

Effect of GA3 as a priming agent on the growth of shallot (*Allium ascalonicum* L.) seedling

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Abstract

This study aimed to evaluate and analyze the effect of priming using GA3 on the growth of various shallot varieties from seeds. This pot study was conducted at Exfarm, Faculty of Agriculture, Hasanuddin University from July to September 2022. The research was arranged in factorial with a randomized complete block design (RCBD) with two treatment factors. The first factor was seed priming consisting of control or no priming (S0), priming with water (S1), 25 mg L⁻¹ GA3 (S2), 50 mg L⁻¹ GA3 (S3) and 75 mg L⁻¹ GA3 (S4). The second factor is the variety consisting of Tuk-Tuk (v1), Sanren F1 (v2), Lokananta (v3), and Maserati (v4). Seed performance testing is carried out by planting prime seeds in pots. Meanwhile, parameters observed included six agronomic parameters and three chlorophyll parameters. The results show that priming with GA3 at concentration of 75 mg L⁻¹ significantly influence the plant height, number of leaves, fresh weight, chlorophyll b content, and total chlorophyll. Variety treatments recorded different responses to various parameters. The Lokananta variety recorded the highest average plant height and dry seedling weight. The Maserati variety recorded the largest average diameter of pseudo stems. Meanwhile, the interaction between GA3 at 75 mg L⁻¹ and the Maserati variety has a significant effect on the root length of shallot seedlings. Then, the interaction between Tuk-Tuk varieties and GA3 75 mg L⁻¹ priming gave the best effect on chlorophyll content.

Keywords: Chlorophyll, Gibberelins, Seed priming, TSS.

Abbreviations: PH_plant height, NL_number of leaves, VSD_visible stem diameter, FWS_fresh weight of seeds, DWS_dry weight of seeds, Chl b_chlorophyll b, Chl tot_total chlorophyll, RL_root length, and Chl a_chlorophyll a.

Introduction

Crop production is greatly influenced by the provision of quality seeds, especially vegetable commodities. Quality seeds will produce plants that have the potential to have high production. Low quality, unhealthy and weak plant seeds may lead to lose an optimal production (Balliu et al., 2017). Shallots are one of the vegetable plants that can be propagated with seeds. The seeds will grow into young plants which will be transplanted once they reach the right age. Seedling growth itself is very important to optimize plant production in the field (Singh et al., 2015).

Cultivating shallots with seeds requires a transplanting process after the seeds grow into seedlings. Transplantation is the process of moving seeds or young plants from the nursery to the field (Hwang et al., 2020). The process of moving seedlings can cause mechanical damage resulting in breaking off the main roots and root hairs, due to the process of moving seedlings from the nursery to the field. In addition, the transplantation process causes stress or a brief shock that causes growth to slow down (Qui and Leskova, 2020).

Sumarno et al. (2021) cultivated shallots from seeds and found that the seeds from these plants only had a survival or germination rate of 83 to 91%. This may affect crop production in the field.

One approach to improve the growth and quality of the produced seeds is to carry out special treatment in the form of priming. Priming is a method of seed hydration which aims to activate seed metabolism until the end of the second germination phase, namely the lag phase, which is then dried again to the initial water content (Corbineau et al., 2023). One of the most important priming materials is GA3. Gibberellic acid (GA3) is a form of phytohormone that plays a role in the plant growth process. Priming using phytochromones can have a direct impact on plant seed growth (Pagano et al., 2023). The use of GA3 as a priming material has been widely used in various types of plants. Priming using GA3 has been known as an alternative in activating germination metabolism and physiological processes during plant growth (Dotto and Silva, 2017). Research conducted by Muruli et al. (2016) who tested

Table 1. Average plant height, number of leaves, and visible stem diameter.

