanced germination, shoot length, root length, and root fresh weight. Coating with both *E. kobei* (isolate 1) and *A. radiobacter* (isolate 3) further increased root length and root fresh weight. These findings indicate that seed coating with *E. kobei* (isolate 1) is an effective method for enhancing germination percentage, seed vigor, seedling vigor, and seedling growth in vegetable soybean.

Keywords: seed quality, organic seed coating, seed enhancement, plant growth-promoting bacteria

1. Introduction

Vegetable soybean (*Glycine max* (L.) Merrill) is a highly nutritious leguminous crop, rich in protein, iron, and calcium. These nutrients are essential components of the human diet (Nair *et al*., 2023). Thailand is one of the countries that cultivate vegetable soybeans for export, with the northern region being the primary cultivation area. However, the cultivation of vegetable soybeans faces challenges due to the high fat content in the seeds, which causes them to deteriorate faster than seeds with a higher starch content. This makes it difficult to maintain seed quality over an extended period. Farmers often need to store vegetable soybean seeds for a period before the next planting season. For instance, when planting at the end of the rainy season around August, farmers typically use seeds harvested in the dry season in April, which need to be stored for approximately four months. Prolonged storage can lead to a decline in seed quality, posing a significant challenge for vegetable soybean cultivation (Mardmai, Banthoengsuk, & Fakthongphan, 2023).

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Plant growth-promoting rhizobacteria (PGPR) enhance seed germination through the production of metabolites, antibiotics, enzymes, and phytohormones (Zörb, Geilfus, & Dietz, 2019). They also facilitate activities such as increased phosphate solubilization in the soil and root colonization. Specifically, gibberellins and indole acetic acid are growth regulators that promote cell elongation and division. These hormones, primarily produced in the apices of coleoptiles, are essential for plant growth (Perez-Garcia, Castañeda-Ramírez, & Lafuente-Rincón, 2019). Additionally, several types of plant growth-promoting bacteria (PGPB) have been reported to produce indole-3-acetic acid (IAA) via tryptophan-dependent or tryptophan-independent pathways. A single bacterium may possess multiple IAA production pathways, with the tryptophan-dependent pathway being the most commonly utilized by microorganisms for IAA synthesis. Tryptophan, a natural exudate of plant roots, is utilized by rhizobacteria to produce IAA as part of their secondary metabolism (Noor *et al*., 2023). These IAA-producing capabilities can significantly enhance the quality of seeds (Rocha *et al*., 2019).

Seed soaking is a preparation method used before planting, where water acts as a carrier to bring various substances into the seed. This initiates biochemical reactions within the seed, resulting in uniform germination (Li, Zhao, & Jiang, 2019; Siri, 2015). Heqin, Zhao, and Jiang (2019) reported that soaking maize seeds with *Bacillus megaterium* strain HX-2 improved several physiological and biochemical parameters in maize seedlings under drought stress, including increased plant height, root length, biomass, and antioxidant enzyme activities. Seed coating, which involves applying a thin layer of polymer to seeds, acts as a medium to deliver beneficial active substances directly to them. This method has been confirmed as effective for adhering bacteria to seeds, thereby enhancing seed germination and seedling growth (Accinelli, Abbas, Little, Kotowicz, & Shier, 2018; Rocha *et al*., 2019). Furthermore, studies have reported that co-coating seeds with *Pseudomonas* and *Bacillus* increases seed vigor and reduces infection levels of *Xanthomonas oryzae* pv. *oryzae* in rice (Palupi, Ilyas, Machmud, & Widajati, 2017), as well as improves the height and biomass of canola in both greenhouse and field conditions (Lally *et al*., 2017). These combined seed treatments with PGPR demonstrate significant benefits for seed germination and plant growth. Therefore, seed soaking and coating with PGPR are demonstrated to provide significant benefits for seed germination and plant growth.

Therefore, this study aims to enhance the quality of vegetable soybean seeds by soaking them with PGPR, which promotes germination and seedling growth. The evaluation will be conducted using the sand and top of paper covered with sand method.

