Research Article

Synergistic Effects of Hormonal Priming and *Trichoderma asperellum* on the Morphological and Physiological Traits of True Shallot Seeds

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Abstract | This study examines the effects of hormonal priming and *Trichoderma asperellum* on the germination and growth traits of true shallot seeds. The research was conducted in two phases: seed testing in a controlled laboratory environment and field experiments. Laboratory studies employed a Completely Randomized Design with four priming treatments: distilled water (control, z0), auxin (z1), cytokinin (z2), and gibberellin (z3), each applied at 100 ppm. The field research utilized a Split Plot Design, with *T. asperellum* dosages as the primary plot factor at three levels (0 kg ha-1, 400 kg ha-1, and 800 kg ha-1) and hormonal priming treatments (distilled water, auxin, cytokinin, and gibberellin) as subplots. There was no significant interaction effect between hormonal priming and *T. asperellum* application. However, gibberellin priming produced the highest values in germination rate (90.60%), sprout vigour index (646.42%), and plumule length (5.44 cm). In terms of *T. asperellum* application, the 800 kg ha-1 treatment resulted in the highest relative growth rate (0.70) and bulb diameter (3.39 cm). The strategic application of hormonal priming and *T. asperellum* at optimal doses can enhance germination and morphological and physiological traits of true shallot seed.

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Introduction

Seeds, as essential reproductive units containing critical food reserves for germination, facilitate the production of new plants. While shallots are typically propagated vegetatively through bulbs, this approach often leads to diminished plant quality over time. The use of true shallot seed (TSS), derived from fully mature shallot flowers, and has gained popularity as a promising alternative due to its potential to enhance germination morphological and physiological traits (Ismarlin *et al*., 2024). Additionally, TSS provides a solution to the limited availability of shallot bulbs, a common limitation in shallot cultivation (Novianti *et al*., 2020). The adoption of true shallot seed (TSS) as an alternative to traditional bulb propagation has

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demonstrated substantial potential for enhancing germination and morphological and physiological traits in shallot cultivation. TSS technology offers distinct advantages over bulb-based methods, including significantly reduced seed requirements (only 3-7.5 kg/ha), decreased production costs, extended seed shelf life, and improved uniformity in seed varieties (Khoyriyah *et al*., 2019). Additionally, TSS cultivation has been shown to yield healthier plants, double productivity, and produce more significant, more uniform bulbs (Nurcharisma *et al*., 2024). However, despite these advantages, the technology's broader implementation is challenged by the need for stringent technical requirements.

The production techniques for true shallot seed (TSS) are comparatively more complex than those for bulb-based propagation, as generative shallot cultivation requires a seedling phase of approximately 30-45 days, followed by field transplantation and an additional 60-70 days until harvest (Syam'un *et al*., 2017). This extended timeline makes TSS cultivation lengthier than that of the vegetative method. Furthermore, TSS is often challenged by low germination rates and uneven growth, necessitating seed enhancement technologies to improve quality (Sharma *et al*., 2023; Ter-Steeg and Gildemacher, 2023). Seed priming, in particular, has emerged as a viable method to address these germination issues (Afzal, 2023; Amir *et al*., 2024). Seed priming, a pre-germination technique designed to enhance seed physiological quality, involves soaking seeds in water or specific solutions to stimulate imbibition and metabolic processes, thereby accelerating germination, increasing germination rates, and producing more uniform, robust seedlings (Fu *et al*., 2024). The early establishment of seedlings, marked by rapid and synchronized root emergence, is critical to successful growth (Pasternak *et al*., 2023). By adjusting solution concentrations or incorporating certain chemicals, various priming treatments can significantly influence germination rates and activate seed metabolism, promoting optimal early growth (Ghimire *et al*., 2023).

