The Function of Bio-invigoration Based on Rhizobacteria in Promoting Shallot (*Allium cepa* L.) Growth in Saline Soil

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Abstract
Shallots are an important commodity whose productivity is often constrained by the use of low-vigor seeds and saline soil conditions. This study aimed to examine the effect of seed bio-invigoration using rhizobacteria and the composition of saline soil media on shallot growth. The study employed a randomized block design with two factors, namely bio-invigoration (no treatment, KLK 05 isolate, and KNU 13 isolate) and saline soil media composition (100%, 75%, 50%, and 25% combined with organic fertilizer). The observed variables included plant height, number of leaves, number of tillers, leaf area, and root length. The results showed that bio-invigoration and the addition of organic fertilizer significantly improved plant growth. The combination of rhizobacteria with moderate to low salinity media produced the best results through increased nutrient uptake, ion balance, and vegetative growth. Therefore, bio-invigoration and organic fertilizer have the potential to be effective strategies for improving shallot growth in saline soils.
Keywords: *Shallot, bio-invigoration, PGPR, salinity and organic fertilizer.*

I. INTRODUCTION

Shallots have been intensively cultivated by farmers for a long time, providing income for smallholder farmers and contributing to national food security [2]. As a vital staple ingredient and seasoning in nearly every Indonesian household dish, especially in Southeast Sulawesi, the demand for shallots continues to rise with population growth and the expansion of the processed food industry. This increasing demand poses a significant challenge if it is not adequately met [16].

Typically, farmers use untreated seeds saved from previous harvests, which tend to lose vigor and viability. Low-vigor seeds lead to uneven early growth, weaker root systems, and reduced resilience to environmental stresses [7]. Additionally, limited fertile land availability forces farmers to cultivate marginal lands, such as coastal saline soils. Soil salinity is a major constraint, causing osmotic stress, disrupting water and nutrient uptake, and leading to toxic ion accumulation (Na^+ and Cl^-), all of which negatively impact shallot growth, bulb yield, and quality [15]. To address these challenges, bio-invigoration techniques and organic fertilizers are employed to enhance seed physiological quality and plant stress tolerance. Bio-invigoration with Plant Growth Promoting Rhizobacteria (PGPR) involves treating seeds with beneficial microbes through conditioning in solid or liquid media. This treatment improves seed viability and vigor, stimulates growth hormone synthesis (such as IAA), and enhances overall plant growth and productivity [17]. Combining organic fertilizer application with bio-invigoration in saline soils is expected to improve soil quality, stimulate root growth, enhance nutrient uptake, and support vegetative growth and bulb formation in shallots. The use of seed bio-invigoration technology to boost vigor and salinity tolerance represents a promising innovation for managing suboptimal lands, potentially leading to higher yields compared to untreated conditions.

II. METHODS

Research Location and Time

This research was conducted at the Field Laboratory of the Faculty of Agriculture and the Agrotechnology Laboratory, Faculty of Agriculture, Halu Oleo University, Kendari, from October 2025 to January 2026.

Materials and Equipment

The materials used in this study included shallot seeds (local Buton variety), indigenous rhizobacterial isolates from saline soil, namely KNU 13 (isolate from Konawe) and KLK 10 (isolate from Kolaka), saline soil media, rice husk charcoal, and Tryptic Soy Agar (TSA) medium. The equipment used included hoes, shovels, label boards, bamboo stakes, polybags (30 cm × 30 cm), analytical balance, inoculation loop (ose needle), Petri dishes, autoclave, Laminar Air Flow Cabinet, Scott bottles, spreader rods, Erlenmeyer flasks, oven, Eppendorf tubes, and a hot plate.

Experimental Design

This study used a factorial Randomized Block Design with two factors: seed bio-invigoration (B) and saline soil media composition (M). The bio-invigoration factor (B) had three levels; without bio-invigoration (B0), KLK 05 isolate (B1), KNU 13 isolate (B2). The saline soil media composition factor (M) had four levels; 100% saline soil (M0), 75% saline soil + 25% organic fertilizer (M1), 50% saline soil + 50% organic fertilizer (M2), 25% saline soil + 75% organic fertilizer (M3). Each block included 12 experimental units (3 bio-invigoration levels × 4 soil media levels), replicated three times for a total of 36 experimental units. Each treatment comprised 6 plants, resulting in 216 plants in total.

