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The Role of Zinc in Botanical Seed Production of Three Local Indonesian Shallot Varieties in the Highlands

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ABSTRACT

Using botanical seeds as planting materials can alternatively assist in overcoming problems associated with seed supply, a crucial factor in shallot cultivation. This research aims to determine and study the response of three local shallot varieties to the application of various doses of zinc in producing botanical seeds. Zn plays an important role in various plant metabolic processes, including those related to flowering and seed yield. The research was carried out in a split-plot design with main plots of varieties are Lokana, Rubaru, and Ambassador 3 Agrihorti, while the subplots, namely the Zn doses, consist of 0 (control), 0.5, 1, 1.5 kg ha^{-1} was repeated three times. Seed testing was carried out using a completely randomized design (CRD), which consisted of 36 experimental units. Results showed an interaction between varieties and Zn doses, which significantly affected harvest time but had no significant impact on seed yield components. The best effect on seed yield components appeared exclusively by the Ambassador 3 Agrihorti variety on the number of capsules per umbel, number of seeds per umbel, seed weight per umbel, seed weight per clump, seed production per hectare and Zn dose of 1.5 kg ha^{-1} to seed weight per clump. Shallot varieties and Zn influenced the quality of seed yield. The Rubaru variety with Zn 1.5 kg ha^{-1} had the best effect on germination percentage and the single variety Ambassador 3 Agrihorti on the germination rate index. Using Zn 1.5 kg ha^{-1} had the best effect on germination time, coefficient velocity of germination, and germination rate index.

Introduction

Shallots are a strategic horticultural commodity with high economic value. They are widely needed by society as a flavoring in various food preparations, as well as diverse secondary metabolite content, which can be used as raw materials for medicine (Abdelrahman et al., 2020). Shallot production in Indonesia in 2022 decreased to 1,982,360 t compared to production in 2021, which reached 2,004,590 t (Central Bureau of Statistics of Indonesia, 2023). Production centers covered ten provinces in Indonesia, namely Central Java, East Java, West Java, West Nusa Tenggara, Yogyakarta, West Sumatera, North Sumatera, South Sulawesi, Bali, and East Nusa Tenggara (Suminartika et al., 2022).

The decline in national shallot production is a big challenge considering that the population is increasing, which is directly proportional to the increase in people's need for shallots and also along with the development of culinary businesses. This is to achieve improvements in shallot cultivation which must always be made to increase production and balance the availability of shallots proportionate to community needs. One of the factors that is a problem in the cultivation process, which often results in a decrease in shallot production, is that the planting material or seeds used are of low quality (Saidah et al., 2020).

In general, farmers in Indonesia often use tubers from previous harvests as planting material, which has various weaknesses, including decreasing quality due to continuous use, the potential to carry disease, and a dormancy period of 4 to 9 weeks, making them suitable for cultivation only sometimes (Agriawati et al., 2022). This also often results in fluctuations in the market, especially when entering the planting season. Efforts can be made to answer the seed problem by switching to seed acquisition from botanical sources or true shallot seeds (TSS), which are quality and disease-free. They have high production potential and maintain high germination power even when stored for 1 to 2 years (Susanto et al., 2022). They also have no dormancy period so that they can be cultivated throughout the year. However, TSS production in Indonesia is limited by low flowering rates, as some varieties flower only at around 30 percent and often fail to produce viable seeds (Pandiangan et al., 2015).

Genetics and the environment are important factors that influence plant growth and yield (Oktaviani et al., 2020), including how the plant can flower and produce well. Several local shallot varieties, including Lokana, Rubaru, and Ambassador 3 Agrohorti, demonstrate high yield potential and are therefore considered highly suitable for development as sources of botanical seed or true shallot seed (TSS). Shallot seed production can be increased by applying more nutrient zinc (Zn) to a specific threshold. Zn is a micronutrient that plays a vital role in various plant growth and development processes. It serves as an enzyme cofactor, helps with chlorophyll biosynthesis, gene expression, signal transduction, and improvements in plant defense system (Hacisalihoglu, 2020). Zinc has an important role in relation to the production of botanical seeds through physiological processes, development, flowering, and seed yield in plants (Singh, 2021). It is necessary for sexual development and evolution during the flowering process and initiation (Souri et al., 2017; Souri and Hatamian, 2019). Zn can also affect metabolic processes and seed maturity levels (Kulthe et al., 2022).

