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The Effect of Matriconditioning Enriched Biofertilizer and Washed Rice Water to Enhance Seed Germination, IAA Content and Seedling Growth on Shallot (*Allium cepa* L.)

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ABSTRACT

Shallot seeds are a healthier option for cultivating shallots, as they reduce seed-borne disease infections, but they have a low germination rate. This study examined the impact of matriconditioning enriched with plant growth-promoting rhizobacteria (PGPRs) and rice washing water (WRW) on increasing germination, IAA content and growth of shallot seeds. The matriconditioning treatments included P0 (control), P1 (PGPR-absent), and PGPR presence in P2 (PGPR-Rhizomax), P3 (PGPR-BenprimA), and P4 (PGPR-FloraOne). The WRW treatments included L0 (0%), L1 (50%), and L2 (100%). The shallot seeds used are deteriorated, with moisture content and germination rates below the quality standards set by the Ministry of Agriculture of the Republic of Indonesia. The research revealed that treatment with P2 was the best result on seed germination and seedling root length. The treatment with P4 was the best result on seedling dry weight. The WRW treatment with L2 exhibited the best result in seed germination, seedling root length, and seedling dry weight. The combination of P2L2 demonstrated the best result on seed germination. The treatment with matricondiitoning increased shallot sprouts IAA content in 13-day-olds compared to controls. Also, the treatment with WRW linearly increased the IAA content in 13-day-old shallot sprouts.

1. Introduction

Indonesian shallot farmers commonly employ bulb-to-bulb planting material due to its perceived ease and practicality. Harti *et al.* (2020) reported that shallot bulb seeds obtained from farmers in various regions of Indonesia were found to be infected with onion yellow dwarf virus, shallot latent virus, and garlic common latent virus. Healthy shallot seedlings can be produced through the utilization of shallot seeds. Saputri *et al.* (2018) reported a lower disease incidence in shallot seeds compared to shallot bulb seeds. Shallot bulb seeds exhibited virus infection rates ranging from 66–100%, while shallot seeds showed 0% infection. However, the use of shallot seed often faces challenges due to low germination rates in the field (Rahayu *et al.* 2019), possibly caused by seed deterioration during storage, leading to decreased physiological quality (Megawati *et al.* 2020). Deterioration of seeds causes leakage of metabolites, decreased respiration, and damage to cell membranes. Starch, sugar, and protein as food reserves in seeds will decrease during seed deterioration (Lakshmi *et al.* 2021).

Panda and Mondal (2020) explained various methods for enhancing seed physiological quality. Seed priming is widely applied as a cost-effective and farmer-friendly technology. It involves controlled hydration of seeds, triggering pre-germination processes, and activating metabolism without inducing radicle emergence. Pagano *et al.* (2023) explained that seed priming enhances the activities of seed metabolism, leading to dormancy release, increasing antioxidant levels, and repairing the Deoxyribonucleic

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Acid (DNA). Pagano *et al.* (2023) also explain that matriconditioning or solid matrix priming is a simple seed priming method that involves mixing seeds with moist solid particle materials. Corbineau *et al.* (2023) explain that the solid carrier possesses a low matric potential, allowing for water absorption and the early stages of germination, inhibiting the radical from breaking through the seed coat. After priming, the seeds are dried to facilitate standard handling, storage, and planting.

Matriconditioning effecting for various seed types is easy to perform and does not cause aeration problems. Furthermore, matriconditioning can be combined with bioagents, phytohormones, nutrients, and pesticides (Pagano *et al.* 2023). Adding phytohormones or Plant Growth Promotor (PGRs) during seed priming enhances crop tolerance to abiotic stress, aiding sustainable crop production in regions susceptible to drought, salinity, and flooding (Corbineau *et al.* 2023). Pagano *et al.* (2023) explains that various media, such as coconut fiber, sand, husks, and other natural fibers, can be used in the matriconditioning technique. Husk charcoal is an easily obtainable material for use as a matriconditioning medium. Several studies report that husk charcoal, when used as a priming or germination medium, can have beneficial or detrimental effects depending on the plant type (Kato *et al.* 1977; Badar and Qureshi 2014; Liu *et al.* 2018; Grafi and Singiri 2022). Anaway *et al.* (2021) reported that matriconditioning treatment using charcoal husk media with *Bacillus* isolate CKD061 and the application of botanical pesticides can enhance the growth and yield of shallot seeds.

The utilization of Plant Growth Promoting Rhizobacteria (PGPR) in combination with seed priming has been extensively studied and proven to enhance the viability and vigor of seeds with physiological deterioration (Ha-tran *et al.* 2021; Sundahri *et al.* 2023). PGPRs play a role in phytohormone production, nutrient provision, and protection, which can enhance seed germination and early seedling growth (Kenneth *et al.* 2019). Furthermore, enhancement of the physiological quality of deteriorated seeds can be achieved through the addition of nutrients to support metabolic processes and enhance seed food reserve accumulation during germination (Damaris *et al.* 2019; Li *et al.* 2022; Nie *et al.* 2022).