The lag phase in germination is a critical stage within the seed priming process, occurring after imbibition when water has entered the seed (Upretee *et al*., 2024; Diya *et al*., 2024). During this phase, essential metabolic processes initiate, including ATP synthesis, activation of the antioxidant system, DNA repair, and the accumulation of photophospholipids and sterols, alongside a notable reduction in water uptake, indicating the transition into the lag phase (Fu *et al*., 2024). The optimal lag phase duration for shallot seeds is approximately 20 hours (Faried *et al*., 2023). Hormonal priming, one effective technique for seed priming, involves soaking seeds in a growth hormone solution before planting to promote faster germination and enhanced resistance to adverse environmental conditions (Rhaman *et al*., 2020a). Commonly used hormones in this technique include gibberellins, auxins, and cytokinins, each contributing to accelerated plant growth (Wong *et al*., 2023). This study examines the role of cytokinins, with a focus on thidiazuron, in triggering suicidal germination of Striga seeds. Although auxin is not explicitly investigated, the findings reveal that cytokinins can substantially boost germination rates, achieving up to 63% germination using cytokinins alone and exceeding 93% when combined with fluridone (Jamil *et al*., 2023). Similarly, research by Agustiansyah *et al*. (2021) has shown that gibberellin priming enhances soybean seed vigour under aluminum stress conditions. For the Lokananta variety of shallot seeds, a gibberellin concentration of 100 ppm has proven effective in increasing the vigour index to 92.5% and germination rate to 93.75% (Elshyana *et al*., 2019). Beyond germination, environmental conditions during transplanting significantly influence plant growth. Beneficial microbes such as *T. asperellum* can effectively address challenges like nutrient deficiencies and disease susceptibility during this stage. Studies on *Trichoderma* sp. have demonstrated robust resistance to fusarium wilt in treated banana seedlings, with no disease symptoms, compared to an 84-85% disease intensity in untreated seedlings (Sudantha and Ernawat, 2014). Moreover, *T. asperellum* protects roots from pathogens and stimulates plant growth, leading to optimized crop yields (Tyśkiewicz *et al*., 2019). These findings underscore the need for further research to elucidate the specific roles of hormonal priming and *T. asperellum* in promoting shallot germination, morphology, and physiology.

Materials and Methods

This research was conducted in two stages: a laboratory-based seed testing phase and subsequent field experiments to evaluate the effects of different priming treatments and *T. asperellum* application rates on true shallot seed growth.

Laboratory experiment

The laboratory experiment was designed using a Completely Randomized Design (CRD) with four priming treatments applied to the seeds at a uniform concentration of 100 ppm: z0: distilled water (control); z1: auxin priming; z2: cytokinin priming; z3: gibberellin priming.

Each treatment was replicated three times, resulting in a total of 12 experimental units. Seeds were primed by soaking in the respective solutions for 12 hours at 25°C, followed by air-drying to their initial moisture content (approximately 12%) at 22°C with 50% relative humidity. After priming, the seeds were stored in sealed containers before conducting germination tests. Germination tests were conducted in a controlled growth chamber set at a constant temperature of 25±2°C, with a relative humidity of 60%, and a photoperiod of 16 hours light / 8 hours dark. Germination parameters, including germination percentage, mean germination time, seedling vigor index, root length, and shoot length, were assessed according to the International Seed Testing Association (ISTA, 2023) protocols.

Field experiment

The field experiment followed a Split Plot Design (SPD) with two factors: Main plot factor: *T. asperellum* application rates at three levels: t0: 0 kg ha^{-1} (control); t1: 400 kg ha^{-1}; t2: 800 kg ha^{-1}. Subplot factor: Four types of hormonal priming treatments: z0: distilled water priming (control); z1: auxin priming (100 ppm); z2: cytokinin priming (100 ppm); z3: gibberellin priming (100 ppm). Each treatment combination was replicated three times, resulting in 36 experimental units, with each plot measuring 2 m × 3 m. The seeds were sown at a depth of 2 cm, with a spacing of 20 cm × 20 cm. The field experiment was conducted under ambient environmental conditions, with an average daytime temperature of 28±3°C and nighttime temperature of 20±2°C, and relative humidity ranging from 60–75%. Irrigation was applied twice a week, and standard agronomic practices, including weed control and pest management, were uniformly maintained across all treatments.

Observed parameters

This study measured parameters including germination power, sprout vigor index, plumula length, relative growth rate, bulb diameter, stomatal density, stomatal aperture, and tension.