Varieties	Parameter		
	PH (cm)	NL	VSD (mm)
Tuk-Tuk	22.53±0.69b	3.42±0.16	2.06±0.11bc
Sanren F1	23.88±0.44b	3.31±0.08	1.98±0.04c
Lokananta	24.71±1.21	3.40±0.14	2.19±0.10ab
Maserati	24.09±0.61a	3.51±0.16	2.24±0.05a
P< (0.05) (sig)	0.006	0.481	0.02
LSD	0.31	-	0.04
Priming	PH (cm)	NL	VSD (mm)
without priming	22.40±0.60b	3.11±0.05b	1.95±0.08
Water Priming	23.37±1.27ab	3.39±0.08ab	2.13±0.08
25 mg L ⁻¹ GA3	23.93±0.67ab	3.50±0.13a	2.15±0.07
50 mg L ⁻¹ GA3	2.48±0.64a	3.50±0.16a	2.15±0.11
75 mg L ⁻¹ GA3	24.82±0.55a	3.56±0.09a	2.21±0.10
P< (0.05) (sig)	0.007	0.025	0.119
LSD	0.39	0.08	-

Notes: The means followed by the same letter in columns (a, b, c) are not significantly different in the DMRT $P < 0.05$ test. PH (plant height), NL (number of leaves), and VSD (visible stem diameter).

GA3 on shallot plants found positive results. The growth performance of shallot seeds at a GA3 concentration of 50 ppm was recorded to increase significantly compared to the control. Research conducted by Adhikari and Subedi (2022) found that there was a positive influence on primed corn seeds using GA3 at a concentration of 50 ppm which were noted to be able to grow optimally, both under normal and stressful conditions. Based on previous research findings, we tested shallot seeds and evaluated the growth of shallot seedlings from seeds that had been primed using GA3 with various concentrations.

Results

Based on the results of the analysis of variance, it shows that variety treatment and seed priming with GA3 alone have a significant effect on plant height (Table 1). The Maserati variety had the highest average plant height (24.09 cm) while it was not significant in Lokananta (24.71 cm) and Sanren F1 (23.88 cm). The Tuk-Tuk had the lowest average plant height (22.53 cm) while was not significant in Sanren F1. For apparent stem diameter, the same results were also obtained, namely the Maserati variety had the largest average apparent stem diameter (2.24 mm) while was not significant in Lokananta. The Sanren F1 variety had the smallest average apparent stem diameter (1.98) while was not significant in Tuktuk (2.06 mm). Based on priming treatment, application of GA3 had a real effect on plant height and number of leaves. It was recorded that applying primer with a concentration of 75 mg L⁻¹ (24.82 cm and 3.56) recorded the highest average plant height and number of leaves compared to other treatments.

The influence of varieties and seed priming on the characteristics of seed fresh weight (FWS), seed dry weight (DWS), chlorophyll b content (Chl b), and total chlorophyll content (Chl tot) can be seen in Table 2. The Lokananta variety showed the best DWS potential with a value of 0.24 g, but this potential was not significantly different from the Maserati variety (0.22 g). Meanwhile, the Tuk Tuk and Sanren F1 varieties are the two lowest varieties with DWS values of 0.16 g and 0.18 g, respectively. However, the Tuk-Tuk variety also has the potential for good total chlorophyll characteristics with

a value of 22.61 µg/mL. Based on the effect of seed priming, administration of 75 mg L⁻¹ GA3 will induce the growth of FWS, Chl b, and Chl tot characters with values of 1.94 g, 16.56 µg/mL, and 25.49 µg/mL, respectively.

The interaction of variety and seed priming with GA3 was recorded to have a significant influence on the parameters of root length and chlorophyll content of the plant (Table 3). The interaction of the Maserati variety with primer using GA3 concentration of 75 mg L⁻¹ recorded the longest rooting. Then the interaction of the Tuk-Tuk variety with priming using GA3 at a concentration of 75 mg L⁻¹ recorded the highest average chlorophyll a compared to other treatment combinations.

