ENHANCING GERMINATION AND SEEDLING GROWTH OF PEANUT TAINAN 9 VARIETY THROUGH SEED COATING WITH *ENTEROBACTER KOBEI*

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(Received: 23 August 2024; Revised: 10 November 2024; Accepted: 11 November 2024)

Abstract

Peanuts can be grown year-round, yet the supply remains insufficient to meet consumer demand. This shortfall is primarily due to high cultivation costs from fertilizer use and poor-quality seeds, which result in uneven germination, weak seedlings, and susceptibility to pests and diseases. To address these issues, seed coating technology is essential for enhancing seed quality before planting. This study aimed to determine the optimal concentration of *Enterobacter kobei* for seed coating and to evaluate its effects on various seed quality parameters. The results indicated that coating seeds with *E. kobei* at 6.60×10^8 CFU/mL significantly improved germination rates and speed of germination compared to non-coated seeds under both sand and greenhouse conditions. Additionally, seeds coated with *E. kobei* at concentrations of 6.60×10^8 CFU/mL, 6.60×10^9 CFU/mL, and 6.60×10^{10} CFU/mL exhibited significantly greater shoot length, root length, seedling length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight in both testing conditions. In conclusion, coating peanut seeds with *E. kobei* at 6.60×10^8 CFU/mL is recommended for enhancing the germination, vigor, and growth of Tainan 9 peanut seedlings.

Keywords: Seed enhancement, Bio-fertilizer, Plant growth-promoting rhizobacteria, Bacteria-producing indole-3-acetic acid.

Introduction

The peanut variety Tainan 9 (*Arachis hypogaea* L.) is significant economically in Thailand due to its ease of cultivation and ability to be grown year–round. The primary cultivation areas are in the northern and northeastern regions of the country. Currently, the domestic production of peanuts is insufficient to meet demand, leading to significant imports from abroad (Agricultural Research Development Agency, 2017). Furthermore, peanut seeds contain a high–fat content, which, under unsuitable environmental conditions, accelerates the oxidation process of the fats in the seeds, leading to rapid deterioration in seed quality. When farmers plant these degraded seeds, it results in uneven germination, weak seedlings, and increased susceptibility to diseases and pests. Consequently, farmers must increase the number of seeds per planting area and invest more in other cultivation factors, thereby raising their overall production costs.

Seed coating technology involves applying a thin layer of polymer around the seed's shell to deliver delivering various active substances directly to the seeds (Siri, 2015). This method is necessary to enhance the seed's germination quality and vigor (Kangsopa, 2019). Particularly, seed coating combined with plant growth–promoting rhizobacteria; PGPR that can fix nitrogen, as well as microorganisms that enhance the availability of phosphorus and potassium, and those that resist plant diseases, is highly beneficial (Inthasan & Dechjiraratthanasiri, 2024). Utilizing diverse PGPR strains demonstrates a symbiotic relationship that mutually supports plant growth and nutrient uptake. Specifically, bacteria that produce indole–3–acetic acid; IAA play a crucial role in stimulating cell division and elongation, thereby promoting rapid and efficient seed germination. Additionally, IAA promotes root proliferation and elongation, enhancing the plant's ability to absorb nutrients and water from the soil (Pérez–García et al., 2023). Scott (1972) discovered that seed coating is an effective method for attaching plant growth–promoting rhizobacteria; PGPR to seeds. This technique provides precise benefits by directly delivering beneficial microorganisms to the seeds, enhancing their growth and overall health. Particularly, *E. kobei* promotes seed germination and seedling growth through the production of phytohormones such as IAA, nitrogen fixation and siderophore production to enhance nutrient absorption, and biofilm formation that protects plants from environmental stress (Kumar & Dubey, 2022; Panneerselvam et al., 2021). However, there are no reports on the efficacy of bacterial seed coating for enhancing the seed quality of the peanut variety Tainan 9.