Zn deficiency can affect growth and significantly reduce plant production (Nandal and Solanki, 2021). Zn foliar application is more effective and can significantly increase crop yields (Ankush et al., 2022). Mumivand et al. (2021) reported that applying Zn through leaves increased growth, seed count, and caused better seed germination in *Satureja khuzistanica*. Zaman et al. (2019) also reported that Zn 0.7 kg ha^{-1} combined with boron 0.3 kg ha^{-1} maximized leaf count, flower count, seed count per umbel, and seed weight per umbel. Research conducted by Laware and Raskar (2014) reported that applying Zn at a concentration of 30 µg mL^{-1} could accelerate the flowering period by 12 to 14 d compared to controls and produce healthy seeds in onion plants. This research aims to determine and study the response of three shallot varieties to the application of various doses of zinc (Zn) in producing botanical seeds (True Shallot Seed).

Material and methods

Study area

The field experiment was conducted in Muntea, Bonto Lojong Village, Ulu Ere District, Bantaeng Regency, South Sulawesi, Indonesia, at an elevation of 1,348 meters above sea level. During this period, the recorded air temperature ranged from 19.52°C to 19.88°C, with soil temperature ranging from 22.75°C to 23.48°C, and soil moisture content from 74% to 83%. Subsequently, laboratory-scale seed testing was conducted at the Plant Breeding and Seed Science Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. The entire research, including both field experiments and laboratory activities, was conducted from October 2022 to March 2023.

Plant materials

This study utilized three local shallot bulb varieties: (1) Lokana from Bantaeng Regency, South Sulawesi Indonesia; (2) Rubaru from Sumenep Regency, East Java Indonesia; and (3) Ambassador 3 Agrihorti from the Vegetable Crops Research Institute (Balitsa), West Bandung, Indonesia. Additional materials included zinc sulfate heptahydrate, benzyl amino purine (BAP), 1 M potassium hydroxide (KOH), chicken manure, NPK and SP-36 fertilizers, herbicide, fungicide, insecticide, distilled water, 70% alcohol, tissue, and filter paper.

Experimental design

The experiment was arranged in a split-plot design, with shallot varieties (V) as the main plot, consisting of Lokana (v_1), Rubaru (v_2), and Ambassador 3 Agrihorti (v_3). The subplot factor was zinc dose (Z), consisting of 0 kg ha^{-1} (z_0), 0.5 kg ha^{-1} (z_1), 1 kg ha^{-1} (z_2), and 1.5 kg ha^{-1} (z_3). Each treatment combination was replicated three times, resulting in a total of 36 experimental units. The research results of shallot seeds in each treatment obtained from the field then taken to the laboratory for seed testing using a completely randomized design (CRD) with the same central plot and subplots in the field.

Procedures

The research began by land preparation and progressed to the postharvest stage, as follows:

Planting preparations

Initial field preparation involved the construction of planting beds measuring 90 cm × 300 cm (plot size 2,7 m) and 30 cm bed height, primary fertilization with SP-36 with 36% P_2O_5 content at a dose of 280 kg ha^{-1} and chicken manure at a dose of 10 t ha^{-1}, installing plastic mulch, installing shade in the form of ultraviolet (UV) plastic and with a UV content of 14%, soaking the tubers with 100 ppm benzyl amino purine (BAP) (Kurniasari et al., 2017) one h before planting, and planting with a spacing of 20 cm × 20 cm.

Zinc applications

Zinc (Zn) was applied as a foliar spray at 15, 30, and 45 days after planting (DAP) using a handheld sprayer. The treatment rates were 0, 0.5, 1.0, and 1.5 kg ha^{-1}, which corresponded to approximately 0.045, 0.090, and 0.135 g per plot per application, respectively. For each application, individual plants received 16.66mL of the spray solution. Foliar spraying was performed in the morning at around 09:00 AM.

Plant maintenance

Maintenance practices included watering, replacement of non-viable plants, application of additional fertilizers, and pest control measures.

Harvesting

Harvesting the botanical seeds was carried out in multiple stages, with harvest times starting from an average age of 112 DAP to an average age of 130 DAP. Botanical shallot seeds were harvested when the plant exhibited harvest criteria, namely, when the capsule had cracked or broken by about 5 to 10%, the color of the capsule skin had turned yellow-brown, and the capsule skin was wrinkled or fleshless (Rosliani et al., 2022).

Post-harvesting

Post-harvest shallot seeds included several stages, namely, withering the umbel stalks to slowly reduce the water content, drying the umbel stalks to dry the capsules so that breaking down the capsule skin can be done quickly and prevent damage to the seeds, and cleaning the seeds to separate the seeds from the dirt that came from the capsules after being threshed (Rosliani et al., 2022).

