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Effects of seed soaking with plant growth regulators combination on the aggregation ability of shallot from seeds

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Abstract

The true seed of shallot (TSS) is an alternative technology to boost high-quality seeds, farming cost efficiency, and shallot productivity in Indonesia. Despite the advantages of TSS, including extended shelf life and lower seed requirements, farmer and consumer acceptance remains limited due to the genetic and physiological constraints leading to single and large-sized bulbs, as an effect of low aggregation ability. This caused shallot bulbs from TSS to have a low price and were not suitable for use as seed bulbs. This research addresses challenges in shallot (*Allium cepa* L. Aggregatum group) production from TSS by investigating the impact of various plant growth regulators (PGRs) treatments and different soaking time on shallot growth and aggregation ability of 'Tuk Tuk' planting from TSS. The study in Yogyakarta employed a split-plot randomized block design from July to November 2018. The main plot varied the PGRs combination (9 treatment), while the subplot used the soaking time of 4 hours and 12 hours. The results indicated that a GA_3 concentration of 100 ppm, in synergy with NAA at 50 ppm for 12 hours, effectively enhanced aggregation compared to another treatment. Notably, the 'Tuk Tuk' shallot, characterized by low aggregation ability, demonstrated improved potential through seed treatment by PGRs, which could raise the number of bulbs from one to an average of two bulbs per plant. This study enhanced shallot aggregation ability, providing valuable insights for research and developing shallot production from true seeds in Indonesia.

INTRODUCTION

Shallot (*Allium cepa* L. Aggregatum group) holds significant prominence as a vegetable crop in Indonesia, serving as a major ingredient cuisine and a vital source of income for farmers. Using the true seed of shallot (TSS) is an alternative technology to boost shallot productivity in Indonesia. Compared to bulb seed, true seed offers several benefits, such as longer shelf life, higher productivity, free bulb-borne pathogens include virus, more accessible storage and distribution, and lower seed requirement of 3–5 kg. ha^{-1}, which can decrease the seed cost by 100% and make the production more cost-effective overall (Pangestuti and Sulistyaningsih, 2011; Adin et al., 2021).

However, the use of TSS still faces challenges in farmer adaptation and consumer acceptance. Shallot bulbs grown from seeds tend to be single and large-sized, which is less preferred by consumers in Indonesia (Irsyad et al., 2018; Adiyoga, 2023). This is due to the low aggregation ability of seed-derived shallot bulbs. Aggregation ability is the ability of shallot plants to form several bulbs into a cluster in one plant, an important characteristic of yield and quality. Seed-derived shallot plants have lower aggregation ability than bulb-derived plants because of genetic and environmental factors

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(Pangestuti et al., 2022). Hence, exploring methods to enhance the aggregation ability of shallot plants derived from seeds is necessary. The mechanism of this limited aggregation ability in seed-derived shallot is still unclear. Rabinowitch and Kamenetsky (2002) suggested the apical dominant theory, which proposed that the loss of apical dominance at the meristem tip triggered the formation of aggregates in shallot. The loss of apical dominance would stimulate the growth of lateral buds that would develop into aggregation bulbs. Krontal et al. (1998) found that the apical meristem in shallot broke down after the first three leaves emerged from the seedling. This indicates that the aggregation process may require more energy to eliminate apical dominance in the initial process of seedling growth. This process could be influenced by seed treatment from the early growth stage.

Sudaryono (2018) reported that the application of soaking TSS in coconut water for 12 hours before sowing increased aggregation ability in TSS 'Trisula'. Coconut water is thought to contain natural growth regulators that enhance growth. These results raised the suspicion that soaking TSS with a combination of growth regulators with the right composition and soaking time as seed treatment could increase the ability to aggregate shallots from seeds. Plant growth regulators (PGRs) could influence specific physiological processes in plants, hence affecting their growth and development. They can function to increase or inhibit many physiological and biochemical processes in plants (Miransari and Smith, 2014), including metabolism in seed dormancy and germination (Graeber et al., 2012).

Soaking seeds in a solution containing 100 ppm gibberellin (GA_3) has demonstrated the potential to enhance growth speed, growth percentage, and seed viability across various plant species (Hosseini et al., 2020; Esan et al., 2020; Sukifto et al., 2020). This positive impact extends to specific plants such as onions (Brar et al., 2020) and shallots (Wahyuni et al., 2021). Yamazaki et al. (2015) showcased the influence of gibberellins on increasing tiller formation in spring onions (*Allium fistulosum*) by watering with 40 ppm GA_3 with 10 mL per plant. Aquino et al. (2023) stated that the most effective cytokinin for inducing shoot proliferation in cereals and grasses was 6-benzyl amino purine (BAP). NAA application at 50 ppm was reported to augment leaf area and leaf area index in pumpkins (Arvindkumar et al., 2014), while NAA at 100 ppm proved to be the optimal treatment for increasing the number of fruit and grape yield per plant (Ghani et al., 2013).