2. Materials and Methods

2.1 Location of experiment, duration, and seed quality

The experiment was conducted at the Seed Technology Laboratory, the Modern Seed Technology Research Center of the Agronomy Program, and the Soil and Advanced Fertilizer Laboratory, Faculty of Agricultural Production, Maejo University. The initial germination rate of the vegetable soybean variety 66-1 was 69%. The experiment was conducted from November 2023 to June 2024.

2.2 Information on bacterial isolates

The study collected bacterial samples from Chiang Muan District, Phayao Province, Thailand. Soil samples were collected from a depth of 10–15 centimeters around the roots of vegetable soybean plants and subjected to serial dilution. The diluted samples were then cultured on potato dextrose agar (PDA) and left to incubate at room temperature for three days. Subsequently, eleven bacterial isolates were identified and purified using the streak plate method on nutrient agar (NA).

2.3 Microbe preparation and determination of IAA production

After obtaining pure bacterial colonies separated on NA, they were cultured in nutrient broth (NB) supplemented with tryptophan (Fluka, Buchs, Japan) at 0.102 g/L. The culture was then agitated at a speed of 125 revolutions per minute (rpm) for 7 days. Subsequently, the cultured bacteria were centrifuged at 5,000 *g* for 5-10 minutes to separate the bacterial cells from the liquid medium. The resulting clear supernatant was collected for analysis to determine the quantity of indole-3-acetic acid (IAA). Indole-3-acetic acid (MW = 175.19) was used as the standard for comparison (Phyto Technology Laboratories, Shawnee Mission KS, United States). A 10 mM IAA stock solution was initially prepared by dissolving the IAA in 50% methanol. This stock solution was then diluted to create a 1 mM IAA solution using 50% methanol. From the 1 mM IAA solution, standard solutions with concentrations of 0, 5, 10, 20, 50, 100, and 150 µM were prepared, each in a volume of 1 mL, adjusted with 50% methanol. For the assay, 1 mL of the clear bacterial sample solution was added to separate test tubes. Van Urk-Salkowski reagent (2 mL) was then added following Salkowski's method (Ehmann, 1977), and the mixture was thoroughly agitated. The solutions were incubated in the dark for 30 minutes, and the absorbance was measured at a wavelength of 530 nm using a spectrophotometer.

2.4 Soaking and coating vegetable soybean seeds

Vegetable soybean seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 1 minute, rinsed three times with sterilized distilled water, and dried to a moisture content of approximately 9%. The three bacterial isolates were prepared for seed soaking and coating by culturing them in 500 mL Erlenmeyer flasks containing 400 mL of NB. Using a sterilized loop, the isolates were inoculated and incubated on a shaker at 125 rpm for 8 days. After incubation, the bacterial concentration was adjusted to 10^9 CFU/mL. Seed soaking was performed by mixing 99 mL of distilled water with 1 mL of bacterial suspension. This process was repeated for all three isolates, and seeds were soaked in the prepared solutions in a growth chamber at 25°C for 30 minutes. Seed coating involved preparing 98.9 mL of

distilled water mixed with 0.1% w/v carboxymethyl cellulose (CMC) as a coating polymer, combined with 1 mL of bacterial suspension. The mixture was homogenized and used to coat the seeds. Fifty grams of seeds underwent nine treatments: no soaking (T1), soaking with distilled water (T2), coating with polymer only (T3), soaking with isolate 1 (T4), soaking with isolate 2 (T5), soaking with isolate 3 (T6), coating with isolate 1 (T7), coating with isolate 2 (T8), and coating with isolate 3 (T9). After treatment, soaked seeds were blotted dry with paper towels and immediately evaluated for seed quality. Coated seeds were dried to a moisture content of approximately 9% before quality assessment, following the same procedure as with the soaked seeds.