Therefore, this experiment aims to determine the optimal concentration of IAA–producing bacteria that can enhance the germination quality, vigor, and growth of Tainan 9 peanut seedlings. The results will provide guidelines for improving seed quality, promoting better cultivation practices, and helping farmers reduce production costs.

Materials and methods

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 (18°53'41.6"N 99°00'39.0"E). The initial germination rate of the peanut variety Tainan 9 was 48%, with a moisture content of 10%. The experiment was conducted from January to April 2024.

2. Information on bacteria

The *E. kobei* DSM 13645 (CP017181) used in this study was sourced from Chiang Muan District, Phayao Province, Thailand, and was identified by sequencing the 16S rRNA gene. Subsequently, the IAA content was determined using the method described by Ehmann (1977), with the results indicating an IAA concentration of 79.15 µg/mL

3. Microbes preparation

E. kobei was cultured by inoculating a single colony into 5 mL of nutrient broth; NB and incubating it for 48 hours with shaking at 170 rpm at 30°C. Subsequently, 100 µL of this culture was transferred into a 250–mL Erlenmeyer flask containing 50 mL of NB and incubated for another 48 hours under the same conditions. After incubation, the bacterial culture was centrifuged at 8,000 rpm for 5 minutes to pellet the bacteria and washed once with 0.85% NaCl (Jomkhame et al., 2022). The bacterial suspension was then mixed with the coating formulation and adjusted to a concentration of 10^7 to 10^{10} CFU/mL. The prepared bacterial coating mixture was subsequently used to coat the peanut seeds.

4. Coating peanut seeds

The peanut seeds were first sterilized on the surface using 0.50% sodium hypochlorite (NaOCl) for 1 minute. After sterilization, they were rinsed three times with sterilized distilled water and dried with sterilized tissue paper. Fifty grams of the peanut seeds were coated with 0.2% w/v methyl cellulose; MC using a rotary pan (model KSC–02D, CERES International

Ltd., Bangkok, Thailand) set at a spinning rate of 32 rpm. The seeds underwent ten different treatments: T1 = non–coated seeds, T2 = seed coating only, T3 = seed coating + *E. kobei* 6.60×10^7 CFU/mL, T4=seed coating + *E. kobei* 6.60×10^8 CFU/mL, T5=seed coating + *E. kobei* 6.60×10^9 CFU/mL and T6=seed coating + *E. kobei* 6.60×10^{10} CFU/mL Following these treatments, the coated seeds from each group were subjected to moisture reduction in a forced–air oven (model KKU40–2) at 33°C for 6 hours.

5. Seed measurement

5.1 Sand testing

Prepare sand with a uniform particle size (<0.05 mm) and sterilize it, followed by further sterilization in an autoclave at 121°C for 15 minutes. Use the sterilized sand as a growth medium for testing 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. Adjust the sand moisture content to approximately 60% and fill the germination boxes to a height of 3 cm. Arrange the seeds on the sand and cover them with an additional 2 cm layer of sand. Place the boxes 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. Abnormal seedlings were evaluated based on the incomplete development of root, stem, and cotyledon structures. Hard seeds were assessed by their hardness, inability to absorb water, and lack of structural change. Dead seeds were identified by their decayed appearance, color change, and possible fungal infestation. Each characteristic was then calculated as a percentage. Germination percentage was assessed using seeds with normal seedlings. The first count was taken on day 5, and the final count was on day 10 (ISTA, 2023). The speed of germination was evaluated by counting the number of seeds that developed into normal seedlings from the 5^{th} to the 10^{th} day, based on the AOSA (1983) method. Mean germination time was calculated by daily assessing normal seedlings for 10 days (Ellis & Roberts, 1980). Shoot length, root length, seedling length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight were assessed on a sample of 20 seedlings, 10 days after sowing, following the method of Jeephet et al. (2022).