Seed testing

Shallot seeds were tested for 7 d at the Plant Breeding and Seed Science Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. Seed testing used the test method on paper. The seeds were first sterilized used 70% alcohol for 3 min, then washed with distilled water and drained on tissue paper. The sterile seeds were then arranged into a petri dish using tweezers. The number of seeds tested was 40 seeds per cup with a total of 36 petri dishes. After that, the seeds were placed in the germinator while paying attention to daily humidity.

Parameters

The parameters included capsule formation percentage (%), harvest time (DAP), number of capsules per umbel, number of seeds per umbel, seed weight per umbel (g), seed weight per clump (g), seed production per hectare (kg ha^{-1}), germination time (d), germination percentage (%), coefficient velocity of germination, and germination rate index (% d^{-1}). The capsule formation percentage was calculated using the formula Siswadi et al. (2022):

$$Capsule\ formation\ (\%) = \frac{(Number\ of\ fruity + no\ fruity\ capsule)}{Number\ of\ flower} \times 100\%$$

seed production per hectare was calculated using the formula:

$$seed\ production\ per\ hectare = \frac{Area\ of\ 1\ ha\ of\ land\ (m^2)}{Plot\ area\ (m^2)} \times seed\ production\ per\ plot$$

Germination time was calculated using the formula Balikai et al. (2019):

$$Germination\ time\ (\%) = \frac{\Sigma(nt)}{\Sigma n}$$

Where:
n = number of seeds germinated (radicle emergence of 2 mm,) at time
t = hours from the beginning of the germination
Σn = final germinate

Germination percentage was calculated using the formula of Pangestuti et al. (2021) as follows:

$$Final\ germination\ percentage\ (\%) = \frac{G}{S} \times 100\%$$

Where:
G = number of normal germinated seeds
S = number of total seeds
The coefficient velocity of germination was calculated using a formula referring to Lazim and Ramadhan (2019):

$$CVG = \frac{\Sigma ni}{niti} \times 100$$

Where:
n = number of seeds germinated on day i
t = number of days from seeding corresponding to n

The germination rate index was calculated using a formula referring to Lazim and Ramadhan (2019), namely as follows:

$$GRI = \frac{G1}{1} + \frac{G2}{2} + \frac{G3}{3} + \frac{G4}{4} + \cdots + \frac{Gn}{n}$$

Where:
G1, G2, …, Gn = germination percentage on a particular day
1, 2, …, n = observation day

Data analysis

The data were analyzed using analysis of variance. In case of significant, the least significant difference test (LSD) with $\alpha_{0.05}$ was performed. All data analysis processes were carried out using Microsoft Excel 2021 software.

Results

The analysis of variance showed that a very significant interaction occurred between the variety treatments and zinc doses that affected harvest time and germination percentage in shallot (Table 1), but no significant interaction occurred on the capsule formation, number of capsules per umbel, number of seeds per umbel, seed weight per umbel, seed weight per clump, seed production, germination time, germination rate index, and coefficient velocity of germination. Single varieties treatment had a significant effect on the number of capsules per umbel, seed weight per umbel, and seed weight per clump and had a very significant effect on the number of seeds per umbel, seed production per hectare, and germination rate index (Table 2). On the other hand, single Zn doses significantly affected the seed weight per clump and Very significantly affected the germination time, coefficient velocity of germination, and germination rate index (Table 3).

Table 1. Effect of varieties and zinc doses interaction on the average harvest time and germination percentage of shallot.

Variety	Zinc doses	Harvest Time (DAP)	Germination percentage (%)
Lokana	0 kg ha^{-1}	130.67$^{a}_{p}$	13.33$^{a}_{q}$
	0.5 kg ha^{-1}	128.00$^{a}_{q}$	15.00$^{b}_{q}$
	1 kg ha^{-1}	127.67$^{a}_{q}$	25.00$^{a}_{p}$
	1.5 kg ha^{-1}	127.00$^{a}_{q}$	20.83$^{b}_{pq}$
Rubaru	0 kg ha^{-1}	120.00$^{c}_{p}$	11.67$^{a}_{q}$
	0.5 kg ha^{-1}	118.66$^{c}_{q}$	16.67$^{b}_{q}$
	1 kg ha^{-1}	114.67$^{c}_{r}$	18.33$^{a}_{q}$
	1.5 kg ha^{-1}	112.89$^{c}_{s}$	35.00$^{a}_{p}$
Ambassador3	0 kg ha^{-1}	127.00$^{b}_{p}$	15.83$^{a}_{q}$
	0.5 kg ha^{-1}	125.67$^{b}_{q}$	30.83$^{a}_{p}$
	1 kg ha^{-1}	125.33$^{b}_{qr}$	24.17$^{a}_{p}$
	1.5 kg ha^{-1}	124.33$^{b}_{r}$	30.00$^{a}_{p}$
LSD Variety ($\alpha_{0.05}$)		1.43	8.38
LSD zinc ($\alpha_{0.05}$)		1.23	8.11