Discussion

Seed priming using GA3 on several shallot varieties had a real influence on various observed parameters. Plant height is influenced individually, each by treatment factors, namely priming and variation. The highest shallot seeds were recorded at a priming concentration of 75 mg L⁻¹ GA3 and the Lokananta variety. This increase in growth is directly influenced by the process of priming the seeds with phytohormones. GA3 supports cell division and enlargement due to the enzyme induction process which can cause the cell wall to soften (Abbaspour et al., 2012). Research by Ma et al. (2018) also found long-term or multi-year effects on seeds prioritized using GA3 on *Leymus chinensis* plants. This treatment is also able to increase the growth of plants cultivated in limited media (pots).

The number of plant leaves also increased significantly in the priming treatment using GA3 with a concentration of 75 mg L⁻¹. This increase is influenced by the role of GA3 itself. GA3 is a type of phytohormone which plays an important role in the plant growth process. Iqbal et al. (2011) explained that gibberellin acid has an important role in plant growth such as increasing the efficiency of photosynthesis, by regulating enzymes that play a role in this process, increasing leaf area index, light interception and playing a role in nutritional use efficiency. Research conducted by Pangestuti et al. (2021) also found an increase in the number of leaves on shallot seedlings from seeds primed with GA3 at a concentration of 100 ppm.

Table 2. Average fresh weight of seeds, dry weight of seeds, chlorophyll b content, and total chlorophyll content.

Varietas	Parameter			
	FWS (g)	DWS (g)	Chl b ($\mu\text{g/mL}$)	Chl tot ($\mu\text{g/mL}$)
Tuk-Tuk	1.66 \pm 0.11	0.16 \pm 0.006b	14.32 \pm 0.14	22.61 \pm 2.37a
Sanren F1	1.66 \pm 0.11	0.18 \pm 0.005b	13.00 \pm 0.78	20.01 \pm 1.02b
Lokananta	1.86 \pm 0.17	0.24 \pm 0.197a	12.97 \pm 1.29	19.81 \pm 1.86b
Maserati	1.84 \pm 0.18	0.22 \pm 0.023a	12.69 \pm 1.06	19.55 \pm 1.65ab
P < (0.05) (sig)	0.115	0.014	0.106	0.005
LSD	-	0.009	-	0.35
Priming	FWS (g)	DWS (g)	Chl b ($\mu\text{g/mL}$)	Chl tot ($\mu\text{g/mL}$)
without priming	1.36 \pm 0.02c	0.19 \pm 0.019	10.92 \pm 0.15d	16.89 \pm 0.03d
Water Priming	1.70 \pm 0.11b	0.18 \pm 0.011	12.19 \pm 0.17cd	18.92 \pm 0.19d
25 mg L ⁻¹ GA3	1.88 \pm 0.06a	0.20 \pm 0.022	13.24 \pm 0.24c	20.50 \pm 0.67c
50 mg L ⁻¹ GA3	1.90 \pm 0.11a	0.21 \pm 0.035	13.31 \pm 0.80b	20.67 \pm 1.39b
75 mg L ⁻¹ GA3	1.94 \pm 0.08a	0.22 \pm 0.023	16.56 \pm 0.69a	25.49 \pm 1.13a
P < (0.05) (sig)	0.000	0.659	0.000	0.000
LSD	0.07	-	0.34	0.44

Notes: Means followed by the same letter in columns (a, b, c, d) are not significantly different in the DMRT P < 0.05 test. FWS (fresh weight of seeds), DWS (dry weight of seeds), Chl b (chlorophyll b), and Chl tot (total chlorophyll).

Table 3. Average of root length, and chlorophyll a.

