Effect of storage time and soaking of natural growth regulators on true shallot seedling

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Abstract. This study aimed to determine the effect of the length of storage and soaking of coconut water growth regulators on TSS shallot seedlings. The research was conducted in Torongrejo Village, Junrejo District, Batu City, from April to May 2023. The experiment used a simple randomised block design (RBD) with the following treatments: The first factor is the freshness of young coconut water consisting of 4 levels, namely S0 = fresh/not stored young coconut water, S2 = young coconut water stored two days at room temperature and S3 = young coconut water stored three days at room temperature. Factor II was the soaking time for TSS consisting of 3 levels, namely P1 = TSS soaked for 1 hour, P2 = TSS soaked for 2 hours, and P3 = TSS soaked for 3 hours. The results showed that coconut water's natural growth regulators contain many substances that help plant growth, including the hormones auxin, cytokinins, and gibberellins, so their effectiveness depends on the length of time the coconut water has been soaked and the coconut water's shelf life. If the coconut water is stored longer, the soaking time will be shorter, namely 1 hour, and if the coconut water is stored, the time required is shorter.

1. Introduction

One of the superior horticultural crops that farmers in Indonesia widely cultivate is shallots (*Allium ascalonicum* L.). Shallots (*Allium cepa* L.) are widely cultivated and consumed worldwide [1]. Shallots are one of the superior horticultural crops and are widely cultivated by farmers in Indonesia. As a food ingredient, shallots are usually served as a cooking spice, such as roasting, boiling, frying, sautéing or steaming. It can be used as an herbal medicine, among other things, to relieve or prevent several common diseases, such as atherosclerosis, asthma, bronchitis and coughs. The health benefits of shallots are mainly attributed to their various bioactive constituents, such as organosulfur compounds, polysaccharide phenolic compounds, and saponins [2] [3]. Allium vegetables are rich in antioxidants, especially quercetin, glycosides and flavonoids [4]. With the many benefits of shallots, the need for shallots is increasing over time, along with population growth. The shallot cultivation currently used by farmers uses bulbs as planting material [5], which come from seed breeders in the vicinity or from previous harvests. The shallot planting area increase continues, so the need for quality seeds from the bulbs also increases.

Providing quality seeds from tubers has obstacles, including (1) the cost of purchasing seeds from large tubers (around IDR 45 million/ha); (2) requires many tubers (around 1.5 t/ha); (3) Requires storage warehouse due to the large number and dormancy period; (4) The period of use of seeds from tubers is short because the quality of the seeds will decrease after four months and will be damaged after six months; (5) distribution of tuber seeds to farmers/regions requires large costs; and (6) high variation in tuber seed quality and low productivity [6]. The disadvantages of vegetative propagation with tubers can be overcome by propagating TSS (True shallot seedling). Therefore, using TSS is an alternative that can be developed to improve the quality of shallot seeds [7]. The problem with using tubers is considered more complicated. So now, the use of TSS seeds is starting to develop. TSS production technology is an alternative to providing seeds in shallot cultivation using seeds as planting material (seed) [8]. The use of TSS has many advantages compared to the use of tubers. TSS seeds have a longer shelf life better seed quality, and what is no less important is low seed-borne pathogen contamination. The use of TSS has many advantages, especially related to the availability of good quality seeds, including having a longer shelf life, low variations in seed quality, high productivity, and rare contamination of seed-borne pathogens [8]. Increasing the acceleration of germination and increasing the viability of TSS can also be done by applying growth regulators. Accelerating germination and increasing TSS viability can also be achieved by the application growth regulator. Growth regulators are substances that promote growth when given in the right concentration.

On the other hand, if given in a concentration that is too high or too low for the plant's needs, it will inhibit and reduce its metabolic process [9]. Using growth regulators combined with auxin, cytokinin and gibberellins can increase the number of leaves, number of tubers and fresh weight of plants [10]. Another ingredient that can be used as a growth regulator is coconut water. Coconut water contains vitamins and minerals [11] and the growth hormones auxin, cytokinin and gibberellin in sufficient concentrations [12]. Coconut water contains the hormone auxin in the form of IAA with levels of around 0.237 ppm; cytokinin in the form of kinetin with a concentration of 0.441 ppm; and Zeatin with a concentration of 0.247 ppm; Gibberellin in the form of GA3, GA5, and GA7 with concentrations of 0.460 ppm, 0.255 ppm, and 0.053 ppm respectively [12]. Coconut water also contains minerals such as N, P, K, Mg, Fe, Na, and Ca [12]. Coconut water can stimulate seed growth and increase germination by soaking the seeds in various concentrations to improve the quality of the sprouts[12]. Thus, this research aims to determine the effect of coconut water as a natural growth regulator on the germination and growth of True shallot seedlings (TSS).