Research Procedures

Preparation of Research Site

The preparation started with cleaning the greenhouse area. Afterward, polybags (30 cm × 30 cm) were prepared and filled with the designated saline soil media.

Preparation of Planting Media

Soil was collected using a shovel and hoe up to a depth of 30 cm. The collected soil was transported to the research site for drying and sieving using a 0.5 mm soil sieve. The soil was then mixed with organic fertilizer according to the treatment dosage and placed into polybags (30 cm × 30 cm). The soil media were loosened and incubated for two weeks before being used as planting media. The filled polybags were then arranged according to the experimental layout.

Preparation of Rhizobacterial Isolates

Indigenous rhizobacterial isolates from saline soil (KNU 13 and KLK 05) used in this study were obtained as stock cultures from the Agronomy Laboratory, Faculty of Agriculture, Halu Oleo University. To reactivate bacterial metabolism, rejuvenation of the KNU 13 and KLK 05 isolates was performed in Eppendorf tubes.

The isolates were multiplied using TSA medium sterilized by autoclaving. Rejuvenation and multiplication were conducted aseptically in a laminar air flow cabinet. The inoculation loop was sterilized over a Bunsen burner flame. One loopful of rhizobacterial isolate was streaked using the quadrant method on TSA media and incubated at room temperature for 48 hours. The 48-hour-old bacterial cultures were then suspended in 100 mL sterile distilled water in an Erlenmeyer flask and shaken at 150 rpm for 24 hours. Following incubation, the 48-hour-old bacterial cultures were suspended in 100 mL of sterile distilled water within an Erlenmeyer flask and shaken at 150 rpm for 24 hours to prepare the bacterial suspension.

Seed Preparation

The rhizobacterial formulation was applied to shallot seeds through seed bio-matriconditioning using a formulation prepared in 250 mL of Tryptic Soy Broth (TSB). Shallot seeds were soaked in the rhizobacterial solution, after which 50 g of rice husk charcoal powder was added as the matriconditioning medium. The seeds and charcoal powder were thoroughly mixed to ensure all seed surfaces were evenly coated, then incubated for 12 hours.

Planting

Once the planting media were prepared, planting was conducted by placing one treated shallot seed into each planting hole at a depth of approximately 3 cm, ensuring that about 1 cm of the seed tip remained visible above the soil surface.

Plant Maintenance

Plant maintenance included watering, weeding, and replanting. Watering was conducted twice daily (morning and afternoon) to maintain field capacity moisture. Weeding was done by removing weeds growing

in the experimental plots to avoid competition for nutrients. Replanting was performed for seeds that failed to grow or plants that died, ensuring all planting holes were filled.

Observed Variables

1. Plant Height (cm): Measured from the base of the stem to the tip of the longest leaf at 14, 28, 42, and 60 days after planting (DAP).
2. Number of Leaves: Counted as the total number of leaves per plant at 14, 28, 42, and 60 DAP.
3. Number of Tillers: Counted as the number of tillers per plant at 14, 28, 42, and 60 DAP.
4. Leaf Area (cm^2): Measured using the cylinder method by destructively sampling the plant. Calculated using the formula:

$$LD = (2\pi r_1 h_1) + (\tfrac{1}{3} \cdot 2\pi r_2 h_2)$$

Where:
LD = Leaf area
r_1 = radius of cylinder
h_1 = height of cylinder
r_2 = radius of cone
h_2 = height of cone

5. Root Length (cm): Measured from the base to the tip of the longest root in a clump using a ruler at 14, 28, 42, and 60 DAP.