2.5 Data collection

2.5.1 Sand testing

Sand with a uniform particle size (<0.05 mm) was prepared and sterilized by heating at 200°C for 2 hours. The sterilized sand was then used as a growth medium to test seed quality. Each treatment was replicated 4 times with 50 seeds per replicate. Plastic boxes (180 mm × 140 mm × 90 mm, L × W × H) were used as containers for the germination test. The sand moisture content was adjusted to approximately 60% and the germination boxes were filled to a height of 3 cm. The seeds were arranged on the sand and covered with an additional 2 cm layer of sand. The boxes were then placed in a germination chamber set to 25°C, with a relative humidity of 80%, a light intensity of 180 μE, and continuous light exposure for 24 hours. Subsequently, the following characteristics were examined. The emergence rate was evaluated by counting the number of visible cotyledons on the 4th day after planting. The speed of emergence was assessed by counting cotyledon emergence daily from day 1 to day 4. Initial germination counts were performed 5- and 8-days post-incubation (ISTA, 2023). The speed of germination was evaluated by counting the number of seeds that developed into normal seedlings from the 5th to the 8th day, based on the method of AOSA (1983). The mean germination time (MGT) was calculated using the equation: $MGT = \sum(n \times d) / N$, where n is the number of seeds germinated each day, d is the number of days from the beginning of the test, and N is the total number of seeds germinated by the end of the experiment (Ellis & Roberts, 1981). Growth was assessed on 10 randomly selected plants 8 days after planting by measuring shoot length, root length, shoot fresh weight, and root fresh weight. Shoots were cut at the base near the seedling medium to measure shoot length and fresh weight (Kangsopa, Thawong, Singsopa, & Rapeebunyanon, 2023).

2.5.2 Top of paper covered with sand testing (TPS)

The TPS method used the same preparation of sand and plastic boxes as the sand testing method. In this test, a germination paper was placed at the bottom of the box, with 50 seeds evenly distributed on top. The seeds were then covered with 2 cm of sand (ISTA, 2022). The box was sealed and placed under the same conditions as the sand testing procedure. Seed quality characteristics were evaluated similarly to the sand testing method.

2.6 Statistical analysis

The percentage of germination was arcsine-transformed to normalize the data before statistical analysis. All data were analyzed using a one-way analysis of variance matching the Completely Randomized Design of experiments, and the differences between treatments were tested using Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1 Quantity of indole-3-acetic acid (IAA)

The isolation of bacteria from Chiang Muan District, Phayao Province, Thailand, led to the identification of 10 bacterial isolates. An evaluation of their indole-3-acetic acid (IAA) production efficiency revealed concentrations of 185, 46, 39, 25, 21, 21, 19, 18, 18, and 18 μg/mL, in the order of isolate numbering. Among these, three isolates (isolates 1–3) exhibited a high capacity for IAA production, with concentrations ranging from 39 to 185 μg/mL when cultured in nutrient broth. Based on these findings, the three isolates with the highest IAA production were selected for further identification through 16S rRNA gene sequencing (Figure 1). The identified species were *Enterobacter kobei* (isolate 1) and *Agrobacterium radiobacter* (isolates 2 and 3) (Table 1). The high IAA production by these three isolates can be attributed to their mechanisms of utilizing tryptophan as a precursor through biochemical pathways such as the indole-3-pyruvate (IPA) pathway and indole-3-acetamide (IAM) pathway. This process is often stimulated by environmental factors, including by compounds released by plants into the rhizosphere, which enhance the growth-promoting effects on associated plants. These mechanisms enable the three isolates to efficiently produce IAA (Alkhalaf, & Ryan, 2015). Consistent with studies on bacteria isolated from the rhizosphere, many bacterial species capable of IAA production have been identified. For example, *Bacillus thuringiensis* and *Pseudomonas aeruginosa* were reported to produce IAA at concentrations ranging from 57 to 288 μg/mL when cultured in media supplemented with tryptophan (Shahab, Ahmed, & Khan, 2009). Similarly, *Bacillus megaterium*, *Lactobacillus casei*, *Bacillus subtilis*, *Bacillus cereus*, and *Lactobacillus acidophilus*, isolated from the rhizosphere of maize, wheat, banana, and cotton, produced IAA in the range from 15 to 65 μg/mL (Mohite, 2013).