2. Material and Methodology

This research was conducted in Klerek Village, Junrejo District, Batu City, Indonesia. Carried out from April to May 2023,

2.1. Experimental material

The TSS shallot seeds used were Maserati, Fungicide Antracol 70WP Zinc, Insecticide Decis 25 EC 500 ml, chemical fertiliser (Urea and NPK 15:15:15) and young coconut water from the local Blitar variety aged eight months as a source of coconut water.

2.2. Research methods

Using a Randomized Block Design with two factors. The first factor is the storage time for coconut water, including:

S1 (coconut water stored for one day)
S2 (coconut water stored for two days)
S3 (coconut water stored for three days)

Meanwhile, the second factor is the length of soaking in coconut water.

P1: TSS seeds are soaked for 1 hour
P2: TSS seeds are soaked for 2 hours
P3: TSS seeds are soaked for 3 hours.

Repetition was carried out three times.

The sowing begins by loosening the soil mixed with leaf compost, then levelling it and planting the seedlings according to the treatment. Followed by covering the seeds sown with husk charcoal, then fine soil mixed with leaf compost and covered with dry pine leaves. Seed maintenance includes watering, weeding, fertilising and controlling pests and diseases. Fertilisation of TSS shallot seeds is carried out at 7 DAP. Fertilisation is done by providing 1g of Phonska NPK fertiliser per plant. Observations on seedling growth variables began at one week of age, with seven-day intervals until 47 HST.

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2.3. Seed soaking *process*

The TSS shallot seeds used in this research were Maserati hybrid seeds. The duration of soaking the seeds depends on the treatment. When soaking, the seeds are placed in water, and the seeds that sink to the bottom of the container are the fruity seeds that are suitable for sowing, while the seeds that float are discarded. After soaking, the seeds are dried on paper towels so the outer skin is not damp.

Figure 1. TSS seed soaking process (1a) TSS seed drying process (1b)

The parameters observed were plant height growth observed a week after sowing up to 6 weeks after sowing when they were transplanted into the field, germination percentage and germination ability.

2.4. Germination percentage (%).

The seed germination percentage was observed by counting the germination seeds in each experimental unit. Observations started the first day after the seeds germinated, spanning 1 – 14 days after planting. If more than 14 days later, the seeds sown are categorised as not germinating. According to [13], the calculation uses the following formula:

$$\text{Germination Percentage} = \frac{\text{Total number of seeds that germinate}}{\text{Number of seeds tested}} \times 100\% \quad (1)$$

2.5. Germination ability test (%).

Germination ability is determined by counting the number of seeds germinating normally within 14 days. By using the formula according to (Kuswanto, 1996):

$$\text{Germination Ability} = \frac{\text{Total number of normal sprouts produced}}{\text{Number of seeds tested}} \times 100\% \quad (2)$$

Data from this study were analysed using Analysis of Variances (ANOVA) uses two-way LSD for planned comparisons without considering the number of treatments. If ANOVA shows a significant difference at the 5% level, proceed with the least significant difference test with an error rate of 5%. (14) where the data were analysed using DSAASTAT software. If ANOVA shows a significant difference at the level of 5%, then proceed with the smallest real difference test with an error rate of 5%. (14), where the data was analysed using DSAASTAT software (Version 1.101).

3. Results and discussion

The analysis of variance showed a real interaction between the storage time treatment and the soaking time of TSS shallot seeds on plant height in the first and second weeks.

Table 1. Average growth of TSS shallot plant seedlings in the first and second weeks

The first week of observation				Second week of observation			
Treatment	P1	P2	P3	Treatment	P1	P2	P3
S0	5,057 cd A	5,057 cd B	5,057 cd C	**S0**	5,94 cde A	5,82 bcde B	5,97 cde C
S4	5,027 cd A	5,69 cd B	4,43 bc BC	**S4**	5,36 bcde A	4,485 bc A	4,90 bcd BC
S5	5,46 d A	5,63 d B	4,19 b B	**S5**	6,27 de A	6,47 e B	4,402 b B
S6	6,5 e B	4,083 b A	0,723 a A	**S6**	5,65 bcde A	5,347 bcde AB	0a A
CV (%)		9,08		CV (%)		15,38	
LSD (5%)		1%		LSD (5%)		1, 31	

[a] The numbers followed by the same lowercase letter denote insignificant differences in the same row. The numbers followed by the same uppercase letter denote insignificant differences in the same column tested at level 5% of LSD and CV, LSD: Least significant difference, CV: Coefficient of variance.