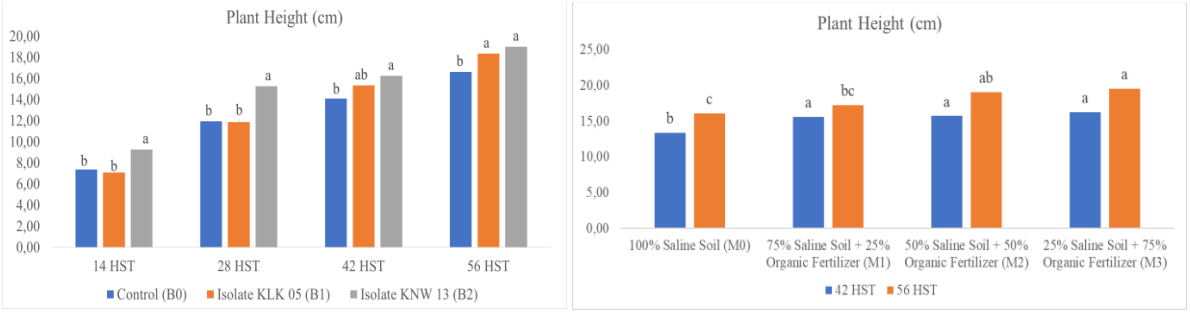
Data Analysis

The collected data were analyzed using Analysis of Variance (ANOVA). When the calculated F value exceeded the critical F table value, the analysis was followed by Duncan's Multiple Range Test (DMRT) to determine significant differences among treatments at a 95% confidence level.

III. RESULTS AND DISCUSSION

Plant Height of Shallots under Bio-invigoration Treatment and Saline Soil Media Composition

The results of this study indicate that bio-invigoration treatment and saline soil media composition physiologically play an important role in enhancing the growth and yield of shallot plants. Bio-invigoration treatment consistently increased plant height compared to the control, indicating that KLK 05 and KNU 13 isolates have a better ability to promote vegetative growth (**Fig. 1a**). In terms of planting media treatment, the highest plant height was observed in the combination of 25% saline soil + 75% organic fertilizer (M3), while the lowest plant height was found in the 100% saline soil treatment (M0) (**Fig. 1b**). High salinity levels can inhibit plant growth, whereas the addition of organic fertilizer can improve soil conditions and support plant growth.



(a) (b)

Fig 1. Graph of the independent effect of seed bio-invigoration treatment on plant height (a) and the independent effect of saline soil media composition on plant height (b).

Inoculation of rhizobacteria originating from saline soils onto plant seeds can increase germination percentage and early seedling growth compared to non-inoculated seeds [18]. This mechanism is associated with the role of rhizobacterial isolates as Plant Growth Promoting Rhizobacteria (PGPR), which are capable of producing phytohormones such as indole-3-acetic acid (IAA), enhancing nutrient availability, and improving root system development [13] [19] [20], as well as alleviating stress through ACC deaminase enzyme activity under environmental stress conditions [3].

Number of Leaves and Leaf Area of Shallots under Bio-invigoration Treatment and Saline Soil Media Composition

The interaction between seed bio-invigoration and saline soil media composition showed that plant vegetative responses to salinity were strongly influenced by their combination, as reflected in the number of leaves and leaf area at 56 days after planting (DAP). Under soil media compositions with moderate to high salinity levels (50%–100%), although environmental conditions inhibited growth due to high osmotic pressure and the accumulation of Na^+/Cl^- ions that reduce the efficiency of water and nutrient uptake, bio-invigoration treatment with KLK 05 (B1) and KNU 13 (B2) isolates resulted in higher leaf number and leaf area compared to the control (**Table 1** and **Table 2**). This indicates that microorganisms can help plants maintain shoot growth even under salt stress conditions.

Table 1. Interaction between seed bio-invigoration treatment and saline soil media composition on the number of leaves at 60 DAP.

Bio-invigoration	Saline Soil				DMRT 0,05
	M0 (100 %)	M1 (75 %)	M2 (50 %)	M3 (25 %)	
Control (B0)	9,89 c Q	17,00 a q	13,22 b r	18,56 a q	
KLK 05 (B1)	12,22 c P	15,78 b q	23,11 a p	21,22 a p	2 = 2,13
KNU 13 (B2)	12,33 b P	20,89 a p	20,22 a q	22,33 a p	3 = 2,24
DMRT 0,05		2 = 2,46	3 = 2,59	4 = 2,66	

Note: Values followed by different letters in the same row (a, b) and column (p, q) indicate significant differences based on the DMRT test at $\alpha = 0.05$.