5.2 Seed testing in greenhouse conditions

Germination testing of both coated and non–coated peanut seeds was conducted in seed trays with peat moss (Klasmann–Deilmann GmbH, Ltd., Germany) used as

the seeding material. Abnormal seedlings, hard seeds, and dead seeds were evaluated similarly to the sand testing method. The first germination evaluations were conducted 5 days after planting, and the final count was recorded 10 days after sowing (ISTA, 2023). The speed of germination was assessed through daily examination of normal seedling emergence from the 5^{th} to the 10^{th} day based on the AOSA (1983) method. Mean germination time was calculated by daily assessing normal seedlings for 10 days (Ellis & Roberts, 1980). Seedling growth was assessed using the same methods as in the sand testing.

6. Statistical analysis

The germination percentage was arcsine–transformed to normalize the data before statistical analysis using the SAS 9.1 program. All data were analyzed by one–way analysis of variance; ANOVA, completely randomized design, and the difference between the treatments was tested using Duncan's multiple range test; DMRT.

Results

1. Seed germination and vigor

Under sand conditions, coating seeds with *E. kobei* at $6.60x10^7$ CFU/mL and $6.60x10^8$ CFU/mL resulted in a significantly higher germination percentage compared to non–coated seeds. The seeds coated with *E. kobei* at $6.60x10^8$ CFU/mL also exhibited a higher speed of germination than non–coated seeds. Furthermore, seeds coated with *E. kobei* at all concentrations (T3–T6) showed a significantly longer mean germination time compared to non–coated seeds. Under greenhouse conditions, the results were consistent with those under sand conditions. Seeds coated with *E. kobei* at $6.60x10^8$ CFU/mL and $6.60x10^9$ CFU/mL exhibited significantly higher germination percentages and speed of germination than non–coated seeds. However, seeds coated with *E. kobei* at $6.60x10^{10}$ CFU/mL and those coated with MC alone demonstrated better mean germination times than other treatments as shown in Table 1.

Table 1 Germination percentage; GE, speed of germination; SGE and mean germination time of peanut seeds after coating with bacteria producing indole–3–acetic acid; IAA, were tested under sand and greenhouse conditions.

Treatment [1]	Sand condition			Greenhouse condition		
	GE (%)	SGE (seedling/day)	MGT (day)	GE (%)	SGE (seedling/day)	MGT (day)
T1	48b	3.63b	7.10a	51b	3.98b	6.97a
T2	44b	3.37b	6.88ab	59ab	4.92ab	6.20b
T3	49b	3.80b	6.65b	51b	4.12b	6.41ab
T4	59a	4.90a	6.51b	64a	5.10a	6.54ab
T5	58a	4.21ab	6.60b	62a	4.22ab	6.72ab
T6	55ab	4.23ab	6.33b	60ab	4.00b	6.22b
F–test	**	*	*	**	**	*
CV.(%)	16.75	21.82	11.60	8.07	13.18	6.42

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

[1] T1 = non–coated seeds, T2 = seed coating only, T3 = seed coating + *E. kobei* $6.6x10^7$ CFU/mL, T4 = seed coating + *E. kobei* $6.60x10^8$ CFU/mL, T5 = seed coating + *E. kobei* $6.60x10^9$ CFU/mL and T6 = seed coating + *E. kobei* $6.60x10^{10}$ CFU/mL

[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.

2. Seed quality

Under sand conditions, the results indicate that peanut seeds exhibit a high percentage of abnormal seedlings, both in coated and non–coated seeds. Coating the seeds with *E. kobei* at $6.60x10^8$ CFU/mL results in a lower percentage of abnormal seedlings compared to other treatments. Coating with *E. kobei* at $6.60x10^{10}$ CFU/mL shows no presence of hard seeds. Non–coated seeds, seeds coated with only MC, and seeds coated with *E. kobei* at $6.60x10^{10}$ CFU/mL exhibit a higher percentage of dead seeds than other treatments as shown in Table 2.

Under greenhouse conditions, it is evident that non–coated seeds have a significantly higher percentage of abnormal seedlings than other treatments. All treatments display a similar percentage of hard seeds, but *E. kobei* at $6.60x10^9$ CFU/mL shows the lowest percentage at just 1%. The greenhouse condition results reveal that the percentage of dead seeds is higher

than the percentage of abnormal seedlings. However, coating seeds with *E. kobei* at 6.60×10^8 CFU/mL results in a lower percentage of dead seeds than other treatments as shown in Table 2.