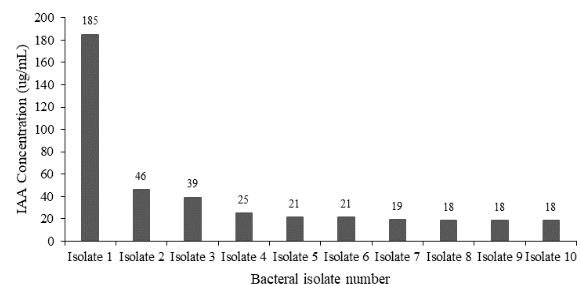

Figure 1. Screening of indoleacetic acid (IAA)-producing strains isolated from the vegetable soybean rhizosphere soil collected from Chiang Muan District, Phayao Province, Thailand

Furthermore, *Stenotrophomonas* sp., isolated from soil surrounding kale roots, was reported to produce 10.78 µg/mL of IAA (Kangsopa & Atnaseo, 2022).

3.2 Seed quality

This experiment employed two seed quality evaluation methods: the sand test, used to simulate natural soil growth conditions, and the top of paper covered with sand test, which was applied to assess moisture control and seed uniformity under controlled environmental conditions. These two methods were utilized to evaluate the quality of vegetable soybean seeds subjected to soaking and coating treatments with IAA-producing bacteria. The experimental results are as follows.

Under sand conditions, the results indicated that seed soaking with *A. radiobacter* (isolate 2) led to a reduction in both emergence percentage and speed of emergence compared to the untreated seeds and other methods (Table 2). Under the top of paper covered with sand conditions, seed coating and seed soaking with all three isolates led to a significantly higher emergence percentage and speed of emergence compared to the untreated seeds. Notably, seed coating with *A. radiobacter* (isolate 3) exhibited the highest speed of emergence, significantly outperforming the untreated seeds (Table 2). Furthermore, these treatments demonstrated improved germination performance. Seed coating with *E. kobei* (isolate 1) and *A. radiobacter* (isolate 3) significantly enhanced the speed of germination compared to the untreated seeds under sand test conditions. Additionally, seed coating with *E. kobei* (isolate 1) resulted in a shorter mean germination time compared to other methods (Table 3). Similarly, seed coating with *A. radiobacter* (isolate 2) demonstrated higher germination percentages and a significantly faster speed of germination, achieving the best mean germination time, which was statistically superior to all other treatments under top of paper covered with sand test conditions (Table 3).

The results of seed soaking and coating with IAA-producing bacteria across all three isolates indicate differential effects on the quality of vegetable soybean seeds. Seed soaking, which is known to stimulate germination by enhancing water absorption and activating enzymes that break down stored reserves (Rocha *et al*., 2022), showed that the addition of IAA-producing bacteria, particularly *E. kobei* (isolate 1), significantly improved seed quality compared to untreated seeds and seeds soaked only in distilled water. This improvement is attributed to the ability of *E. kobei* to produce phytohormones, especially IAA, and growth-promoting substances that enhance enzymatic activity, leading to more efficient and rapid seed germination (Panneerselvam *et al*., 2021; Sun, Shahrajabian, & Soleymani, 2024).

Conversely, seed coating with IAA-producing bacteria from all three isolates demonstrated greater potential for improving seed germination quality and seedling vigor compared to seed soaking. Seed coating involves applying a thin polymer layer around the seed, which serves as a carrier for beneficial bacteria (Rocha *et al*., 2022). Studies have shown that associating bacteria with seeds enhances factors that promote germination, primarily due to the beneficial actions of the bacteria. The experiment revealed that seed coating with *A. radiobacter* (isolate 3) significantly increased

Table 1. Identification of IAA producing bacteria by DNA sequencing of 16S rDNA

Isolate number	Identified bacterium	% Similarity	GenBank
Isolate 1	*Enterobacter kobei*	99.38	CP017181
Isolate 2	*Agrobacterium radiobacter*	99.16	NR_116306
Isolate 3	*Agrobacterium radiobacter*	100	NR_116306

Table 2. Emergence percentage (EMER) and speed of emergence (SPE) of vegetable soybean seeds after soaking and coating with bacteria producing indole-3-acetic acid (IAA), tested under sand and top of paper covered with sand conditions