Moderate salinity conditions (50%) combined with bio-invigoration techniques using rhizobacteria, are able to create a more balanced growing environment for plants, both in terms of water and nutrient availability. Rhizobacteria, as Plant Growth-Promoting Rhizobacteria (PGPR), can enhance plant tolerance to salinity through improved ionic balance, increased nutrient uptake, and stimulation of phytohormone production such as auxin, which plays a role in cell expansion and leaf development [6] [8]. Through these mechanisms, plants are able to maintain leaf formation and expand their photosynthetic surface more optimally even under moderate to high salinity stress conditions.

Table 2. Interaction between seed bio-invigoration treatment and saline soil media composition on leaf area at 60 DAP.

Bio-invigoration	Saline Soil				DMRT 0,05
	M0 (100 %)	M1 (75 %)	M2 (50 %)	M3 (25 %)	
Control (B0)	71,71 b p	75,16 b p	87,14 a q	84,34 a q	
KLK 05 (B1)	64,61 d p	78,55 c p	98,17 b p	113,29 a p	2 = 6,98
KNU 13 (B2)	65,66 d p	80,96 c p	103,81 b p	113,25 a p	3 = 7,33
DMRT 0,05		2 = 8,06	3 = 8,47	4 = 8,72	

Note: Values followed by different letters in the same row (a, b) and column (p, q) indicate significant differences based on the DMRT test at $\alpha = 0.05$.

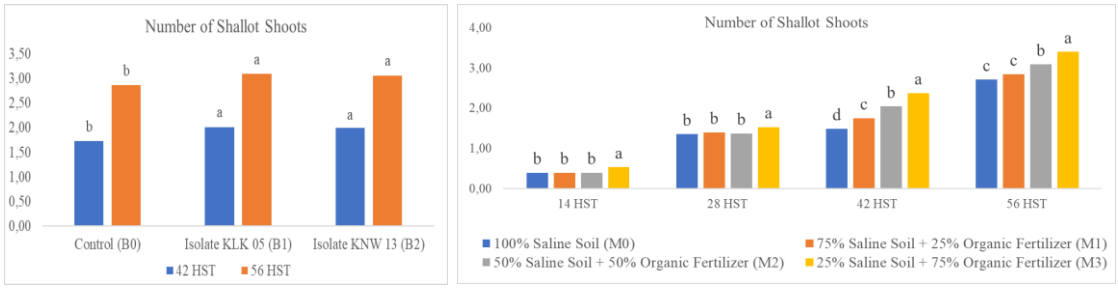
Rhizobacteria can enhance the uptake and accumulation of K^+ and Ca^{2+} in leaves, reflecting improved selective ion transport and nutrient delivery to metabolically active tissues. This improvement is closely associated with increased K^+/Na^+ and Ca^{2+}/Na^+ ratios in both roots and shoots,

better nutrient status, and enhanced photosynthetic performance, ultimately contributing to improved plant growth and salinity tolerance [5][11][21].

Number of Tillers of Shallots under Bio-invigoration Treatment and Saline Soil Media Composition

The results of this study on the number of tillers (**Fig. 2**) showed that bio-invigoration treatment, particularly with KLK 05 and KNU 13 isolates, was able to increase the number of shallot tillers at 42 and 56 days after planting (DAP) compared to the control. Meanwhile, saline soil media at 25–50% consistently produced a higher number of tillers at all observation times compared to the control.

Although under high salinity conditions (75–100%), osmotic pressure and the accumulation of toxic ions limit plant growth, rhizobacterial isolates still demonstrated better performance than untreated plants. This indicates that PGPR application is an effective biological strategy to enhance early growth of shallots in saline soils. The presence of PGPR in the rhizosphere is capable of regulating the expression of salinity stress tolerance genes related to nitrogen (N), phosphorus (P), and plant growth hormone metabolism [6].