Washed rice water, a household waste that contains high levels of starch and protein, is commonly used as a nutrient source for plants. Rice contains mainly carbohydrates (77-89%), with 77.6% being starch also proteins, fats, amylose, and vitamin E found in the pericarp, aleurone, and testa layers that are washed away during rice cleaning (Pereira *et al.* 2021). Nabayi *et al.* (2021) reported that washed rice water contains various micronutrients and macronutrients. The utilization of washed rice water as a nutrient source to enhance seed germination is still limited. Based on these statements, combining matriconditioning with a mixture of PGPR biofertilizer and the use of washed rice water during germination has the potency to improve the germination and seedling growth of shallot seeds.

In this study, the role of plant growth-promoting rhizobacteria in combination with matriconditioning is extensively discussed for its function as a phytohormone producer with the potential to enhance seed germination and early growth of shallot plants. The role of PGPR as a biofertilizer comes into play during root inoculation. In this research, the influence of Washed rice water is primarily explored as a nutrient provider for seeds, aiming to enhance their germination capacity and stimulate early growth. The chosen onion seed variety for this study is 'Lokananta', which is known for its adaptability to lowland cultivation.

2. Materials and Methods

2.1. Materials

The used materials included shallot seeds of the Lokananta variety (BM8705) produced by PT East West Seed Indonesia, PGPR-biofertilizer RhizomaX (contain *Rhizobium* sp. 10^{10} cfu/ml, *Bacillus polymixa* 10^{10} cfu/ml, and *Pseudomonas fluorescens* 10^{10} cfu/ml), PGPR-biofertilizer BenprimA (contain *Bacillus polymixa* 10^{7} cfu/ml and *Pseudomonas fluorescens* 10^{7} cfu/ml), PGPR-biofertilizer FloraOne (contain *Rhizobium* sp. 3.4×10^{10} cfu/ml, *Pseudomonas fluorescens* 9.3×10^{10} cfu/ml, *Azospirilum* sp. 7.3×10^{8}, *Aspergillus niger* 3.4×10^{7}, and *Trichoderma harzianum* 1.3×10^{7}). The matriconditioning medium utilized is husk charcoal. The stock solutions of PGPR biofertilizer were 10 g/l (BenprimA), 10 g/ l (RhizomaX), and 15 ml/l (FloraOne). The stock solution of rice water was obtained by washing

500 grams of white rice (Ciherang variety) with 500 ml of water, with 4 replications.

2.2. Experimental Design

The research treatments consisted of two factors: matriconditioning treatment with rice husk charcoal as the medium and a mixture of PGPR-biofertilizer (M+PGPR), consisting of five levels: control or without matriconditioning treatment (P0), matriconditioning without PGPR biofertilizer (P1), M+PGPR-RhizomaX (P2), M+PGPR-BenprimA (P3), and M+PGPR-FloraOne (P4), and washed rice water (WRW), with three levels: control (L0), 50% concentration (L1), and 100% concentration (L2).

The preliminary test aims to determine the moisture content (MC) and germination percentage (GP) of the seed lots used in the research. The germination test was carried out using the test on paper method using opaque stencil paper and incubated in a germinator with a temperature of 20–30°C and tested on sands (Ministry of Agriculture of the Republic of Indonesia, 2017). The germination test used 400 seeds with 4 replications (100 seeds for each replication). The seed moisture content test used 4-5 grams of seeds with two replications. Shallot seeds were baked at 103±2°C for 17±1 hour.

The matriconditioning media consisted of 50 grams of rice husk charcoal, 20 ml of distilled water (control) or PGPR-biofertilizer (according to the research treatment), and 20 ml of rice wash water. Three grams of shallot seeds were mixed with the matriconditioning media to treat the seeds and then incubated for 24 hours in a dark room. The washed rice water (40 ml) was applied 1-5 days after cultivation.

The germination testing of the shallot seeds was carried out using the sand method, and germinated in a greenhouse with a daily temperature range of 30–40 C. The research design for the seed germination test used a split-plot design with a factorial completely randomized design, repeated four replications, with each replication consisting of 100 seeds.

The shallot seeds were planted in a mixture of soil and compost (ratio 1:1). Ten treated shallot seeds were planted on polybags (15 × 15 cm) with a depth of 0.5 cm. The seed holes were covered with rice-husk charcoal. The research design for seedling growth testing used a factorial randomized block design with three replications.

2.3. Observation

The observed parameters included germination percentage (the percentage of normal germination on the 12th day) (ISTA 2012; Ministry of Agriculture of the Republic of Indonesia 2017), seed value index (describing the speed of seed germination (%.etmal^{-1}) (ISTA 2012), estimated normal germination per 24-hour period until the 12th day of germination), Indole Acetic Acid (IAA) content of the shallot sprout, seedling height (cm), seedling leaf number (pieces), seedling root length (cm), and dry weight of the seedlings (g).