Treatment combinations	RL (cm)	Chl a ($\mu\text{g/mL}$)
Tuk-Tuk + without priming	2.26 \pm 0.04b	6.46 \pm 0.19h
Tuk-Tuk + Water Priming	2.94 \pm 0.40ab	6.84 \pm 0.34efgh
Tuk-Tuk + 25 mg L ⁻¹ GA3	3.56 \pm 0.40ab	8.68 \pm 0.56bcd
Tuk-Tuk + 50 mg L ⁻¹ GA3	3.67 \pm 0.19ab	9.61 \pm 0.35ab
Tuk-Tuk + 75 mg L ⁻¹ GA3	3.57 \pm 0.98ab	9.86 \pm 0.32a
Sanren F1 + without priming	3.00 \pm 0.15ab	6.76 \pm 0.12fgh
Sanren F1 + Water Priming	3.19 \pm 0.09ab	6.74 \pm 0.38fgh
Sanren F1 + 25 mg L ⁻¹ GA3	2.81 \pm 0.23b	6.53 \pm 0.25h
Sanren F1 + 50 mg L ⁻¹ GA3	3.31 \pm 0.20ab	7.18 \pm 0.48efgh
Sanren F1 + 75 mg L ⁻¹ GA3	3.89 \pm 0.20ab	7.88 \pm 0.63def
Lokananta + without priming	2.74 \pm 0.30b	6.15 \pm 0.09h
Lokananta + Water Priming	2.50 \pm 0.15b	6.46 \pm 0.22h
Lokananta + 25 mg L ⁻¹ GA3	2.29 \pm 0.18b	6.91 \pm 0.06efgh
Lokananta + 50 mg L ⁻¹ GA3	2.50 \pm 0.18b	7.75 \pm 0.29defg
Lokananta + 75 mg L ⁻¹ GA3	2.79 \pm 0.24b	8.98 \pm 0.46abcd
Maserati + without priming	2.38 \pm 0.02b	6.55 \pm 0.25gh
Maserati + Water Priming	2.34 \pm 0.02b	6.86 \pm 0.05efgh
Maserati + 25 mg L ⁻¹ GA3	2.59 \pm 0.07b	6.93 \pm 0.15efgh
Maserati + 50 mg L ⁻¹ GA3	2.53 \pm 0.20b	7.99 \pm 0.64cde
Maserati + 75 mg L ⁻¹ GA3	4.56 \pm 0.08a	9.03 \pm 0.29abc
P < (0.05) (sig)	0.013	0.021

Notes: Means followed by the same letter in columns (a, b, c, d) are not significantly different in the DMRT P < 0.05 test. RL (root length), and Chl a (chlorophyll a).

priming treatment with GA3 had no effect on real stem diameter, while the varieties had a significant effect. It is known that the largest trunk diameter was recorded in the Maserati variety. In general, the pseudo stem diameter of shallot seedlings ranges from 1.98 to 2.24 mm. Furthermore, by observing root length, it was discovered that there was an interaction between variety and priming using GA3. It was recorded that the Maserati variety primed with 75 mg L⁻¹ of GA3 had the longest roots. This is not far from the influence of GA3 itself which is able to regulate plant growth and development. Gibberellin acid is a growth regulator that promotes flowering, cell division and seed growth after germination. Gibberellins also stimulate stem elongation by stimulating cell division and elongation (Castro-Camba et al., 2022).

Fresh weight and dry weight of seedlings were influenced by both treatment factors separately. Fresh weight of seedlings is influenced by GA3 priming. The tendency is that the higher the GA3 concentration, the seed weight also increases. The increase in seed weight is of course influenced by the number of leaves. The large number of leaves supports extensive light capture for the photosynthesis process. A similar thing was also found by Anwar et al. (2020) who noted an increase in the fresh and dry weight of seedlings by priming using GA3. Observations of plant dry weight were not influenced by priming, but the use of varieties had a different effect. The heaviest seed dry weight was recorded in the Lokananta variety. The dry weight of seedlings in different varieties ranges from 0.16 to 0.24 g.

Table 4. Information of soil characteristics in this study.