Table 1. Showed the growth of shallot seedlings. TSS in the first week after sowing shows a significant interaction between treatments. The interaction of S6P1 resulted in the highest growth. The level below was the S5P1 and S5P2 treatments. From the table we can also compare that soaking for 1 hour (P1) was the highest in the coconut water treatment that was stored for three days (S6). Meanwhile, soaking for 3 hours (P3) was highest when the seeds were treated with fresh coconut water without being stored (S0).

The interaction between coconut water stored for three days (S6) and soaked for 3 hours (P3) on shallot TSS seeds resulted in the lowest growth in the first week and death in the second week. This outcome is possible because the concentration of young coconut water is too thick (lacks fluid cells). This situation can disrupt the metabolic system of shallot seed cells, which can ultimately inhibit their growth. According to [15], a too dense concentration results in a high difference in solution concentration between the seed cells and the growth medium. This difference in concentration also allows the fluid in the seed cells to be absorbed out of the cells so that the seed cells can become dehydrated.

Seedling growth in the second week showed that S5P2 produced high growth similar to S5P1, S4P1, S6P1, S0P1, S0P2 and S0P3. It can be seen that soaking for one hour (P1), two hours (P2) and three hours (P3) in all types of coconut water, whether fresh (S0), is stored for one day (S1), two days (S2) and three days (S3) is not significantly different. Meanwhile, soaking for 2 hours (P2) in coconut water that was stored for two days was not significantly different from coconut water that was stored for three days, resulting in higher growth compared to soaking for 2 hours (P2) in fresh coconut water and that which was stored for one day. Furthermore, soaking for 3 hours (P3) in fresh coconut water and stored for one day gave higher growth than soaking for 3 hours (P2) in coconut water stored for two days, which was not significantly different from coconut water stored for three days. Young coconut water contains many hormones, auxin and cytokinin, because auxin and cytokines are active in dividing cells in meristematic cell tissue.

This statement is reinforced by the opinion of [16], who said that mineral content, vitamins, sucrose, and growth regulators induced in coconut water will decrease along with increasing the age of the coconut. This statement is strengthened by the opinion of [17], who said that the content of minerals, vitamins, sucrose and growth regulators induced in coconut water will decrease as the coconut ages.

Table 2. Average growth of TSS shallot plant seedlings in the third and fourth weeks

Third week of observation				Fourth week of observation			
Treatment	P1	P2	P3	Treatment	P1	P2	P3
S0	11,86 de BC	10,89 cde B	11,25 cde B	**S0**	15,81 defg B	14,42 cd A	15,17 cdef B
S4	10,64 cd A	9,92 bc AB	11,33 de B	**S4**	12,5 b A	14,58 cde A	16,30 fg BC
S5	12,28 e C	10,58 cd B	11,17 cde B	**S5**	17,36 g C	16,11 efg B	17,22 g C
S6	12,17 e C	8,75 b A	0a A	**S6**	16,67 fg BC	13,64 bc A	0a A
CV (%)	7,17			CV (%)	6, 26		
LSD (5%)	1, 22			LSD (5%)	1, 49		

[a] The numbers followed by the same lowercase letter denote insignificant differences in the same row. The numbers followed by the same uppercase letter denote insignificant differences in the same column tested at level 5% of LSD and CV, LSD: Least significant difference, CV: Coefficient of variance.

The results of the analysis of variance in Table 2 show a significant interaction between storage time treatment and soaking time for TSS shallot seeds on plant height in the third and fourth weeks. Table 2 shows the growth of shallot seedlings. TSS in the third week after sowing shows that the S6P1 and S5P1 treatments produced high growth and were not significantly different from S5P3, S4P3 and S0 at P1, P2 and P3. The level below is the S4P1 and S5P2 treatments. From the table you can also compare that soaking for 1 hour (P1) was highest in the coconut water treatment that was stored for three days (S6) and two days (S5). While soaking for 2 hours (P2) was highest in the treatment of seeds with fresh coconut water without storage (S0), storing coconut water for one day (S4) was no different from 2 days (S5), where this also happened with a soaking period of 3 days.