The analysis of Indole Acetic Acid (IAA) was performed when the shallot sprouts were 13 days after germination (DAG). The IAA analysis followed a modified method based on the study by Patten & Glick (2002). The extraction of Indole Acetic Acid began by grinding 0.1 gram of a fresh leaf sample using a mortar and pestle. The extracted solution was then transferred to a microtube and diluted with sterile distilled water to a volume of 1.5 ml. The extraction solution was centrifuged at a speed of 10,000 x g and a temperature of 4°C for 10 minutes. After centrifugation, 0.5 ml of the resulting supernatant was collected using a micropipette and transferred to another microtube. To this supernatant, 1 ml of Salkowsky reagent was added, mixed by vortexing, and incubated in a dark room for 15 minutes. The incubated solution was then measured for its absorbance using a spectrophotometer at a wavelength of 520 nm. The concentration of IAA hormone was determined by calculating the regression equation derived from the standard curve. The standard curve for the IAA hormone was generated using concentrations of 0, 10, 20, 40, and 60 mg/L. The preparation of the IAA hormone standard curve involved using a stock solution of IAA with a concentration of 100 mg/L, which had been previously diluted with distilled water.

2.4. Data Analysis

The results of the preliminary test are presented quantitatively without statistical analysis to compare the moisture content and germination of shallot seeds against the quality standards for shallot seeds according to the Ministry of Agriculture of the Republic of Indonesia. IAA content is also presented in a quantitative descriptive manner without comparative statistical analysis. The data of shallot seed germination and early shallot seedling growth analysis was performed using Analysis of Variance (ANOVA). If the ANOVA results showed a significant effect at the 5% level, further analysis was conducted using Duncan's Multiple Range Test (DMRT) at a significance level of 5%. Correlation analysis was conducted to determine the relationship between seed vigor and viability with the growth of shallot seedlings up to 6 weeks after cultivation.

3. Results

3.1. Preliminary Test

Shallot seeds sourced for germination testing had a moisture content of 15.17%, while seeds used for seedling growth testing had a moisture content of 9.09%. The seed source used for the seedling growth test exhibited a germination percentage of 67%. Meanwhile, the germination rate of the seed source used for the germination test was 52.25% (Table 1).

3.2. Shallot Seed Germination

The matriconditioning treatment with the Rhizomax PGPR-biofertilizer (P2) exhibited the most favorable trend in accumulated seed germination capability compared to the other treatment groups (Figure 1), also showed the highest germination rate when compared to

Table 1. Results of moisture content and germination percentage testing of shallot seed

Sample of seed	MC (%)	GP (%)	GP test method	Seed source	SQ of MC* (%)	SQ of GP* (%)	Seed conditioning before treatment
Seedling test	15.17±0.02	67.00±2.45	TP	Commercial (certified seed)	8.00	70.00	The seeds are left in an open space
Germination test	9.09±0.00	52.25±14.77	TS	Commercial (certified seed)	8.00	70.00	No conditioning

The data are presented as mean (4 replicates) ± standard deviation. TP: test on paper, TS: test on sand, SQ: standard quality, GR: germination rate, MC: moisture content; * = standard quality based Ministry of Agriculture of the Republic of Indonesia (2017)