Data analysis

The collected data were analyzed using analysis of variance (ANOVA) to determine the significance of treatment effects. Mean comparisons were conducted using the Least Significant Difference (LSD) test at a 5% significance level. Statistical analyses were performed using STAR version 2.0.1 software developed by the International Rice Research Institute (IRRI), and data visualization was carried out using OriginPro 2024 (Version 10.1).

Results and Discussion

Germination

Analysis of variance showed that priming treatment with various growth regulators significantly affected germination parameters such as germination power, Vigour index of sprouts, and plumula length.

Germination ability

Analysis of variance showed that priming treatment with various types of growth regulators had a very significant effect on germination power. The average germination (%) in priming treatment with various growth regulators is presented in Figure 1. The results of the LSD test at the 5% significance level, as presented in Figure 1, indicate that the gibberellin priming treatment achieved the highest average germination rate of 90.60%. This rate is significantly different (P ≤ 0.05) from those observed in the distilled water priming treatment (80.60%), auxin treatment (83.00%), and cytokinin treatment (81.20%).

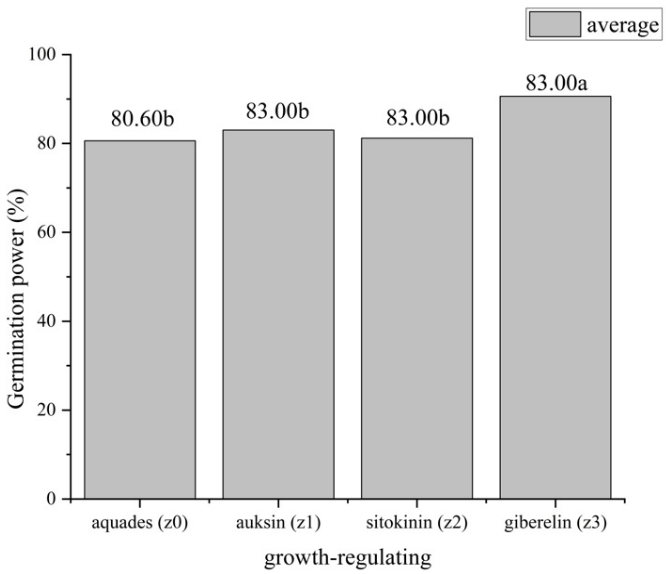

Figure 1: *Average germination (%) in priming treatment of various types of growth regulators.*
Description: Values followed by identical letters (a, b) indicate no significant difference according to the BNT test at α = 0.05

Table 1: *Correlation of variables of germination variables.*

	GP	VIS	PL
GP	1,000		
VIS	0.936 tn	1,000	
PL	0.971*	0.989*	1,000

Description: (*) significant at 0.05 level, r = 0.950, r = 0.990, (tn) not significant at 0.05 and 0.01 levels.

Vigour index of sprouts

The analysis of variance revealed that priming treatment with different growth regulators had a highly significant effect on the sprout vigor index. The average sprout vigor index for the various priming treatments involving different growth regulators is presented in Figure 2. The results of the LSD test at the 5% significance level, as shown in Figure 2, demonstrate that the gibberellin priming treatment (z3) resulted in the highest average sprout vigor index of 646.42. This value was significantly higher (P ≤ 0.05) compared to the distilled water priming treatment (z0) at 452.51, auxin treatment (z1) at 534.25, and cytokinin treatment (z2) at 407.82