Table 2 Abnormal seedling, hard seeds and dead seeds of peanut seeds after coating with bacteria producing indole–3–acetic acid; IAA, were tested under sand and greenhouse conditions.

Treatment[1]	Sand condition			Greenhouse condition		
	Abnormal seedling (%)	Hard seed (%)	Dead seed (%)	Abnormal seedling (%)	Hard seed (%)	Dead seed (%)
T1	25ab[2,3]	1ab	26a	17a	5ab	27ab
T2	23ab	2ab	31a	10b	3ab	28ab
T3	31a	4a	16bc	12b	4ab	33a
T4	19b	3ab	11c	11b	4ab	21b
T5	23ab	1ab	18bc	12b	1b	25ab
T6	27ab	0b	26a	10b	7a	23b
F–test	*	*	**	*	**	**
CV.(%)	**17.40**	**12.60**	**21.00**	**17.14**	**19.08**	**13.82**

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

[1] T1 = non–coated seeds, T2 = seed coating only, T3 = seed coating + *E. kobei* 6.6×10^7 CFU/mL, T4 = seed coating + *E. kobei* 6.60×10^8 CFU/mL, T5 = seed coating + *E. kobei* 6.60×10^9 CFU/mL and T6=seed coating + *E. kobei* 6.60×10^{10} CFU/mL

[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.

3. Seedling growth

Under sand conditions, the results revealed that coating seeds with *E. kobei* at concentrations of 6.60×10^8–6.60×10^{10} CFU/mL (T4–T6) significantly enhanced seedling growth. Specifically, seeds coated with *E. kobei* at 6.60×10^8 CFU/mL exhibited the highest shoot length among all treatments, while those coated at 6.60×10^{10} CFU/mL showed the greatest root length. Additionally, seedling length assessment indicated that seeds coated with *E. kobei* at 6.60×10^8 CFU/mL (T4) and 6.60×10^{10} CFU/mL (T6) resulted in longer seedlings than other

treatments, showing a statistically significant difference compared to non–coated seeds as shown in Table 3. Figure 1 illustrates that eight–day–old seedlings from seeds coated with *E. kobei* at $6.60x10^8$–$6.60x10^{10}$ CFU/mL (T4–T6) exhibited superior growth than other treatments. Seeds coated with *E. kobei* at $6.60x10^8$ CFU/mL had the highest shoot fresh and dry weights, whereas those coated at $6.60x10^{10}$ CFU/mL demonstrated superior root fresh and dry weights as show in Table 4.

Under greenhouse conditions, coating seeds with *E. kobei* at $6.60x10^{10}$ CFU/mL resulted in the highest shoot length, with statistically significant differences compared to other treatments. Seeds coated with *E. kobei* at $6.60x10^8$ CFU/mL showed significantly greater root length than non–coated seeds. The seedling length results mirrored those under sand conditions, with seeds coated with *E. kobei* at $6.60x10^8$ (T4) and $6.60x10^{10}$ CFU/mL (T6) having the highest seedling length as show in Table 3. All coating treatments resulted in higher shoot fresh weights compared to non–coated seeds. Coating with *E. kobei* at $6.60x10^8$ CFU/mL yielded the highest shoot dry weight, root fresh weight, and root dry weight, showing statistically significant differences compared to non–coated seeds as shown in Table 4.

Table 3 Shoot length, root length and seedling length of peanut seeds after coating with bacteria producing indole–3–acetic acid; IAA, tested under sand and greenhouse conditions.