Mean values followed by the same letter are not significantly different according to the least significant different test (LSD $\alpha_{0.05}$).

The LSD $\alpha_{0.05}$ test results showed that the fastest average harvest time was the Rubaru variety treated with a Zn dose of 1.5 kg ha^{-1} (112.89 DAP), which was significantly different from other treatments of variety and zinc doses. The LSD $\alpha_{0.05}$ test showed that the highest germination percentage was the Rubaru variety, with a Zn dose of 1.5 kg ha^{-1} (35%), which was significantly different from the Lokana variety and also significantly different from the other zinc treatments, but not significantly different from the Ambassador 3 Agrihorti variety.

Table 2. Effect of variety on the average of shallot seed yield and quality components.

Varieties	Capsule formation (%)	Number of capsules per umbel	Number of seeds per umbel	Seed weight per umbel (g)	Seed weight per clump (g)	Seed production (kg ha^{-1})	Germination rate index (% d^{-1})
Lokana	42.49	30.05^{b}	29.83^{b}	0.05^{b}	0.05^{b}	0.76^{b}	4.05^{b}
Rubaru	45.68	54.42^{a}	121.74^{a}	0.19^{a}	0.41^{ab}	29.47^{b}	4.28^{b}
Ambassador3	49.52	58.81^{a}	182.98^{a}	0.30^{a}	0.67^{a}	155.09^{a}	6.76^{a}
LSD ($\alpha_{0.05}$)	Ns	17.33	67.86	0.12	0.36	34.56	1.29

Means followed by the same letter (a, b) are not significantly different according to the least significant different test (LSD $\alpha_{0.05}$). ^{ns}; (non-significant) based on analysis of variance.

The highest average capsule formation percentage in the varieties treatment was in the Ambassador 3 Agrihorti variety (49.52%). Meanwhile, the LSD $\alpha_{0.05}$ test results showed that the highest average number of capsules per umbel, number of seeds per umbel, seed weight per umbel, and seed weight per clump were the Ambassador 3 Agrihorti varieties (58.81, 182.98, 0.30, 0.67 g) and was significantly different from the Lokana variety but not significantly different from the Rubaru variety. The highest average seed production per hectare and germination rate index was the Ambassador 3 Agrihorti varieties (155.09 kg ha^{-1} and 6.76% d^{-1}) and was significantly different from the other treatments.

Table 3. Effect of zinc doses on the average of shallot seed yield and quality components.

Zinc doses	**Capsule formation (%)**	**Seed weight per clump (g)**	**Germination Time (d)**	**Coefficient velocity of germination**	**Germination rate index (% d^{-1})**
0 kg ha^{-1}	46.65	0.27$_{q}$	6.11$_{p}$	16.40$_{q}$	2.28$_{r}$
0.5 kg ha^{-1}	47.33	0.30$_{q}$	5.69$_{p}$	17.91$_{q}$	4.49$_{q}$
1 kg ha^{-1}	44.76	0.39$_{pq}$	5.72$_{p}$	17.64$_{q}$	4.90$_{q}$
1.5 kg ha^{-1}	44.85	0.55$_{p}$	4.68$_{q}$	22.23$_{p}$	8.45$_{p}$
LSD ($\alpha_{0.05}$)	Ns	0.19	0.64	2.83	1.92

Means followed by the same letter (p, q) are not significantly different according to the least significant different test (LSD $\alpha_{0.05}$). ^{ns}; (non-significant) based on analysis of variance.