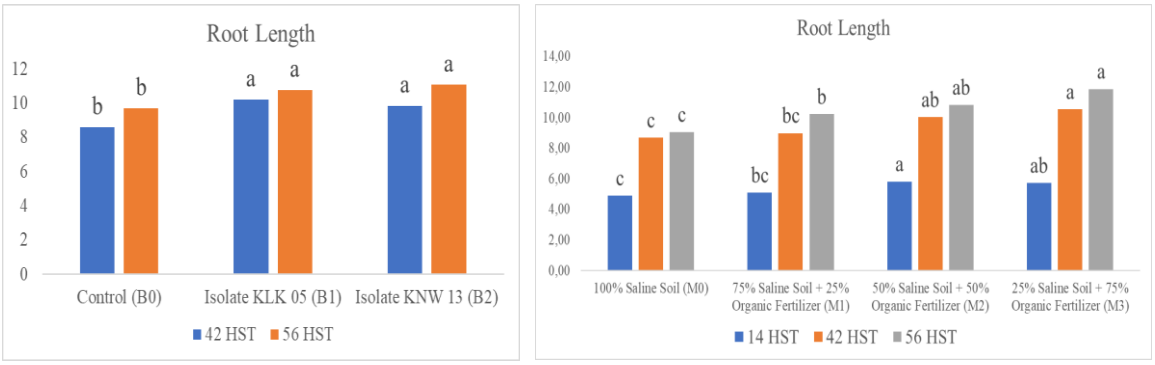
(a) (b)

Fig 2. Graph of the independent effect of seed bio-invigoration treatment on the number of tillers (a) and the independent effect of saline soil media composition on the number of tillers (b).

The increase in the number of tillers can be explained by the ability of rhizobacteria to produce IAA, a phytohormone that stimulates cell division and differentiation at lateral growth points, thereby accelerating the formation of shoots and tillers. Plants inoculated with rhizobacteria tend to produce more tillers to increase their chances of survival under stress conditions [1]. In addition, rhizobacteria enhance the efficiency of nutrient uptake, such as nitrogen and phosphorus, which are essential for root growth and tiller formation even under stress conditions [10].

Root Length of Shallots under Bio-invigoration Treatment and Saline Soil Media Composition

The improvement in shallot growth performance can be explained by the role of rhizobacteria as plant growth-promoting rhizobacteria (PGPR) in enhancing plant tolerance to salinity stress. PGPR are known to contribute to improved K^+/Na^+ ion homeostasis in plants subjected to salt stress by increasing selectivity and regulating ion transport in the roots [14]. In addition, PGPR indirectly support ionic balance through enhanced acquisition of essential nutrients and reduction of oxidative stress caused by the accumulation of reactive oxygen species [21]. Furthermore, rhizobacteria play a role in strengthening cell wall structure and increasing the plant's capacity to maintain tissue water status, enabling plants to better mitigate the negative impacts of environmental stress [12].



(a) (b)

Fig 3. Graph of the independent effect of seed bio-invigoration treatment on root length (a) and the independent effect of saline soil media composition on root length (b).

In addition to these physiological effects, PGPR also trigger systemic immune responses known as induced systemic resistance (ISR) in plants. This occurs when microorganisms respond to root signals and produce molecules that are translocated to the leaves to activate systemic plant defenses. Changes in root exudation patterns due to stress or pathogen attack lead to the selection of ISR-inducing bacteria in the rhizosphere, thereby strengthening the overall plant defense system [4].

Fig 4. Growth performance of shallot plants subjected to seed bio-invigoration treatment and saline soil media composition.

Bio-invigoration with PGPR triggers ISR signaling that enhances plant growth and development through modifications of plant physical and chemical properties, particularly under salinity stress, thereby supporting better productivity and physiological resilience [9]. Therefore, the combination of these physiological and biochemical mechanisms explains why bio-invigoration treatment produces better growth responses compared to the control, especially under saline media conditions.

IV. CONCLUSION

Seed bio-invigoration using rhizobacteria and saline soil media composition significantly affects shallot growth. The KLK 05 and KNU 13 isolates were able to improve all growth parameters compared to the control. Media supplemented with organic fertilizer under moderate to low salinity conditions produced the best results. The combination of both treatments effectively enhanced plant tolerance to salinity stress and vegetative growth.

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