Generally, the regulation of genes influencing plant growth processes is intricate, involving multiple growth hormones, suggesting the potential necessity for interactions between hormones (Miransari and Smith, 2014; Yang et al., 2004). A combination of auxin and gibberellin, with a GA_3 concentration of 20 ppm + NAA 100 ppm, has enhanced plant height, primary branches, and leaf development in cucumbers (Dalai et al., 2015). The PGRs 0.5 mg L^{-1} NAA and 1 mg L^{-1} BAP were reported could increase aggregation of micro bulbs in Lily (Deswiniyanti and Dwipayani, 2020). However, the effect of PGRs treatment to bulb aggregation ability in shallots planting from seeds has not been widely reported. This study was undertaken to investigate the impacts of various combinations of PGRs treatments and soaking durations on the growth, yield, and aggregation capability of shallots derived from seeds.

MATERIALS AND METHODS

The study took place on the sandy coastal terrain, Samas Beach, within Sri Gading Village, Sanden District, Bantul Regency, Yogyakarta (5 m above sea level/ASL) from July to November 2018, in the dry season with no rainy days from July to September and average of rainfall 1.2 mm and 29.6 mm in October and November, respectively. Plant analysis was conducted in the Plant Science and Environmental Ecology Laboratory, Faculty of Agriculture, Universitas Gadjah Mada. Research used wooden planting boxes with 59 × 42 × 33 cm dimension lined with plastic sacks, caliper, digital scale (ACIS AD-I series), analytical balance (Sartorius), oven (Winder WTC) and uv-vis spectrophotometer (Genesis 10S, China). The materials used were TSS 'Tuk Tuk' (low aggregation ability), dolomite, SP-36 fertilizer (PT Petrokimia Gresik), ZA (PT Petrokimia Gresik), NPK 16-16-16 (PT. Saprotan Utama, Indonesia), NPK 15-9-20 (PT. Meroke Tetap Jaya, Indonesia), KCl (PT. Sentana Adidaya Pratama Indonesia), fungicide with active ingredient Mancozeb 80% (Dithane-M45, PT. Dow Agroscience Indonesia), propineb 70% (Antracol 70WP, PT. Bayer Indonesia), Difenoconazole 125 g.L^{-1} and Azoxystrobin 200 g.L^{-1} (Amistartop, PT. Syngenta Indonesia), insecticide with active ingredient Methomyl 40 g.L-1 (Metindo, PT. MKD, Indonesia), Chlorantraniliprole 50 g.L^{-1} (Prevathon, PT. DuPont, Indonesia), herbicide with active ingredient Isopropyl Amine Glyphosate 486 g.L^{-1} (Round Up, PT. Nufarm, Indonesia), citronella

oil, Gibberellic Acid (GA_3) (Merck, Germany); Naphthalene Acetic Acid/NAA (Serva, USA), Benzylaminopurine/BAP (Duchefa, Nedherland), distilled water, and young coconut water (type of coconut with an estimated age of 6–7 months after the appearance of flowers, with soft fruit flesh resembling jelly) (Sudaryono, 2017, personal communication). The research used sand as the growing media with planting distance of 15 × 10 cm, with two plants per planting hole. Thus, the population per box was 32 plants (1 box represented one replication per treatment). Each observation unit consisted of 2 plants. A split-plot randomized block design was used for the study, the soaking seed as main plot and soaking time as subplot (Table 1) with three replication. The total population was 9 × 3 × 3 × 32 = 2.592 plants.

Application of seed treatment with plant growth regulators (PGRs)

Seed treatment with PGRs was carried out by soaking shallot seeds in the PGRs mixture according to the treatments presented in Table 1. The proportion of seed weight to the PGRs solution was 20% (w/v). Following the soaking process, the seeds were dried on paper towels for a duration of 4–12 hours until they achieved air dryness, then mixed with fungicide containing 80% mancozeb until the fungicide covered the entire surface of the seeds. Fungicides are shown to reduce the condition of damping off seedlings (Lamichhane et al., 2017).

Seedling preparation, transplantation, plant maintenance and harvesting

The nursery bed in the screen house had a width of 100 cm and 600 cm long and was filled with a mixture of sand, rice husk, charcoal, and manure in a 4:2:1 (v/v/v) ratio as the sowing medium. The shallot seeds were planted in 10 cm-spaced rows with a rate of 1 $g.m^{-2}$. The seedlings were kept until 42 days after sowing (DAS). The nursery plants were watered twice a day or as needed. To prevent damping-off and basal rot diseases, a fungicide containing 80% mancozeb was applied 2 $g.L^{-1}$ at 10 and 28 DAS, continued with propamocarb hydrochloride 722 $g.L^{-1}$ fungicide (PT. Bayer, Indonesia) at 30 DAS. At 28 DAS, NPK 16-16-16 fertilizer was given at 5 $g.L^{-1}$ (1 liter per m^2 plant). Weeds were removed manually by hand weekly when they were still small to protect the seedling roots from damage.