The highest average capsule formation percentage Zn treatment was at a Zn dose of 0.5 kg ha^{-1} (47.33%). The LSD $\alpha_{0.05}$ test showed that the highest average seed weight per clump was caused by the Zn treatment dose of 1.5 kg ha^{-1} (0, 55 kg ha^{-1}), which was significantly different from the Zn treatment dose of 0 kg ha^{-1} and 0.5 kg ha^{-1} but not significantly different from the Zn treatment dose of 1 kg ha^{-1}. Meanwhile, the fastest average germination time and highest coefficient velocity of germination were highest in the Zn treatment, namely in the Zn treatment at a dose of 1.5 kg ha^{-1} (4.68 d and 22.23), which was significantly different from other treatments. The highest germination rate index was the Zn treatment dose of 1.5 kg ha^{-1} (8.45% d^{-1}), which was significantly different from the other treatments.

Discussion

The results showed an interaction between varieties and Zn dose, which significantly affected harvest time but had no significant impact on the seed yield components. Several factors influenced the success of capsule formation, the most important of which were environmental factors and pollinating insects. The capsule formation phase in this study occurred from December 2022 to January 2023, simultaneous with the rainy season. Rainfall in December 2022 was 611 mm (very high category), which lasted for 22 d, and January rainfall was 119 mm (medium category), which lasted for 12 d (Meteorology, Climatology and Geophysics, 2023), which was also accompanied by strong winds. These factors resulted in many flowers falling and not succeeding in forming capsules. Another consequence of the influence of bad weather is the need to pollinate insects in the research area, so the pollination process was not optimal. Pollinating insects are an essential factor influencing the success of capsule formation. As stated by Fahrianty et al. (2020), the activity of pollinating insects impacts the success of capsule formation because shallot flowers are dichogamous and partly self-incompatible.

The Rubaru variety with the Zn treatment had the fastest average on ripening time, compared to the other two varieties, namely Lokana and Ambassador 3 Agrihorti. The non-uniform harvest time for each variety was influenced by the different genetic abilities of each varieties and the suitability of the growing environment. This is to the opinion of Tuhuteru et al. (2023) that the differences in response shown by each variety is influenced by the genetic ability supported by appropriate environmental conditions. The faster harvest time for the Rubaru with the Zn treatment was also influenced by Zn's ability to control growth and development, affecting the harvest time. This is supported by research results by Tayade et al. (2018), who found that the flowering and first harvest time in tuberose plants applied with Zn was faster than those without Zn treatment.

The highest average seed weight per clump was significantly influenced by each variety and Zn doses alone, with the most increased seed weight per clump being the Ambassador 3 Agrihorti variety and the Zn treatment at the highest dose. The seed weight which was influenced by the varieties, was closely related to the genetic ability of the Ambassador 3 Agrihorti varieties, with a more significant number of capsules and seeds. Thus, the effect on seed weight was directly proportional. The Ambassador variety showed superior genetic characteristics and was in harmony with the environment where it was cultivated to produce botanical shallot seeds compared to the other two varieties, namely the Rubaru and Lokana. The different genetic characteristics of each variety can trigger differences related to morphological characters and yield between shallot varieties. This is the opinion of Yeshiwas et al. (2023) who stated that plant growth and yield components are greatly influenced by varieties, growing location, and planting season. Apart from being affected by varieties, the weight of seeds per clump of shallots was also influenced by Zn. From these results, it was known that Zn can increase the weight of shallot seeds. The same thing was reported through the research results of Yousefi et al. (2023) who found that seed yield in *Phaseolus vulgaris* plants was significantly influenced by applying Zn on the leaves. Yousefi et al. (2023) also added that micronutrients are important in increasing leaf area, light absorption, and plant dry matter accumulation.

Conclusion

Shallot varieties and Zn influence the quality of shallot botanical seeds. Zn significantly affected all components of germination parameters in shallot seeds, with the best results obtained in the highest dose of Zn treatment. Zn also has a role in improving the quality of plant, affecting the quality of seed germination, which was also better than other treatments. Zn is a micronutrient needed for better metabolic activity and plays an important role in ensuring plant quality and productivity. One indicator that shows the quality of seeds was the ability of the seeds to germinate or have good germination power. A better germination rate index and seed vigor with Zn treatment are closely related to Zn's ability to synthesize proteins from source to sink, as well as faster seed germination, which was possibly caused by the presence of metabolites that higher, which helps the resumption of embryo growth during the germination period. Overall, varieties influenced the components of seed yield, while Zn had an influence on the quality of seed yield of shallot. Further research needs to be carried out regarding the use of Zn doses higher than 1.5 kg ha^{-1} and application of other technologies, especially in relation to improving the quality of shallot seed yield, while evaluating the ability of seeds to grow in the field and the shelf life of shallot seeds before planting.

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Conflict of Interest

The authors indicate no conflict of interest in this work.

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