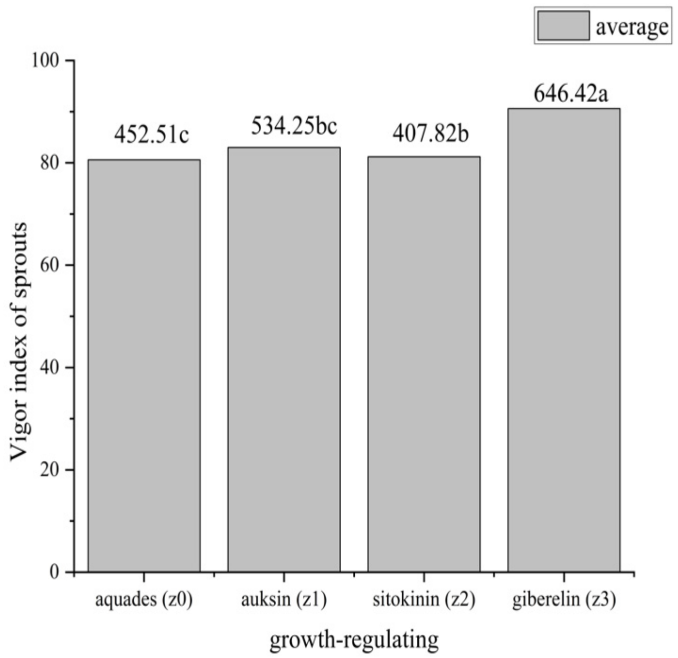

Figure 2: *Average vigour index of sprouts in hormonal priming treatment; Description: Values followed by identical letters (a, b, c) indicate no significant difference according to the BNT test at α = 0.05*

Plumula length

The analysis of variance revealed that priming treatment with different growth regulators had a highly significant effect on plumula length. The average plumula length for the various priming treatments involving different growth regulators is presented in Figure 3. The results of the LSD test at the 5% significance level, as shown in Figure 3, demonstrate that the gibberellin priming treatment (z3) resulted in the highest average plumula length of 5.44 cm. This value was not significantly different from the auxin treatment (z1) at 4.98 cm. However, it was significantly higher (P ≤ 0.05) compared to the distilled water priming treatment (z0) at 4.26 cm and the cytokinin treatment (z2) at 3.75 cm. The results of the correlation analysis in Table 1 illustrate specific relationships among seed germination traits. Germination power (GP) exhibited a strong positive correlation with plumula length (PL) (r= 0.971) while showing a non-significant inverse correlation with the vigor index of sprouts (VIS) (r= 0.936). Furthermore, the vigor index of sprouts was highly significantly correlated with seedling plumula length (r = 0.989).

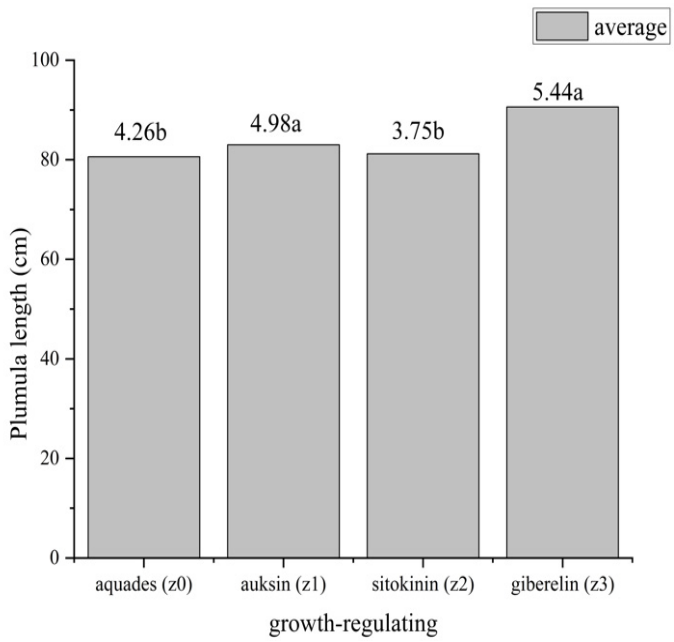

Figure 3: *Average plumula length (cm) on priming treatment of growth regulators.*

Description: Values followed by identical letters (a, b) indicate no significant difference according to the BNT test at α = 0.05

Seed priming is a simple and inexpensive seed treatment technique that can enhance plant growth even under abiotic stress. According to Rhaman *et al*. (2020a), the results of the correlation analysis in Table 1 illustrate a specific correlation among seed germination traits. Early germination success is characterized by rapid and uniform root emergence and growth. Various treatments can be applied to the germination phase, including differences in solution osmotic potential, duration, external temperature, and certain chemical compounds. Efficient treatments trigger metabolic processes during the germination phase (De Paiva Gonçalves *et al*., 2024; Jhanji *et al*., 2024). The effectiveness of seed priming is fundamentally indicated by the softening of the endosperm, which facilitates the mobilization of stored nutrients, promotes embryo cell elongation, and enhances overall seed quality and performance across diverse environmental conditions (Adhikary *et al*., 2021). The results showed that hormonal priming treatment had a significant effect on germination power, vigor index of sprouts, and plumula length. Hormonal priming can increase seed viability, characterized by an increase in seed germination (Fu *et al*., 2024; Pangestuti *et al*., 2021).