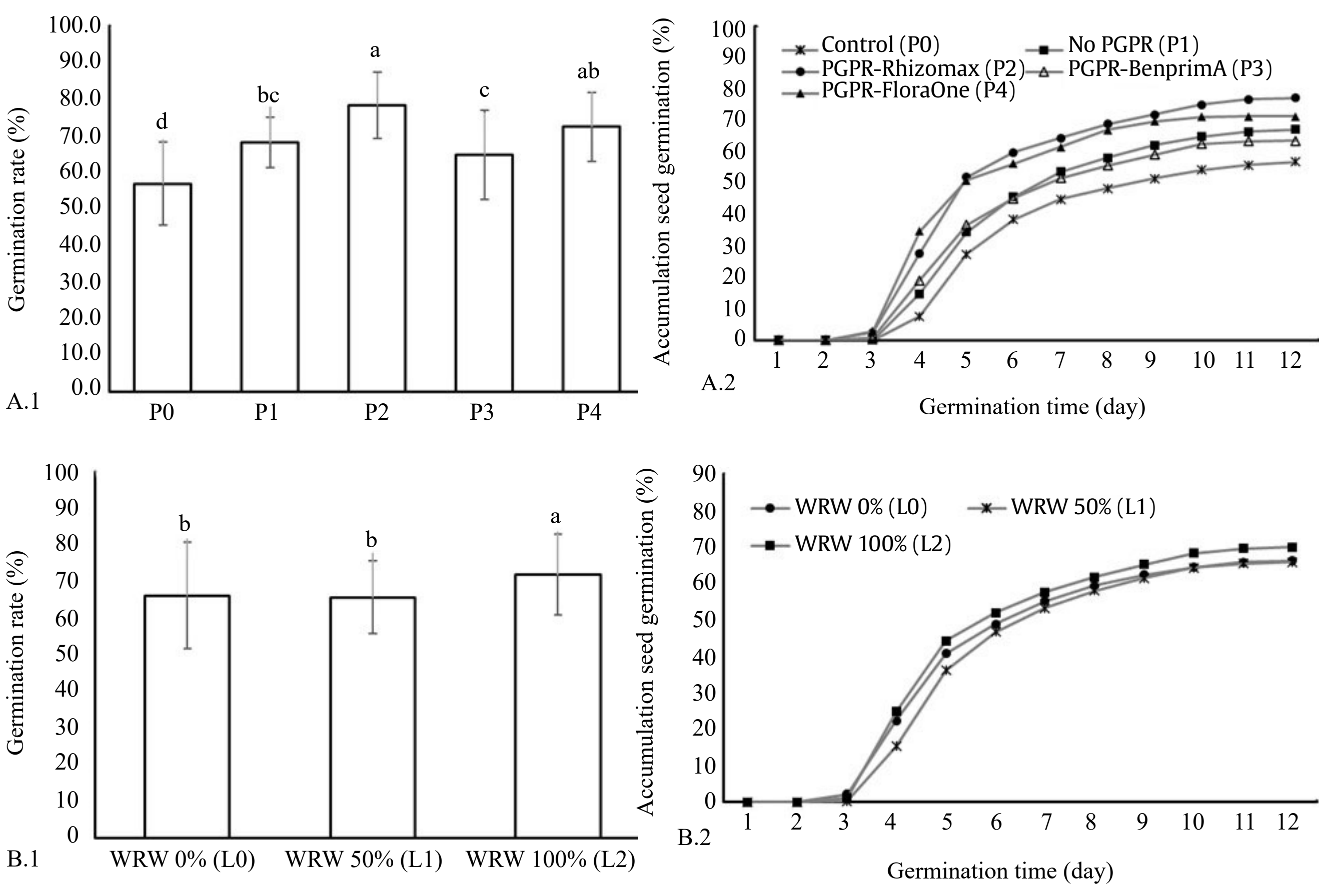

Figure 1. The Effects of matriconditioning (A) and washed rice water (B) treatment on germination rate (1) and accumulation seed germination (2). Graphs A.1 and B.1 have value (mean with 4 replications) followed by the same letter indicating no significant difference based on Duncan's test (p<0.05). Graphs A2 and B2 also represent the mean with 4 replications. P0: control, P1: matriconditioning without PGPR-biofertilizer, P2: matriconditioning with the Rhizomax PGPR-biofertilizer, P3: matriconditioning with the BenprimA PGPR-biofertilizer, P4: matriconditioning with the FloraOne PGPR-biofertilizer. L0: control or without washed rice water, L1: 50% washed rice water, L2: 100% washed rice water

the other treatment groups ($p<0.05$), reaching 78.2%. The matriconditioning treatment with the PGPR-FloraOne biofertilizer displayed the second-highest results in both accumulated seed germination and germination rate. In contrast, the control treatment (P0) displayed the lowest germination percentage at 56.75% among other treatment groups ($p<0.05$).

Washed rice water at a concentration of 100% displayed the most favorable trend in accumulated seed germination capability compared to the other treatment groups (Figure 2). Also, it demonstrated the highest germination rate among the various groups, reaching 71.85% ($p<0.05$). Washed rice water at a concentration of 50% (L1) and the control (L0) exhibited similar effects on the germination capability of red onion seeds, with no significant differences observed.

The treatment group of matriconditioning and washed rice water demonstrated an interaction as a result of the seed value index (Table 2). Generally, the washed rice water treatment showed a positive impact on increasing the seed value index. However, the data indicated a decreasing trend in the washed rice water treatment when combined with PGPR-Floraone (P4). Nevertheless, this decreasing trend did not exhibit a significant influence. The combined treatment with

Table 2. Effects of the interaction between matriconditioning treatment and washed rice water on the seed value index of shallot seeds

Treatment		Seed value index (% day^{-1})
Matriconditioning	Washed rice water	
Control (P0)	WRW 0% (L0)	9.52±2.25^{gh}
	WRW 50% (L1)	8.46±1.09^{h}
	WRW 100% (L2)	10.85±3.20^{fg}
PGPR-absent (P1)	WRW 0% (L0)	12.77±2.07^{cdef}
	WRW 50% (L1)	11.48±1.94^{efg}
	WRW 100% (L2)	12.97±2.54^{cdef}
PGPR-Rhizomax (P2)	WRW 0% (L0)	15.66±2.02^{ab}
	WRW 50% (L1)	14.06±2.60^{bcd}
	WRW 100% (L2)	17.25±1.92^{a}
PGPR-BenprimA (P3)	WRW 0% (L0)	9.53±2.39^{gh}
	WRW 50% (L1)	12.02±2.44^{def}
	WRW 100% (L2)	14.81±1.80^{abc}
PGPR-FloraOne (P4)	WRW 0% (L0)	17.03±2.40^{a}
	WRW 50% (L1)	13.93±2.13^{bcde}
	WRW 100% (L2)	14.8±2.38^{abc}
P-value		
Matriconditioning		0.001
Washed rice water		0.040
Matriconditioning*washed rice water		0.036