Treatment[1]	Sand condition			Greenhouse condition		
	Shoot length (cm)	Root length (cm)	Seedling length (cm)	Shoot length (cm)	Root length (cm)	Seedling length (cm)
T1	9.23b[2]	9.52b	18.75b	7.85c	13.68b	21.53c
T2	8.97b	8.95b	17.92b	8.60b	14.76ab	23.36ab
T3	9.88b	8.79b	18.67b	8.73b	14.93ab	23.66ab
T4	11.25a	10.30ab	21.55a	8.92b	16.87a	25.79a
T5	10.02ab	10.03ab	20.05ab	8.32bc	14.66ab	22.98bc
T6	10.80ab	11.08a	21.88a	9.53a	14.72ab	24.25a
F–test	**	**	**	**	**	**
CV.(%)	**11.02**	**12.18**	**15.31**	**5.34**	**10.05**	**7.68**

** : significantly different at P≤0.01.

[1] T1 = non–coated seeds, T2 = seed coating only, T3 = seed coating + *E. kobei* $6.60x10^7$ CFU/mL, T4 = seed coating + *E. kobei* $6.60x10^8$ CFU/mL, T5 = seed coating + *E. kobei* $6.60x10^9$ CFU/mL and T6 = seed coating + *E. kobei* $6.60x10^{10}$ CFU/mL

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

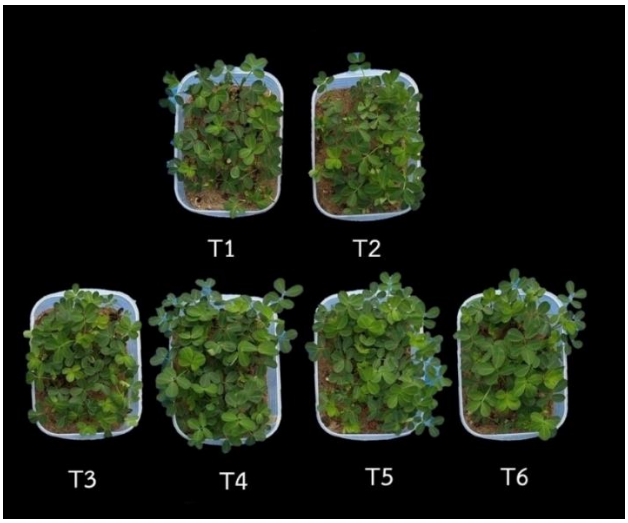

Figure 1 Seed quality testing using the sand test at 10 days after sowing. T1 = non–coated seeds, T2 = seed coating only, T3 = seed coating + *E. kobei* $6.60x10^7$ CFU/mL, T4 = seed coating + *E. kobei* $6.60x10^8$ CFU/mL, T5 = seed coating + *E. kobei* $6.60x10^9$ CFU/mL and T6 = seed coating + *E. kobei* $6.60x10^{10}$ CFU/mL

Table 4 Shoot fresh weight; SFW, root fresh weight; RFW, shoot dry weight; SDW and root dry weight; RDW of peanut seeds after coating with bacteria producing indole–3–acetic acid; IAA, tested under sand and greenhouse conditions.

Treatment[1]	Sand condition				Greenhouse condition			
	SFW (mg)	RFW (mg)	SDW (mg)	RDW (mg)	SFW (mg)	RFW (mg)	SDW (mg)	RDW (mg)
T1	1631b[2]	734b	286b	244b	1446b	1332b	263b	78b
T2	1734ab	979b	320ab	253b	1856a	1532ab	274ab	83ab
T3	1698b	892b	324ab	307ab	1837a	1561ab	287ab	89ab
T4	2011a	1025ab	379a	322ab	2009a	1679a	315a	91a
T5	1985ab	1013ab	335ab	314ab	1905a	1581ab	295ab	81ab
T6	2008a	1265a	355ab	354a	1927a	1567ab	289ab	87ab
F–test	**	**	**	**	**	*	*	*
CV.(%)	16.95	14.81	10.49	14.94	10.18	12.33	8.03	9.24

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

[1] T1 = non–coated seeds, T2 = seed coating only, T3 = seed coating + *E. kobei* $6.60x10^7$ CFU/mL, T4 = seed coating + *E. kobei* $6.60x10^8$ CFU/mL, T5 = seed coating + *E. kobei* $6.60x10^9$ CFU/mL and T6 = seed coating + *E. kobei* $6.60x10^{10}$ CFU/mL