Seedling growth in the fourth week showed that S5P1 and S5P3 produced high growth that was not significantly different from S5P2, S4P3, S6P1 and S0P1. It can be seen that soaking for one hour (P1) using coconut water stored for two days (P2) is similar to storing for three days (S6). Likewise, soaking for two hours (P2) using coconut water with storage for two days (S5) resulted in higher growth. Likewise, soaking for three hours (P3) resulted in higher growth in coconut water stored for one day (S4) and two days (S5). Coconut water is a natural ingredient that contains hormones such as cytokinin 5.8 mg/l, the hormone auxin 0.07 mg/L, and a small amount of gibberellin and several other compounds that can stimulate germination and plant growth [18]. According to [19], coconut water contains minerals, cytokinin, auxin, phosphorus and kinetin, which stimulate cell division and shoot and root growth.

Table 3. Average growth of TSS shallot seedlings in the fifth and sixth weeks

Fifth week of observation				The sixth week of observation			
Treatment	P1	P2	P3	Treatment	P1	P2	P3
S0	19,75 cde B	15,44 b A	15,89 bc B	**S0**	19,75 bc A	20,91 cd B	18,33 bc B
S4	17,39 bcde AB	19,25 bcde BC	17,36 bcde B	**S4**	23,45 de B	25,39 e C	23,06 de C
S5	19,57 cde B	20,08 de C	21,07 e C	**S5**	24,11 e B	23,47 de BC	23,42 de C

S6	15,89 bc A	16,53 bcd AB	0a A	**S6**	17,19 b A	17,45 b A	0a A
CV (%)		12,21		CV (%)		8,4	
LSD (5%)		3, 42		LSD (5%)		2, 81	

[a] The numbers followed by the same lowercase letter denote insignificant differences in the same row. The numbers followed by the same uppercase letter denote insignificant differences in the same column tested at level 5% of LSD and CV, LSD: Least significant difference, CV: Coefficient of variance.

The results of the analysis of variance in Table 3 show a significant interaction between storage time and soaking time for TSS shallot seeds on plant height in the fifth and sixth weeks. Table 3 shows the growth of TSS shallot seedlings in the fifth and sixth weeks after sowing, showing that the S5P3 treatment produces high growth and is not significantly different from S5P1, S5P2, then S4P1, S4P2 and S4P3 along with S0 both at P1, P2 and P3.

From the table, you can also compare the highest soaking treatment for 1 hour (P1) with the coconut water treatment stored for two days (S5), which is no different from storing coconut water for one day (S4) and fresh coconut water (S0). Meanwhile, soaking for 2 hours (P2) was highest in the treatment of seeds with coconut water stored for two days (S5), whereas this also occurred with the highest soaking time of 3 hours (P3) in the treatment of seeds with coconut water stored for two days (S5).

Seedling growth in the sixth week showed that the S5P1, S5P2 and S5P3 treatments produced high growth that was not significantly different from S4P1. S4P2 and S4P3 . It can be seen that soaking for one hour (P1) using coconut water stored for one day (P2) is similar to storing for two days (S6). Likewise, the soaking time was three hours (P2) using coconut water with storage for two days (S5).

Applying growth regulators or nutrients during pre-soaking, priming and pre-sowing in many crops will improve seed survival rates and growth performance, which can result in overall plant growth and productivity [20]. Among the natural sources used, plant extracts and those containing endogenous growth regulators such as coconut water are gaining much traction [21]. Coconut water is a natural plant product that is locally available and can be used to increase germination rates and is the cheapest, freely accessible and environmentally friendly source of nutrition. Some of coconut water's most important and useful components are cytokinins (kinetin trans-zeatin, a class of phytohormones) [22]. The other is indole-3-acetic acid (IAA), the main auxin of plants and other components such as sugars, alcohols, lipids, amino acids, nitrogen compounds, organic acids, and enzymes [23]. The results of our research align with the findings of [24], indicating that coconut water treatment produces shoots faster and in greater numbers. This outcome is because coconut water contains the hormones auxin, cytokinin, and gibberellin. Auxin is a plant hormone that can regulate physiological processes in plants, such as growth, cell division and differentiation, and protein synthesis. Auxin also functions to form roots and shoots, encouraging cell division and elongation, thereby encouraging shoots to emerge taller.