Data represent mean (4 replication) ± standard deviation. The value followed by the same letter indicates no significant difference based on Duncan's test ($p<0.05$)

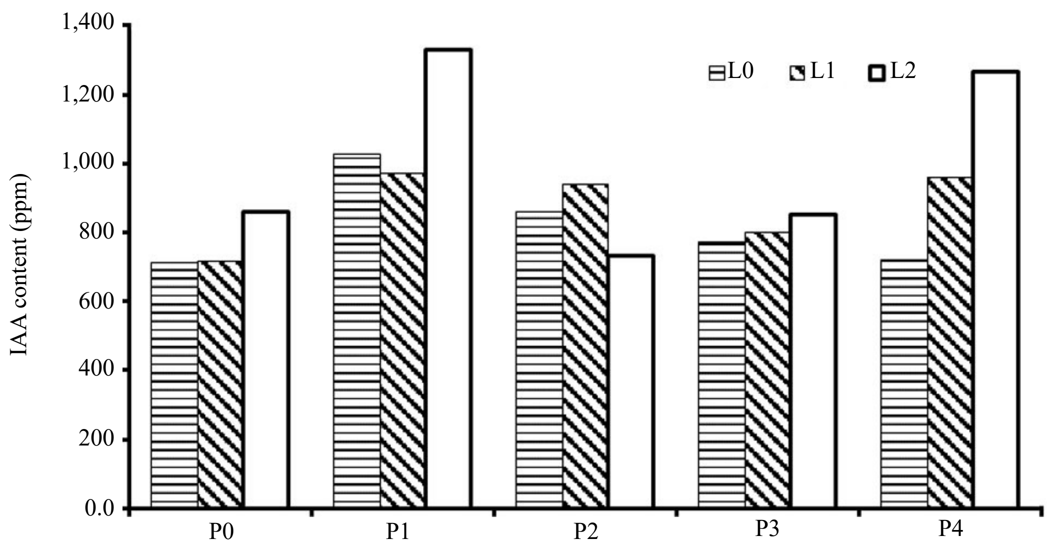

Figure 2. The endogenous IAA content at 13-day-old shallot sprouts. Data is presented without replication. P0: control, P1: matriconditioning without PGPR-biofertilizer, P2: matriconditioning with the Rhizomax PGPR-biofertilizer, P3: matriconditioning with the BenprimA PGPR-biofertilizer, P4: matriconditioning with the FloraOne PGPR-biofertilizer. L0: control, L1: Washed rice water at a concentration of 50%, L2: Washed rice water at a concentration of 100%

P2L2 (PGPR-Rhizomax and WRW 100%) yielded the highest seed value index for shallot seeds at 17.25% days^{-1}. On the other hand, the lowest seed value index for shallot seeds was observed in the treatment with P0L1 at 8.46% day^{-1}.

3.3. IAA Content of Shallot Sprouts

The highest IAA content of shallot sprouts treated with matriconditioning enriched PGPR and washed rice water was observed in the combination of P1L2 at 1330.5 mg/L. The next highest IAA content was found in the P4L2 combination, at 1265.5 mg/L. The lowest IAA content was observed in the treatment with P0L0, at 710.5 mg/L (Figure 2).

3.4. Shallot Seedling Growth

The washed rice water treatment had a significant when the shallot seedlings were 4 weeks after cultivation, but the subsequent growth of shallot seedling heights was not significantly different. The effects of matriconditioning treatment and washed rice water treatment on shallot seedling height are presented in Table 3.

The influence of the matriconditioning treatment and washed rice water on the leaf number of shallot seedlings is presented in Table 4. Also, the combination of both treatments showed similar results in terms of leaf number but influenced significantly at 6 weeks after cultivation (Table 5).

The treatment with P4 (M+PGPR-FloraOne) resulted in the best root length of shallot seedlings (14.54 cm), while treatment with P2 (M+PGPR-Rhizomax) had the second-best root length (14.45 cm). There was no significant difference between P4 and P2 in terms of root length (Table 6). The treatment with L2 (WRW 100%) showed the best results for root length and dry weight of shallot seedlings, measuring 13.91 cm and 0.169 grams, respectively (Table 6). The control of treatment (L0) yielded the lowest values for root length and dry weight, measuring 13.30 cm and 0.166 grams, respectively.

Table 4. The influence of matriconditioning and washed rice water on the shallot on the shallot leaf number

Treatment	Leaf number	
	4 weeks	5 weeks
Matriconditioning		
Control (P0)	3.26±0.28^{b}	3.63±0.31^{a}
PGPR-absent (P1)	3.26±0.36^{ab}	3.89±0.41^{a}
PGPR-Rhizomax (P2)	3.22±0.33^{a}	4.11±0.47^{a}
PGPR-BenprimA (P3)	3.19s±0.24^{a}	4.00±0.37^{a}
PGPR-FloraOne (P4)	3.44±0.37^{a}	4.22±0.33^{a}
Washed rice water		
WRW 0% (L0)	3.38±0.35^{a}	4.04±0.53^{a}
WRW 50% (L1)	3.29±0.33^{a}	3.93±0.42^{a}
WRW 100% (L2)	3.16±0.25^{a}	3.93±0.29^{a}
P-value		
Matriconditioning	0.456	0.018
Washed rice water	0.165	0.624
Matriconditioning *washed rice water	0.722	0.882

Data represent mean (with 4 replications) ± standard deviation. The value followed by the same letter indicates no significant difference based on Duncan's test ($p<0.05$)

3.5. Correlation of Traits in Between Shallot Seed Germination and Early Seedling Growth

Shallot germination affected seedling growth. The influence of the germination of shallot seed on seedling growth was reinforced by the correlation analysis results (Table 7).