Treatment[1]	Sand		Top of paper covered with sand	
	EMER (%)[2]	SPE (seedlings/day)	EMER (%)[2]	SPE(seedlings/day)
T1	27 ab[3]	6.83 ab	13 d	8.06 f
T2	38 ab	9.50 ab	58 bc	17.33 bcd
T3	36 ab	9.00 ab	65 ab	15.33 de
T4	45 a	11.33 a	69 ab	20.44 abc
T5	16 b	4.00 b	45 c	12.67 e
T6	25 ab	6.17 ab	57 bc	16.33 cde
T7	43 a	8.50 ab	75 a	21.72 ab
T8	37 ab	7.50 ab	78 a	21.28 ab
T9	46 a	11.50 a	79 a	22.44 a
F-test	**	*	**	**
CV.(%)	12.47	16.36	9.12	15.50

*, ** : significantly different at P≤0.05 and P≤0.01, respectively

[1] T1 = untreated seeds, T2 = seed soaking only, T3 = seed coating only, T4 = soaking + isolate 1, T5 = soaking + isolate 2, T6 = soaking + isolate 3, T7 = coating + isolate 1, T8. = coating + isolate 2 and T9 = coating + isolate 3

[2] Data are transformed by the arcsine before statistical analysis and back transformed data are presented.

[3] Means within a column followed by the same letter are not significantly at P ≤ 0.05 by DMRT.

Table 3. Germination percentage (GE), speed of germination (SGE), and mean germination time (MGT) of vegetable soybean seeds after soaking and coating with bacteria producing indole-3-acetic acid (IAA), tested under sand and top of paper covered with sand conditions

Treatment[1]	Sand			Top of paper covered with sand		
	GE (%)	SGE (seedlings/day)	MGT (day)	GE (%)	SGE (seedlings/day)	MGT (day)
T1	69 ab	6.78 b	6.14 ab	65 bc	2.67 bc	6.19 a
T2	66 b	6.73 b	6.24 ab	70 abc	3.12 bc	5.32 bc
T3	69 ab	6.71 b	6.41 a	69 abc	2.81 bc	5.62 b
T4	71 a	7.41 ab	6.07 ab	61 c	3.82 b	5.36 bc
T5	59 b	6.66 b	5.98 ab	73 abc	3.42 bc	5.50 b
T6	63 b	6.84 b	6.06 ab	79 ab	3.25 bc	5.26 bc
T7	75 a	8.73 a	5.91 b	84 a	4.75 a	5.61 b
T8	61 b	6.70 b	6.18 ab	83 a	4.86 a	4.71 d
T9	74 a	8.45 a	6.11 ab	73 abc	1.49 c	5.02 bc
F-test	**	**	*	*	**	**
CV.(%)	9.08	11.95	3.64	8.94	27.39	3.61

*, ** : significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively

[1] [1] T1 = untreated seeds, T2 = seed soaking only, T3 = seed coating only, T4 = soaking + isolate 1, T5 = soaking + isolate 2, T6 = soaking + isolate 3, T7 = coating + isolate 1, T8. = coating + isolate 2 and T9 = coating + isolate 3

[2] Data are transformed by the arcsine before statistical analysis and back transformed data are presented.

[3] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

seed emergence within the first four days of testing under both conditions. This improvement is likely attributable to the production of IAA by *A. radiobacter*, a plant hormone crucial for stimulating enzyme production, such as amylase, which is essential for breaking down starch stored in the endosperm into sugars used as energy during seed germination and early seedling growth (Naderi, Etesami, Alikhani, & Arani, 2022). Further assessment at eight days post-germination showed that seed coating with *E. kobei* (isolate 1) resulted in a higher germination percentage and speed of germination compared to untreated seeds under both methods. *E. kobei*, also an IAA producer, plays a vital role as a signaling molecule in the germination process, coordinating cell division, root growth, and the development of other seedling structures (Zhang *et al*., 2021). It also stimulates early radicle emergence and facilitates the breakdown of stored reserves, such as starch, proteins, lipids, oils, and phytate, thereby providing the necessary energy for germination and seedling growth (Kumar, & Dubey, 2022; Panneerselvam *et al*., 2021).