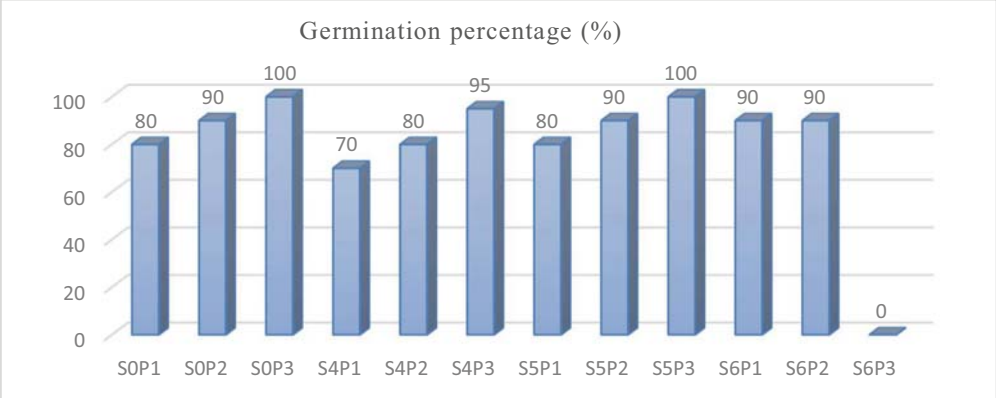

Figure 2. Percentage of germination of TSS shallot plant seedlings in the fifth and sixth weeks

The application of natural growth control substances in coconut water has been proven to increase the germination percentage of TSS shallots. It is shown in Figure 1 that soaking fresh coconut water for 3 hours and soaking coconut water stored for two days for 3 hours can produce 100% germination. The level below is the treatment of fresh coconut water, which is soaked for 2 hours, and the treatment of coconut water stored for two days, soaked for 2 hours. The coconut water soaking treatment showed a low germination percentage, which was kept for one day by soaking for one hour. Meanwhile, soaking for 3 hours using coconut water stored for three days produced a very low percentage. Seeds are the main element for plant growth and production, which can have a major influence on success or failure, both natural and artificial [25]. Germination from TSS seeds is a critical stage for plant survival. Pre-germination treatment is very important to accelerate germination, strengthen seed growth, increase the germination percentage, and shorten the germination period needed to reach optimal levels [22].

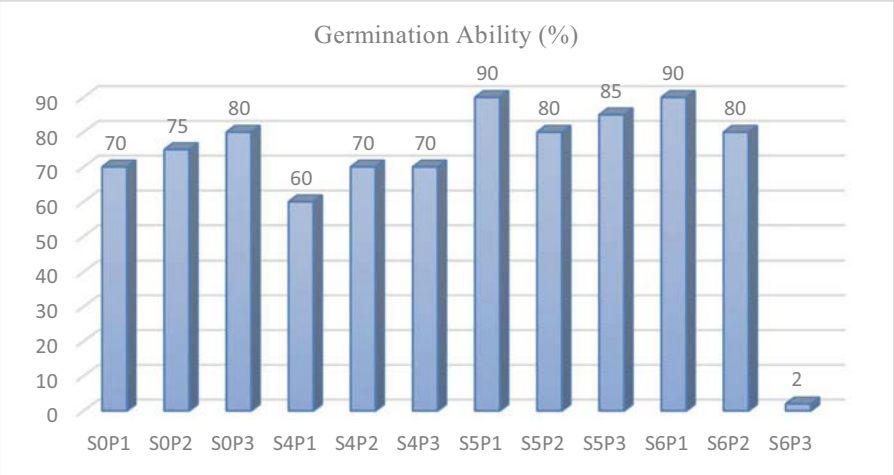

Figure 3. Germination ability of TSS shallot plant seedlings in the fifth and sixth weeks

Figure 3 shows that soaking coconut water stored for two days for 1 hour produces a germination capacity of 90%, as well as soaking coconut water stored for three days for 1 hour. The soaking treatment in coconut water stored for one day and soaked for 1 hour resulted in low germination ability. According to [26], the application of Growth Regulator Substances has been proven to increase the germination capacity of TSS Shallots.

4. Conclusion

From the research results, the natural growth regulator of coconut water contains many substances that help plant growth, including the hormones auxin, cytokinin and gibberellins. The effectiveness of coconut water is proven to be more real if the length of soaking in the coconut water and the shelf life of the coconut water are adjusted. The research here proved a real interaction between the storage of coconut water and the length of soaking. Storing coconut water for 1 to 2 days with a soaking time of between 1 and 3 hours can produce better plant growth. The treatment of fresh coconut water without storage requires a longer time, namely 3 hours of soaking, which can produce better TSS shallot germination.

Acknowledgement

The authors thank the Agricultural and Food Research Organization National Research and Innovation Agency for the financial support of the JRC program house (no. B-855/III.11/PR.03.06/2/2023B) for this research.

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