Parameter	pH H ₂ O	C-Organic	total nitrogen	C/N	P Olsen	Cation exchange capacity
unit		%	%		ppm	me/100g
Value	6.68	2.85	0.22	13	12.72	24.38

Priming seeds with GA3 in several varieties also affects plant chlorophyll levels. Chlorophyll has an important role in the photosynthesis process. There are two types of chlorophyll that are dominant in the photosynthesis process, namely chlorophyll a and b (Mandal and Dutta, 2020). Chlorophyll a, b and total Chlorophyll increased significantly using GA3 when compared to the control. Research conducted by Ramteke et al. (2016) also found something similar, where priming using GA3 was able to increase chlorophyll levels in papaya seedlings.

Materials and Methods

Plant materials and experimental design

Screen house scale research was conducted at the Exfarm, Faculty of Agriculture, Hasanuddin University. This research was carried out from July to September 2022. The average temperature in the screen house during the research was $34.27 \pm 4.72^\circ\text{C}$ and the average humidity was $60.33 \pm 14.22\%$. Meanwhile, information of soil characteristic has shown in Table 4.

The research was structured in a randomized complete block design (RCBD) with two treatment factors and three replications. The first factor, namely seed priming, consists of control or no priming (S0), priming with water (S1), 25 mg L⁻¹ GA3 (S2), 50 mg L⁻¹ GA3 (S3) and 75 mg L⁻¹ GA3 (S4). The second factor is the variety consisting of Tuk-Tuk (v1), Sanren F1 (v2), Lokananta (v3), and Maserati (v4). These two factors resulted in 20 treatment combinations, each treatment being repeated 3 times so that there were 60 observation units.

The seed priming process

The seed priming process by GA3 was carried out in stages according to Katriani et al. (2023). The seeds provided were placed into GA3 solutions with various concentrations. The ratio of the number of seeds to the volume of solution is 1:5 (w/v). After putting the seeds and solution in a plastic container, we attached a hose connected to the aerator and left it for 20 hours. After that, we removed the seeds and drained them on tissue paper until drying.

Seed performance testing

Seed performance testing was carried out by planting prime seeds in pots with size 11 x 12 cm. Previously, a pot was provided containing a medium consisted of soil and compost fertilizer from chicken waste with a ratio of 1:1 (w/w). The seeds were then immersed 1 cm deep in the pot. The number of seeds planted was 5 per pot. Then maintenance was carried out in the form of watering and weeding until 40 days after planting. Meanwhile, the fertilizer just used the nutrition in media without chemical fertilization.

Observation and data analysis

Parameters observed included plant height (cm), number of leaves, apparent stem diameter (mm), root length (cm), fresh weight of seedlings (g), dry weight of seedlings (g), levels of chlorophyll a, chlorophyll b and total chlorophyll (µg/mL). All parameters were measured at seedling age 40 days after planting. Determination of chlorophyll level was done according to Liu et al. (2020) with spectrophotometric methods. Chlorophyll a, b and total levels were determined using the following formula:

$$\text{Chl a} = 12,72A663 - 2,59A645 \quad (1)$$

$$\text{Chl b} = 22,88A645 - 4,67A663 \quad (2)$$

$$\text{Chl tot} = \text{Chl a} + \text{Chl b} \quad (3)$$

The collected data was then analyzed using ANOVA and if there was a real effect it was tested further using the duncan's multiple range test with $\alpha = 0.05$.

Conclusion

We concluded that the priming interaction using GA3 concentration of 75 mg -L and the Maserati variety has a real influence on the root length of shallot seedlings. Then the interaction of the Tuk-Tuk variety with 75 mg L⁻¹ GA3 priming had the best effect on chlorophyll a levels. Priming treatment using GA3 concentration of 75 mg L⁻¹ had a significant effect on plant height, number of leaves, fresh weight, chlorophyll b content and total chlorophyll. The treatment variations recorded different responses to various parameters. The Lokananta variety has the highest average plant height and the largest dry weight of seeds. The Maserati variety recorded the largest average stem diameter.