Moreover, the seed coating method effectively controls the gradual absorption of water by the seed, allowing moisture to slowly permeate through the seed coat (Paravar, Piri, Balouchi, & Ma, 2023; Rocha *et al*., 2019). As the seed absorbs moisture, the coating gradually dissolves, enabling the seed to absorb water containing bacteria through small openings on the seed surface, such as the hilum and micropyle (Rocha *et al*., 2019; Siri, 2015). This water, infused with both nutrients and IAA-producing bacteria, facilitates the transport of IAA into the seed. Once inside, the IAA is transported into the seed's cells via transport proteins in the cell membrane, activating various enzymatic functions and cellular processes, thereby enhancing the efficiency of seed germination (Fiodor, Ajijah, Dziewit, & Pranaw, 2023; Pérez-García *et al*., 2023). Additionally, the absorbed IAA penetrates various seed tissues, including meristematic tissues, and further stimulates processes critical to seedling germination and growth, resulting in improved overall development. However, the effectiveness of bacterial phytohormone production is influenced by the surrounding environment (Fiodor *et al*., 2023; Rocha *et al*., 2019). Consequently, the results suggest that seed coating with IAA-producing *E. kobei* offers superior improvement in the quality of vegetable soybean seeds compared to other methods.

3.3 Seedling growth

The results of seed treatment experiments, involving soaking and coating under sand conditions, indicate that neither treatment significantly improved shoot length when compared to untreated seeds, except for soaking in distilled water, which resulted in a notably reduced shoot length. Seed soaking and coating with *E. kobei* (isolate 1) promoted significantly longer root length compared to untreated seeds. Additionally, coating with *A. radiobacter* (isolate 2) and *A. radiobacter* (isolate 3) also resulted in longer roots than those of untreated seeds. Seed soaking alone resulted in a reduction in shoot fresh weight compared to all other treatments. Additionally, seed soaking combined with *A. radiobacter* (isolate 2) was found to result in a reduction in root fresh weight compared to all other methods (Table 4). When considering Figure 2, seed coating with *A. radiobacter* (isolate 2) demonstrated a more developed root system; however, there was no significant difference compared to seed soaking with *E. kobei* (isolate 1), seed coating with *E. kobei* (isolate 1), and seed coating with *A. radiobacter* (isolate 3), as reflected in the root length results in Table 4. However, the changes in root fresh weight across all treatments were noticeably better than those observed for seed soaking with *A. radiobacter* (isolate 2).

Under top of paper covered with sand conditions, seed coating with *E. kobei* (isolate 1) significantly increased both shoot length and shoot fresh weight compared to untreated seeds, with statistically significant differences (Table 5). The results suggest that seed soaking and coating can enhance seedling root length. Specifically, seedlings treated with *E. kobei* (isolate 1) showed markedly higher root

Table 4. Shoot length, root length, shoot fresh weight and root fresh weight of vegetable soybean seeds after soaking and coating with bacteria producing indole-3-acetic acid (IAA), tested under sand conditions

Treatment[1]	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)
T1	13.07 a	20.67 b	22.40 ab	14.88 ab
T2	11.45 b	21.18 ab	19.70 b	13.40 ab
T3	13.17 a	21.14 ab	20.35 ab	13.61 ab
T4	13.25 a	22.18 a	21.24 ab	14.92 a
T5	12.68 ab	20.13 b	20.94 ab	12.88 b
T6	12.87 ab	20.96 ab	22.28 ab	13.27 ab
T7	14.35 a	22.43 a	21.16 ab	15.33 a
T8	12.66 ab	22.08 a	21.27 ab	14.35 ab
T9	13.48 a	22.45 a	22.75 a	15.38 a
F-test	**	**	**	**
CV.(%)	6.57	8.53	6.91	19.41

** : significantly different at $P \leq 0.01$
[1] [1] T1 = untreated seeds, T2 = seed soaking only, T3 = seed coating only, T4 = soaking + isolate 1, T5 = soaking + isolate 2, T6 = soaking + isolate 3, T7 = coating + isolate 1, T8. = coating + isolate 2 and T9 = coating + isolate 3
[2] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