Table 3. The influence of matriconditioning and washed rice water on the shallot seedling height

Treatment	Seedling height, Mean (cm) ± Standard Deviation			
	3 week	4 week	5 week	6 week
Matriconditioning				
Control (P0)	17.53±1.27	24.76±0.72	26.82±1.61	31.20±1.75
PGPR-absent (P1)	17.31±0.87	23.99±2.03	27.60±1.12	32.20±1.34
PGPR-Rhizomax (P2)	17.39±1.27	24.07±1.60	27.65±1.22	32.23±1.55
PGPR-BenprimA (P3)	16.71±0.77	24.31±1.01	27.50±2.19	31.05±0.67
PGPR-FloraOne (P4)	17.24±1.42	24.81±1.26	27.64±1.53	31.67±1.67
Washed Rice Water				
WRW 0% (L0)	17.45±1.20	24.91±1.50^{a}	27.46±1.00	32.20±1.40
WRW 50% (L1)	17.30±1.30	24.55±1.20^{ab}	27.81±1.70	31.53±1.40
WRW 100% (L2)	16.97±0.80	23.70±1.30^{b}	27.06±1.80	31.29±1.50
P-value				
Matriconditioning	0.596	0.542	0.781	0.308
Washed rice water	0.488	0.044	0.45	0.229
Matriconditioning *washed rice water	0.568	0.57	0.951	0.71

Value (mean with 4 replications) followed by the same letter indicates no significant difference based on Duncan's test ($p<0.05$)

Table 5. The influence of interaction between matriconditioning and washed rice water on the shallot on the shallot leaf number at 6 weeks after cultivation

Treatment		Leaf number
Matriconditioning	Washed rice water	
Control (P0)	WRW 0% (L0)	3.89±0.19^{d}
	WRW 50% (L1)	3.89±0.38^{cd}
	WRW 100% (L2)	4.56±0.39^{ab}
PGPR-absent (P1)	WRW 0% (L0)	4.56±0.39^{ab}
	WRW 50% (L1)	4.22±0.51^{abcd}
	WRW 100% (L2)	4.22±0.39^{abcd}
PGPR-Rhizomax (P2)	WRW 0% (L0)	4.67±0.34^{a}
	WRW 50% (L1)	4.44±0.20^{abc}
	WRW 100% (L2)	3.89±0.19^{cd}
PGPR-BenprimA (P3)	WRW 0% (L0)	4.33±0.34^{abcd}
	WRW 50% (L1)	4.00±0.00^{bcd}
	WRW 100% (L2)	4.33±0.34^{abcd}
PGPR-FloraOne (P4)	WRW 0% (L0)	4.22±0.19^{abcd}
	WRW 50% (L1)	4.33±0.34^{abcd}
	WRW 100% (L2)	4.33±0.34^{abcd}
P-value		
Matriconditioning		0.563
Washed rice water		0.437
Matriconditioning*washed rice water		0.034

Data represent mean (with 4 replications) ± standard deviation. The value followed by the same letter indicates no significant difference based on Duncan's test ($p<0.05$)

Table 6. Seedling root length and seedling dry weight of shallot seeds treated with M+PGPR and rice washed water

Treatment	Dry weight (gram)	Root length (cm)
Matriconditioning	0.14±0.03^{b}	12.78±1.15^{b}
Control (P0)	0.18±0.02^{a}	13.52±1.03^{ab}
PGPR-absent (P1)	0.18±0.03^{a}	14.45±1.40^{a}
PGPR-Rhizomax (P2)	0.17±0.02^{a}	12.94±0.92^{b}
PGPR-BenprimA (P3)	0.16±0.02^{a}	14.54±1.29^{a}
PGPR-FloraOne (P4)		
Washed rice water	0.17±0.02	13.30±1.16
WRW 0% (L0)	0.17±0.03	13.73±0.62
WRW 50% (L1)	0.17±0.01	13.91±1.08
WRW 100% (L2)		
P-value		
Matriconditioning	0.009	0.006
Washed rice water	0.916	0.348
Matriconditioning*washed rice water	0.169	0.134

Data represent mean (with 4 replications) ± standard deviation. The value followed by the same letter indicates no significant difference based on Duncan's test ($p<0.05$)

4. Discussion

The results of the preliminary test for both sources of shallot seeds showed that the moisture content and germination percentage did not meet the quality standards for certified seed. The seeds used in the study had undergone deterioration, indicated by their low germination rate and high moisture content. Seed deterioration during storage is negatively associated with seed viability, characterized by metabolic leakage, reduced respiration, and cell membrane damage (De Vitis *et al.* 2020; Lakshmi *et al.* 2021). Deterioration also depletes food reserves like sugars, amino acids, and lipids within seeds (Lakshmi *et al.* 2021; Farooq *et al.* 2022), impacting seed quality and subsequently reducing plant growth and yield (Xue *et al.* 2021).