References

- Abbaspour J, Ehsanpour A A, Amini F (2012) The role of gibberellic acid on some physiological responses of transgenic tobacco (*Nicotiana tabacum* L.) plant carrying Ri T-DNA. *J Cell Mol Res.* 3(2): 75-80.
- Adhikari S, Subedi R (2022). Effect of seed priming agents (GA3, PEG, Hydropriming) in the early development of maize. *RJOAS.* 9: 113-120.
- Anwar A, Yu X, Li Y (2020) Seed priming as a promising technique to improve growth, chlorophyll, photosynthesis, and nutrient contents in cucumber seedlings. *Not Bot Horti Agrobot.* 48(1): 116-127.
- Balliu A, Marsic N K, Gruda N S (2013) Seedling Production. In: Baudoin W, Nersisyan A, Shamilov A, Hodder A, Gutierrez G, de Pascale S, Nicola S, Gruda N, Urban L, Tanny J (eds) *Good agricultural Practices for greenhouse vegetable production in*

- the South East European countries - Principles for sustainable intensification of smallholder Edition: Plant Production and Protection. FAO, Paris.
- Castro-Camba R, Sánchez C, Vidal N, Vielba J M (2022) Plant development and crop yield: The role of gibberellins. *Plants*. 11(19): 2650.
- Corbineau F, Ozbingol N T, Bouteau H E (2023) Improvement of seed quality by priming: Concept and biological basis. *Seeds*. 2(1): 101-115.
- Dotto L, Silva V N (2017) Beet seed priming with growth regulators. *Semin Cienc Agrar*. 38(4): 1785-1798.
- Hwang S J, Park J H, Lee J Y, Shim S B, Nam J S (2020). Optimization of main link lengths of transplanting device of semi-automatic vegetable transplanter. *Agronomy*. 10(12): 1938
- Iqbal N, Nazar R, Khan M I R, Masood A, Khan N A (2011) Role of gibberellins in regulation of source-sink relations under optimal and limiting environmental conditions. *Curr Sci*. 100(7): 998-1007.
- Liu N, Xing Z, Zhao R, Qiao L, Li M, Liu G, Sun H (2020) Analysis of chlorophyll concentration in potato crop by coupling continuous wavelet transform and spectral variable optimization. *Remote Sens*. 12(17): 2826.
- Ma H Y, Zhao D D, Ning Q R, Wei J P, Li Y, Wang M M, Liu X L, Jiang C J, Liang Z W (2018) A multi-year beneficial effect of seed priming with gibberellic acid (GA3) on plant growth and production in a perennial grass, *Leymus chinensis*. *Sci Rep*. 8: 13214
- Mandal R, Dutta G (2020). From photosynthesis to biosensing: Chlorophyll proves to be a versatile molecule. *Sens Int*. 1: 100058
- Muruli C N, Bhanuprakash K, Channakeshava B C (2016) Impact of seed priming on vigour in onion (*Allium cepa* L.). *J Appl Hortic*. 18(1): 68-70.
- Pagano A, Macovei A, Balestrazzi A (2023) Molecular dynamics of seed priming at the crossroads between basic and applied research. *Plant Cell Rep*. 42: 657-688.
- Pangestuti R, Sulistyarningsih E, Kurniasih B, Murti R H (2021) Improving seed germination and seedling growth of true seed shallot (TSS) using plant growth regulator seed priming. *IOP Conf Ser: Earth Environ Sci*. 883: 012024
- Qui K, Lesokvar D I (2020) Humic substances improve vegetable seedling quality and post-transplant yield performance under stress conditions. *Agriculture*. 10: 1-18.
- Ramteke V, Paithankar D H, Baghel M M, Kurrey V K (2016) Impact of GA3 and propagation media on growth rate and leaf chlorophyll content of Papaya seedlings. *Res J Agric Sci*. 7(1): 169-171.
- Singh H, Jassal R K, Kang J S, Grewal K (2015) Seed priming techniques in field crops - A review. *Agric Rev*. 36: 251-264.
- Sumarno J, Hiola FSI, Nur A (2021) Study on application of TSS (true shallot seed) shallot technology in Gorontalo E3S Web Conf. 232: 03011