Table 5. Shoot length and shoot fresh weight of vegetable soybean seeds after soaking and coating with bacteria producing indole-3-acetic acid (IAA), tested under top of paper covered with sand conditions

Treatment	Shoot length (cm)	Shoot fresh weight (g)
Untreated seeds	10.83 c	19.07 bc
Seed soaking only	13.79 bc	22.07 ab
Seed coating only	14.74 bc	20.97 abc
Seed soaking + isolate 1	14.08 bc	18.65 bc
Seed soaking + isolate 2	14.19 bc	17.61 c
Seed soaking + isolate 3	15.14 b	21.84 ab
Seed coating + isolate 1	16.15 a	23.64 a
Seed coating + isolate 2	14.26 bc	19.22 bc
Seed coating + isolate 3	14.91 bc	19.75 bc
F-test	**	*
CV.(%)	17.67	8.89

*, ** : significantly different at $P \leq 0.05$ and $P \leq 0.01$, respectively
[1] Means within a column followed by the same letter are not significantly at $P \leq 0.05$ by DMRT.

Figure 2. Growth of vegetable soybean seedlings at 8 days after sowing, following seed soaking and coating with IAA-producing bacteria, tested under sand conditions. T1 = untreated seeds, T2 = seed soaking only, T3 = seed coating only, T4 = soaking + isolate 1, T5 = soaking + isolate 2, T6 = soaking + isolate 3, T7 = coating + isolate 1, T8. = coating + isolate 2 and T9 = coating + isolate 3

length and root fresh weight than the untreated seeds. *E. kobei* (isolate 1) is an IAA-producing bacterium that plays a crucial role in promoting root growth and cell division. IAA facilitates root cell elongation, resulting in longer roots and a more robust root system (Etesami & Glick, 2024; Panneerselvam *et al*., 2021). Longer roots increase the absorption area for water and nutrients from the soil, providing seedlings with the necessary resources for optimal growth, thereby contributing to the overall strength and development of the seedlings (Etesami & Glick, 2024; Zhang *et al*., 2021). Moreover, under the top of paper covered with sand conditions, where seeds receive moisture from the sand above, coating with *E. kobei* (isolate 1) increased shoot length and shoot fresh weight by 49% and 24%, respectively, compared to untreated seeds. This could be attributed to *E. kobei*'s ability to produce IAA, which is crucial for stimulating

cell elongation in meristematic tissue, leading to longer shoot cells, and consequently increased shoot length in seedlings (Zhang *et al*., 2021). In addition to promoting cell elongation, IAA produced by *E. kobei* also enhances cell division in meristematic tissue, particularly in the shoot apex. Increased cell division results in a larger number of cells in the shoot apex, contributing to longer shoot lengths (Kumla, Suwannarach, Matsui, & Lumyong, 2020; Zhang *et al*., 2021)

However, *A. radiobacter* (isolate 2) and *A. radiobacter* (isolate 3) also play a significant role by producing IAA that stimulates cell division in the root tip. The increased cell division leads to a higher number of cells in the roots, resulting in longer and stronger roots. Therefore, it is evident that seed coating with IAA-producing bacteria effectively enhances the root length of vegetable soybean seedlings.

4. Conclusions

The evaluation of seed soaking and coating with IAA-producing bacteria concluded that seed coating with *E. kobei* (isolate 1) significantly improved the germination percentage compared to untreated seeds under top of paper covered with sand conditions. Soaking seeds with *E. kobei* (isolate 1) enhanced germination percentage, shoot length, root length, and root fresh weight when tested under sand conditions. Furthermore, both seed soaking and coating with *E. kobei* (isolate 1), as well as coating with *A. radiobacter* (isolate 3), were shown to significantly increase root length and root fresh weight compared to untreated seeds. Therefore, seed coating with *E. kobei* (isolate 1) is recommended as an effective method for enhancing germination percentage, seedling vigor, and growth in vegetable soybean seedlings.

Additionally, *A. radiobacter* (isolate 2) and *A. radiobacter* (isolate 3) were found to improve germination, seedling vigor, and root length, suggesting that these isolates could serve as viable alternatives for enhancing the quality and growth of vegetable soybean seedlings further.

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