In this research, matriconditioning treatment with husk rice subtracts on shallot seeds conduced seed germination and early growth of seedlings better than control. Zhai *et al.* (2022) reported that matriconditioning seeds with a sand substrate enhances metabolism, respiration, and stimulates the seed germination of *Pinus massoniana*. Solid matrix priming using rice husk media is rarely conducted. Some studies have reported that rice husk can act as a germination inhibitor due to its chemical compounds that generally inhibit seed germination (Kato *et al.* 1977; Liu *et al.* 2018; Grafi and Singiri 2022). Conversely, other studies have reported the opposite result, indicating that rice husk can facilitate early plant growth (Badar and Qureshi 2014; Purwantoro 2016; Grafi and Singiri 2022). Rice husk can facilitate the activity of seed coat-degrading bacteria, thereby promoting water absorption during the initial stages of germination and subsequently inhibiting radicle emergence through the seed coat (Yokota *et al.* 2014). This represents the initial step in initiating priming using solid media such as rice husk (Corbineau 2023). Badar and Qureshi (2014) also reported that a combination of rice husk compost with *Rhizobium* sp-I (JUR1) effectively enhances the early growth of sunflowers.

Matriconditioning with PGPR biofertilizers Rhizomax (P2) and FloraOne (P4) enhances shallot seed germination more than PGPR-absent

Table 7. Correlation between observed variables

Correlation coefficient	GP	SVI	SH	LC	SRL	SDW	IAA
GP	1.000						
SVI	0.966*	1.000					
SH	0.366^{ns}	0.327^{ns}	1.000				
LC	0.230^{ns}	0.230^{ns}	0.596*	1.000			
SRL	0.569*	0.603*	0.016^{ns}	0.242^{ns}	1.000		
SDW	0.518*	0.416^{ns}	0.579*	0.675*	0.440^{ns}	1.000	
IAA	0.180^{ns}	0.152^{ns}	0.107^{ns}	0.371^{ns}	0.456^{ns}	0.598*	1.000

N = 15; GR = Germination rate; SVI = Seed value index; SH = Seedling Height; LC = Seedling Leaf number; SRL = Seedling Root Length; SDW = Seedling Dry Weight; IAA = Indole Acetic Acid; CC =; * = Significant correlation between variables at 5% level; ns = not significant

matriconditioning (P1). Matriconditioning with biofertilizer BenprimA (P3) mirrors the effects of PGPR-absent matriconditioning (P1) on shallot seed germination. Lee *et al.* (2022) explained that rhizobacteria acted as regulators in enhanced seed germination through their mechanism of producing phytohormones. Phytohormones such as auxins, cytokinins, abscisic acid, and gibberellins were noted for their efficacy in boosting germination, modulating oxidative stress, regulating antioxidant enzymes, fostering nutrient uptake, increasing antioxidants, and promoting continuous plant growth from germination (Rhaman *et al.* 2020; Mitra *et al.* 2021). The effectiveness of rhizobacteria in generating seed germination-enhancing phytohormones, such as IAA, cytokinins, and gibberellins, was heightened when operating within a consortium with broader variations (Kenneth *et al.* 2019; Lebrazi *et al.* 2020; Sritongon *et al.* 2023). Saeed *et al.* (2021) also explain that the effectiveness of rhizobacteria in enhancing seed germination in deteriorated seeds varies by bacterial type and abilities.

In this study, analysis of phytohormone production by PGPR was not conducted, so the comparison of results between matriconditioning treatments was solely based on the composition of the fertilizer products. The biofertilizer Rhizomax contains *Rhizobium* sp., *B. polymixa*, and *P. fluorescens*, where the PGPR consortium composition in Rhizomax biofertilizer was more complex compared to BenprimA (*B. polymixa* and *P. fluorescens*). Rhizobium present in the Rhizomax fertilizer has the potential to produce IAA, cytokinin, and gibberellins (Fahde *et al.* 2023). However, the biofertilizer FloraOne had a complete content of PGPR isolates, including *Rhizobium* sp. (3.4×10^{10} cfu/ml), *P. fluorescens* (9.3×10^{10} cfu/ml), *Azospirillum* sp. (7.3×10^{8} cfu/ml), and *Aspergillus niger* (3.4×10^{7} cfu/ml); conversely it also contained *Trichoderma harzianum* (1.3×10^{7} cfu/ml). Sánchez-Montesinos *et al.* (2020) explained that the use of *Trichoderma* for seed priming reduces seed germination in some horticultural crops, but in contrast, it can also suppress seed-borne diseases.