According to Rajiman and Megawati (2022), seeds exhibiting a germination rate exceeding 80% have high productivity potential, as germination is a critical indicator of successful crop establishment and subsequent cultivation outcomes. Furthermore, gibberellin priming demonstrated superior outcomes, reflected by a vigor index of 646.42 and a plumule length of 5.44 cm, significantly higher than other treatments. The application of growth regulators during seed imbibition in the priming process directly influences seed metabolic activity (Rhaman *et al*., 2020b). Seeds may experience dormancy, a phase in which germination is inhibited even under optimal environmental conditions (Klupczyńska and Pawłowski, 2021; Pandian *et al*., 2024). Wu *et al*. (2024) stated that gibberellin treatment could alleviate dormancy by reducing abscisic acid levels, a hormone responsible for inducing dormancy in seeds. Moreover, gibberellin aids in catalyzing the hydrolysis of starch reserves in the endosperm, converting them into glucose and amino acids that act as primary energy sources and substrates for synthesizing organic compounds essential for initial seedling establishment (Zhang *et al*., 2023).

Growth rate and bulb diameter

The analysis of variance showed that *T. asperellum* treatment had a significant impact on the growth and productivity of shallots, particularly on parameters such as relative growth rate and bulb diameter. This indicates that the treatment effectively enhances these key growth characteristics. These findings underscore the potential of *T. asperellum* in improving shallot cultivation, offering a promising approach for agricultural productivity. Tables 2 and 3 present the average values for these parameters across different treatment levels.

Table 2: *Average relative growth rate in hormonal priming and Trichoderma asperellum treatments.*

Trichoderma asperellum	**Priming type**				**Average**	**NP BNT**
	Aquadest	**Auxin**	**Cytokinins**	**Gibberellin**		
0 kg ha^{-1}	0.52	0.54	0.50	0.57	0.53b	0.06
400 kg ha^{-1}	0.52	0.53	0.63	0.64	0.58b	
800 kg ha^{-1}	0.67	0.71	0.69	0.72	0.70a	

Description: Values followed by identical letters (a, b) indicate no significant difference according to the BNT test at α = 0.05

Table 3: *Average bulb diameter (cm) in the treatment of hormonal priming and T. asperellum.*

Trichoderma asperellum	**Priming type**				**Average**	**NP BNT**
	Aquadest	**Auxin**	**Cytokinins**	**Gibberellin**		
0 kg ha^{-1}	3.04	3.01	3.15	3.30	3.12b	0.17
400 kg h^{-1}	3.18	3.18	3.20	3.20	3.19b	
800 kg ha^{-1}	3.22	3.56	3.31	3.46	3.39a	

Description: Values followed by identical letters (a, b) indicate no significant difference according to the BNT test at α = 0.05

Relative growth rate

The analysis of variance demonstrated that *T. asperellum* treatment had a highly significant effect on the relative growth rate, as shown in Table 2. The results of the LSD test at the 5% significance level, as presented in Table 2, indicate that the application of *T. asperellum* at 800 kg ha^{-1} (t2) resulted in the highest relative growth rate of 0.70. This value was significantly higher ($P \leq 0.05$) compared to the 400 kg ha^{-1} treatment (t1) with a rate of 0.58 and the control treatment (t0) at 0 kg ha^{-1}, which achieved a growth rate of 0.53.