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

Discussion

The assessment of seed quality across both non–coated and coated treatments (T2–T6) highlights a critical distinction in initial seed viability. Non–coated seeds exhibited poor initial quality, characterized by high percentages of abnormal and dead seeds under both testing conditions. This is consistent with the inherent vulnerability of seeds with thin coats, such as peanuts, to rapid moisture absorption, which can lead to internal cellular damage and incomplete germination (Halmer, 2008; Pedrini et al., 2017; Vidak et al., 2022). Conversely, seed coating treatments, particularly those involving *E. kobei* at 6.60×10^8 CFU/mL, showed a notable reduction in the incidence of abnormal and dead seeds. The potential role of IAA produced by *E. kobei* in improving seed quality is suggested by its ability to mobilize nutrients, regulate gene expression, and interact with other hormones, thereby supporting the transition from dormancy to germination and promoting the growth of healthy seedlings (Rocha et al., 2019).

In terms of seed germination and vigor, the experimental data revealed that the initial germination rate of non–coated seeds was only 48%. However, seeds coated with *E. kobei* demonstrated enhanced germination rates, faster germination speeds, and improved mean germination times, particularly at a concentration of 6.60×10^8 CFU/mL The improved performance can be attributed to the multiple phytohormonal activities of *E. kobei* especially its production of IAA, which facilitates the breakdown of stored nutrients within the seed, providing the energy necessary for germination (Rocha et al., 2019). Additionally, the nitrogen–fixing capability of *E. kobei* plays a crucial role in enhancing root development and nutrient absorption, further contributing to the vigor of the seedlings (Ludueña et al., 2019; Roslan et al., 2020). The positive effects of seed biopriming with *Enterobacter* spp. have been observed in other studies with crops like lettuce (Jeephet et al., 2024).

Finally, the impact of *E. kobei* on seedling growth is evident in the significant improvements observed across different coating concentrations. Coating seeds with *E. kobei*, particularly at 6.60×10^8 CFU/mL, not only promoted seedling growth through IAA production but also through the production of other phytohormones like cytokinins, which enhance cell division and elongation, thereby strengthening root and shoot development (Anzuay et al., 2023; Pérez–García et al., 2023). The production of siderophores by *E. kobei* further facilitated the iron solubilization, a critical element in chlorophyll synthesis and electron transfer during

plant growth (Rocha et al., 2019). Additionally, the formation of biofilms on seed and root surfaces by *E. kobei* provided a protective environment, enhancing nutrient exchange and offering resilience against environmental stresses. This multifaceted interaction underscores the potential of *E. kobei* as a biopriming agent to improve overall seed quality, germination, vigor, and seedling growth, supporting the findings of previous research (Roslan et al., 2020; Sarron et al., 2018).

Conclusions

The experimental results demonstrated that coating seeds with *E. kobei* at concentrations of $6.60x10^8$ CFU/mL, $6.60x10^9$ CFU/mL, and $6.60x10^{10}$ CFU/mL significantly enhanced the germination rate of the seeds. Additionally, improvements were observed in shoot length, root length, seedling length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight compared to non–coated seeds. Therefore, it can be concluded that coating peanut seeds with *E. kobei* at a concentration of $6.60x10^8$ CFU/mL is the most suitable for enhancing germination, seedling vigor, and seedling growth in the Tainan 9 peanut variety. However, *E. kobei* at concentrations of $6.60x10^9$ CFU/mL and $6.60x10^{10}$ CFU/mL demonstrated efficacy similar to that of $6.60x10^8$ CFU/mL This suggests that these concentrations could potentially be applied to improve the seed quality and growth of other leguminous crop varieties.

Acknowledgement

The author is very grateful for the scholarship from the Graduate School Department, Office of Academic Administration and Development of Maejo University for the Academic Year 2021 in support of this research. Gratitude is also given to the Agronomy Program, Faculty of Agricultural Production, Maejo University for providing the experimental venue and equipment.

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