The research results showed that treatment with L2 (100% washed rice water) resulted in better shallot seed germination compared to the other washed rice water treatments. Additionally, treatment with L2 exhibited the best root length and seed dry weight, although there was no significant difference compared to L0 (control) and L1 (50%). Treatment with L2 also tended to produce the highest seedling leaf count during the growth of shallot seedlings but resulted in the lowest seedling height at various observation periods. In general, washed rice water contains various macro and micronutrients, including S, N, K, Mg, C, B, P, Cu, Ca, and Zn (Nabayi *et al.* 2021). Macronutrients and micronutrients in rice that are removed during washing can produce a solution that enhances seed germination. Addition of exogenous macro and micronutrients such as $CaCl_2$, NaCl, KCl, KNO_3, K_3PO_4, KH_2PO_4, $MgSO_4$, Mo, B, Zn, and Mn through seed priming helped repair damaged DNA structure, enhanced metabolism, simulated the antioxidant system, induced resistance to oxidative stress, and maintained ion balance in seeds (Martínez-Ballesta *et al.* 2020).

The research results indicated that the combination treatment with matriconditioning with washed rice water significantly affected the seed value index and leaf count (6-week-old seedling). Non-fermented washed rice water also contained several rhizobacter strains, including *Bacillus, Enterobacter, Pantoea, Klebsiella*, and *Stenotrophomonas* (Nabayi *et al.* 2021), that combined with PGPR biofertilizers can form a more complex consortium of rhizobacteria. The synergy of the consortium in PGPRs biofertilizers needs to be considered due to the potential antagonistic relationships between microorganisms, which could cause instability and may hinder the expected functions of the consortium as plant growth-promoting agents (Denaya *et al.* 2021). The synergistic effects of rhizobacter activity were demonstrated in the analysis of endogenous IAA content in shallot seedlings.

The analysis of endogenous IAA content at 13-day-old shallot sprouts showed that it was relatively different. The application of washed rice water to shallot sprouts linearly increased the IAA content of shallot seedlings (Figure 2); this indicates that the washed rice water used contains IAA-producing rhizobacteria. In general, the addition of exogenous IAA to the seeds can increase the endogenous IAA levels during germination (El-Mergawi & Abd El-Wahed 2020). However, treatment with L2 showed a decrease in the endogenous IAA content in shallot seedlings when combined with treatment with P2L2. The IAA hormone does not solely control germination but is also regulated by the activities of gibberellin, cytokinin, and ethylene hormones (Xue *et al.* 2021). The decrease of endogenous IAA content in shallot seedlings was suspected to be caused by the high uptake of exogenous IAA by the seeds or the presence of antagonistic rhizobacterial activities that potentially reduced the endogenous IAA content in plants (Dhungana and Itoh 2019).

Zhao and Zhong (2013) reported that the exogenous soaking of IAA decreased the endogenous IAA content in the roots of Chinese pear seedlings. Zhao and Zhong (2013) also found that soaking Chinese pear seeds with exogenous IAA showed the best germination performance at a concentration of 10^{-4} M, then decreased at a concentration of 10^{-3} M. (Lee *et al.* 2022) also reported that excessive accumulation of auxin inhibits the metabolism of rice under anaerobic conditions, resulting in hindered seedling growth. Dhungana and Itoh (2019) stated that the host plant conditions, microorganisms, and environmental factors determine the level of IAA in plants. Dhungana and Itoh (2019) further explained that certain strains of PGPR bacteria from the groups *B. cereus, P. aeruginosa, Ralstonia metallidurans, P. aureofaciens*, and *B. megaterium* decrease the endogenous IAA levels in plants through direct IAA degradation or tryptophan degradation mechanisms.
The root length of the shallot seedling showed a significant correlation with the germination variables of shallot seeds. Radicle induction occurs prior to shoot formation during germination, emphasizing the importance of root elongation under optimum conditions (Hourston *et al.* 2020; Obroucheva 2021). High seed vigor and viability resulted in better root growth compared to seeds with low vigor and viability (Reed *et al.* 2022). Seed priming is an advanced technology for enhancing seed vigor and viability and stimulating vegetative growth under various environmental conditions (Shelar *et al.* 2021; Pagano *et al.* 2023).

The dry weight of the seedlings showed a significant correlation with germination percentage, seedling height, leaf count, and IAA content. Germination percentage represents the percentage of successful seedlings in producing essential plant structures within a batch. The success of seed germination indicates the presence of enzymatic activities such as alpha-amylase, protease, and dehydrogenase (Carrera-Castaño *et al.* 2020). These enzymes play a crucial role in mobilizing food reserves, maintaining water balance, enhancing nutrient uptake, and generating energy during germination (Xue *et al.* 2021; Nie *et al.* 2022). Additionally, seeds that exhibit good germination ability demonstrate the presence of antioxidant activities, including superoxide dismutase, catalase, and peroxidase, which protect the seeds from oxidative stress under suboptimal conditions (García-Caparrós *et al.* 2020; Xue *et al.* 2021).

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