Bulb diameter

The analysis of variance further indicated that *T. asperellum* treatment had a significant effect on shallot bulb diameter, as detailed in Table 3. The results of the LSD test at the 5% significance level, as shown in Table 3, demonstrate that the application of *T. asperellum* at 800 kg ha^{-1} (t2) produced the highest average bulb diameter of 3.38 cm. This value was significantly greater ($P \leq 0.05$) than that observed in the 400 kg ha^{-1} treatment (t1), which achieved an average diameter of 3.19 cm, and the control treatment (t0) at 0 kg ha^{-1}, with an average diameter of 3.12 cm. These findings highlight the significant impact of *T. asperellum* treatment on key growth parameters, including relative growth rate and bulb diameter in shallots. *T. asperellum* was an effective biocontrol agent, enhancing plant resilience against diseases (Guzmán-Guzmán *et al*., 2023). The highest application rate of *T. asperellum* (800 kg ha^{-1}) yielded optimal results across several growth metrics, with an average relative growth rate of 0.70 and a bulb weight of 28.02 g, along with an average bulb diameter of 3.38 cm. Additionally, applying *T. asperellum* as a biofertilizer has demonstrated its effectiveness in improving soil quality. This fungus produces specific enzymes that break down complex organic compounds, releasing essential nutrients for plant uptake. Furthermore, *T. asperellum* enhances soil physical and chemical properties, including structure, water retention capacity, and nutrient availability, leading to improved plant growth (Galung, 2021).

The symbiotic interaction between plant roots and *T. asperellum* enhances nutrient bioavailability by facilitating the dissolution of otherwise inaccessible mineral compounds through fungal enzyme activity, remarkably increasing phosphorus and iron uptake essential for plant growth (Contreras-Cornejo *et al*., 2024). In this mutualistic exchange, plants release root exudates, supplying a carbon source critical for *T. asperellum* proliferation (Dutta *et al*., 2023). Furthermore, the application of *T. asperellum* optimizes soil health by decomposing organic matter through enzymatic activities involving cellulase, endoglucanase, cellobiohydrolase, and glucosidase, thereby promoting biosynthesis of organic compounds and bolstering plant biomass (Karuppiah *et al*., 2021).

Seed priming, a pre-sowing hydration treatment, involves soaking seeds in specific solutions under controlled conditions for a designated period and then re-drying to the original moisture level to prevent radicle emergence (Jarrar *et al*., 2024). This process activates essential metabolic pathways that promote germination, repair seed damage, and ultimately enhance crop vigor and yield potential. As an accessible and cost-effective agronomic practice, seed priming has improved seedling quality, uniformity, early flowering, and crop yields, mainly through prolonged soaking in either aerated or low-potential water solutions (Doran *et al*., 2021). Research findings indicate that gibberellin priming significantly enhances shallot yield, reaching 1.99 kg/m^2, due to gibberellin's ability to promote generative growth in shallot plants (Cokrosudibyo *et al*., 2023). Gibberellin, a vital plant hormone, is critical in regulating physiological processes such as seed germination, stem elongation, flowering, and fertilization (Faruk *et al*., 2023). Its application stimulates a range of positive responses that contribute to higher yields and productivity, not only by increasing bulb quantity but also by enlarging bulb size through enhanced cell division and accumulating food reserves within the bulbs (Zhumanova *et al*., 2024). The combination of *T. asperellum* and hormonal priming treatment did not significantly affect crown and bulb ratio parameters and harvest index. The application of *T. asperellum* 800 kg ha^{-1} and hormonal priming gibberellin showed better results on the bulb crown ratio and harvest index of 0.16 and 0.88, respectively, compared to other treatments, although not statistically significant. This result may be attributed to suboptimal environmental conditions, as factors such as temperature, soil pH, light intensity, and nutrient availability are essential for enzyme activity involved in protochlorophyllide catalysis, a process highly dependent on favorable environmental conditions.

Stomatal parameter

The analysis of variance revealed that *T. asperellum* treatment did not have a significant effect ($P > 0.05$) on stomatal parameters, including stomatal density (stomata mm^{-2}), stomatal aperture (μmol m^{-2}), and stomatal aperture tension (mmol $H_2O/m^2/s$), as presented in Table 4. The average values for these parameters across different treatments are detailed in Table 4. Table 4 demonstrates that the control treatment recorded an average stomatal density of 48.70, serving as the baseline. The auxin treatment increased stomatal density to an average of 52.65, while the cytokinin treatment exhibited the highest average value at 53.22, indicating its significant impact. Gibberellin treatment had a similar effect to auxin, with an average stomatal density of 52.66. The control treatment (0 kg ha^{-1}) averaged 10.26 for stomatal aperture, whereas the 400 kg ha^{-1} treatment recorded the highest average aperture of 11.03, demonstrating optimal enhancement. However, the 800 kg ha^{-1} treatment of *T. asperellum* resulted in a reduced average aperture of 9.12, suggesting diminished effectiveness at higher concentrations. Similarly, stomatal aperture tension followed the same trend, with the 400 kg ha^{-1} treatment achieving the highest average of 11.03, compared to 10.25 in the control treatment and 9.12 in the 800 kg ha^{-1} treatment of *T. asperellum*.

Table 4: *Stomatal parameters under the treatment of hormonal priming and T. asperellum.*

Parameter	*Trichoderma asperellum*	Priming type				Average
		Aquades	Auksin	Sitokinin	Giberelin	
Stomatal density (stomata mm-2)	0 kg ha^{-1}	50.96	57.75	61.15	47.56	54.36
	400 kg ha^{-1}	47.57	52.65	45.86	50.96	49.26
	800 kg ha^{-1}	47.56	47.56	52.64	59.45	51.80
	Average	48.70	52.65	53.22	52.66	
Stomatal aperture (µmol m-2)	0 kg ha^{-1}	9.92	8.96	11.42	10.72	10.26
	400 kg ha^{-1}	11.02	10.37	12.1	10.64	11.03
	800 kg ha^{-1}	7.87	10.99	7.52	10.1	9.12
	Average	9.60	10.11	10.35	10.49	
Stomatal aperture tension (mmol $H_2O/m^2/s$)	0 kg ha^{-1}	9.92	8.96	11.42	10.72	10.25
	400 kg ha^{-1}	11.02	10.37	12.10	10.64	11.03
	800 kg ha^{-1}	7.87	10.99	7.52	10.10	9.12
	Average	9.60	10.10	10.34	10.49	

These findings highlight that the 400 kg ha^{-1} treatment most effectively enhances stomatal performance across all parameters. In contrast, cytokinin treatment proved to be the most effective priming treatment for increasing stomatal density. Cytokinins are recognized as potent priming agents that significantly enhance stomatal density, contributing to improved drought tolerance and more rapid stomatal responses under stress conditions (Yang *et al*., 2023). Plants treated with cytokinins demonstrate faster stomatal closure and reopening, critical for maintaining hydration and optimizing gas exchange during drought recovery (Hai *et al*., 2020). However, while cytokinins effectively improve stomatal density, they may not uniformly enhance all aspects of stomatal function. This underscores the importance of adopting tailored strategies based on specific environmental conditions and crop requirements (Bouvier and Kelly, 2024).

The analysis of stomatal aperture (µmol m^{-2}) revealed that under the control treatment (0 kg ha^{-1}), the aperture ranged from 8.96 to 11.42, with an average of 10.26. Cytokinins demonstrated the highest aperture (11.42), while auxins showed the lowest (8.96) at this level. The 400 kg ha^{-1} treatment recorded the highest average stomatal aperture at 11.03, ranging from 10.37 to 12.10. Cytokinins again displayed the highest aperture (12.10), indicating its strong effect, followed by distilled water (11.02), while auxins were slightly lower at 10.37. Conversely, the 800 kg ha^{-1} treatment of *T. asperellum* exhibited the lowest average aperture of 9.12, ranging from 7.52 to 10.99. Auxins performed best at this level (10.99), while distilled water recorded the lowest aperture (7.87). On average, gibberellin demonstrated the highest overall performance (10.49), followed by cytokinins (10.35), auxins (10.11), and distilled water (9.60). These findings suggest that the 400 kg ha^{-1} treatment of *T. asperellum* is the most effective application rate for improving stomatal aperture, with cytokinins consistently showing the most substantial enhancement across all treatment levels. Conversely, the 800 kg ha^{-1} treatment of *T. asperellum* resulted in diminished performance, potentially indicating adverse effects at higher application levels. *T. asperellum* has been found to enhance leaf stomatal conductance, a critical factor for CO_2 assimilation and photosynthesis (Yang *et al*., 2023). The activation of genes involved in growth hormone synthesis in treated plants suggests that *T. asperellum* stimulates cytokinin production, facilitating the stomatal opening and promoting overall plant vigor (Wang *et al*., 2023).

The analysis of stomatal aperture tension (mmol $H_2O/m^2/s$) revealed that under the control treatment (0 kg ha^{-1}), values ranged from 8.96 to 11.42, with an average of 10.25. Cytokinins demonstrated the highest value (11.42), while auxins showed the lowest (8.96) at this level. The 400 kg ha^{-1} treatment recorded the highest average tension of 11.03, ranging from 10.37 to 12.10. Cytokinins again achieved the highest value (12.10), followed by distilled water (11.02), while auxins showed slightly lower performance (10.37). Conversely, the 800 kg ha^{-1} treatment of *T. asperellum* exhibited the lowest average tension of 9.12, ranging from 7.52 to 10.99. Auxins performed the best at this level (10.99), while cytokinins recorded the lowest value (7.52). On average, gibberellin demonstrated the highest overall stomatal aperture tension (10.49), followed by cytokinins (10.34), auxins (10.10), and distilled water (9.60). These findings suggest that the 400 kg ha^{-1} treatment is the most effective for enhancing stomatal aperture tension, with cytokinins showing strong performance at optimal levels. However, the 800 kg ha^{-1} treatment of *T. asperellum* resulted in reduced performance, indicating potential stress or adverse effects at higher concentrations. Cytokinins can affect guard cell turgor and solute fluxes, both of which are essential for regulating stomatal movement. For example, the introduction of synthetic light-activated K+ channels in guard cells has been demonstrated to accelerate stomatal responses, enhancing water use efficiency while maintaining carbon fixation (Zhuo *et al.*, 2024). Stomatal aperture is a vital physiological process in plants, regulating the exchange of gases, including carbon dioxide and water vapor, between the plant and its environment (Harrison *et al.*, 2020). The opening and closing of stomatal pores are driven by the movement of guard cells, which are influenced by various environmental and internal factors, such as water availability, light intensity, carbon dioxide levels, and phytohormones. Notably, cytokinins have been identified as key phytohormones that contribute to increasing stomatal aperture tension (Roux and Leonhardt, 2018).

Conclusions and Recommendations

The synergistic effects of hormonal priming and *T. asperellum* on the morphological and physiological traits of true shallot seeds, with gibberellin priming and *T. asperellum* application at 800 kg ha^{-1} demonstrating the most significant improvements; however, the findings are limited to specific environmental conditions and treatment concentrations, suggesting the need for further research to explore broader applications and long-term impacts for sustainable agricultural practices.

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Novelty Statement

This study presents a pioneering approach by exploring the synergistic effects of hormonal priming and *T. asperellum* on true shallot seeds' morphological and physiological traits. The novelty lies in the integration of gibberellin, cytokinins, and *T. asperellum* as a combined treatment, which is a unique strategy for improving seedling performance. While previous research has examined the individual effects of hormonal priming or biological treatments like Trichoderma, this research uniquely demonstrates their synergistic application in shallots. This combined approach advances our understanding of seed priming techniques and introduces an innovative method to enhance crop establishment, resilience, and productivity, providing a promising tool for sustainable agriculture in shallot cultivation. The findings also offer a new pathway for optimizing seed treatments and crop management practices in other horticultural crops.

Author's Contribution

Syatrianty A. Syaiful and Katriani Mantja: Wrote the first draft of the manuscript.
Syatrianty A. Syaiful, Katriani Mantja and Farida Faisal: Designed the experiment.
Irmayanti Irmayanti, Purnama Isti Khaerani and Muhammad Fuad Anshori: Analyzed the data.
Syatrianty A. Syaiful, Katriani Mantja, Farida Faisal, Irmayanti Irmayanti, Purnama Isti Khaerani and Muhammad Fuad Anshori: Finalized the manuscript. All authors reviewed and approved the final manuscript.

Conflict of interest

The authors have declared no conflict of interest.

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