The seedlings were transplanted at 42 DAS; when the seedling stems were enlarged and hardened, the roots were well formed, and the seedlings had 3–4 leaves. The seedlings were pulled out, and then their leaves were cut, leaving about 8–10 cm from the base of the stem to reduce evapotranspiration and stress on the seedlings. Planting was done in sandboxes in field condition with a planting distance of 15 × 10 cm per hole with 2 plants. The basic fertilizer was given 3 days before planting using 20 tons. ha^{-1} of manure and 130 $kg.ha^{-1}$ SP-36. Additional fertilization was applied 7, 28, and 42 days after transplantation (DAT) with an NPK 16-16-16 100 kg dose.ha^{-1}, ZA 86 $kg.ha^{-1}$, NPK 15-9-20 as much as 150 $kg.ha^{-1}$, ZA 100 $kg.ha^{-1}$, NPK 15-9-20 as much as 150 $kg.ha^{-1}$, and KCl 75 $kg.ha^{-1}$. Watering was done every day, morning, and evening, or as needed. Pest control was done by spraying insecticides and fungicides alternately once a week and spraying citronella oil as an insect repellent. Harvesting was done at 110 DAS.

Table 1. Seed treatment in shallot seed 'Tuk Tuk'

Factors	Treatment
Soaking seed	1. Without soaking seed (control)
	2. Soaking seed with GA_3 100 ppm
	3. Soaking seed with $GA_3$100 ppm and NAA 50 ppm
	4. Soaking seed with GA_3 100 ppm and NAA 100 ppm
	5. Soaking seed with GA_3 100 ppm and BAP 50 ppm
	6. Soaking seed with GA_3 100 ppm and BAP 100 ppm
	7. Soaking seed with GA_3 100 ppm, NAA 50 ppm and BAP 50 ppm
	8. Soaking seed with $GA_3$3GA_3 100 ppm, NAA 100 ppm and BAP 100 ppm
	9. Soaking seed with young coconut water
Soaking time	1. Four (4) Hours
	2. Twelve (12) Hours
	3. Without soaking (control)

Observation and measurement

Variables of observation were plant height and number of leaves in seedling phase (2−6 week after showing (WAS)/14, 28, 42 DAS) and 12 WAS (84 DAS). Chlorophyll contents (a, b and total) were observed at 84 DAS. Meanwhile, the number of bulbs per plant, total bulb weight per plant, and bulb dimension (height and diameter) were observed after harvest (110 DAS). The chlorophyll analysis employed the spectrophotometer technique with an 80% acetone solvent. A leaf sample weighing 0.5 grams was crushed and mixed with 10 ml of 80% acetone, then filtered through a paper filter. The absorbed solvent was measured using a UV-Vis spectrophotometer at 645 nm and 663 nm wavelengths. The chlorophyll concentration in the leaf was calculated using the provided formula (Rajalakshmi and Banu, 2015):

Chlorophyll a = 12.7 (A663) – 2.69 (A645)
Chlorophyll b = 22.9 (A645) – 4.68 (A663)
Total Chlorophyll = 20.2 (A645) + 8.02 (A663)

Data analysis

All variables and outcome components underwent analysis through analysis of variance (ANOVA) employing a factorial design at a 5% error level. Subsequently, additional testing was conducted using DMRT (Duncan's multiple range test) at α=5% error level. The data analysis utilized SAS software version 9.2.

RESULTS AND DISCUSSION

Seedlings and shallot plants from observed seeds could grow well in the sand medium, providing a sterile and neutral environment that eliminates growth bias factors from planting media. Planting in sand medium ensured that the impact of seed treatment did not arise from unidentified elements within the planting medium, particularly when soil was used. Previous research has highlighted the role of endophytic bacteria in soil, contributing to plant growth and various functions such as nitrogen fixation (Kandel et al., 2015), phosphate solubilization (Otieno et al., 2015), enhanced nutrient availability, nutrient absorption facilitation (Robinson et al., 2015), and the production of phytohormones like cytokinins (Kudoyarova et al., 2014), auxin (Khan et al., 2014), gibberellins (Khan et al., 2014) and abscisic acid (Sgroy et al., 2009).

Seed treatment with Plant Growth Regulators (PGRs) could potentially enhance seedling height, with noticeable effects emerging 28 DAS (Figure 1). While all treated seedlings exhibited increased height compared to the control, not all differences were statistically significant. The divergence in seedling height became more pronounced at 42 DAS (ready for transplant phase). Generally, soaking seeds for 12 hours resulted in heightened seedling growth compared to the control and soaking seeds for 4 hours of treatment. This was significant in treatments

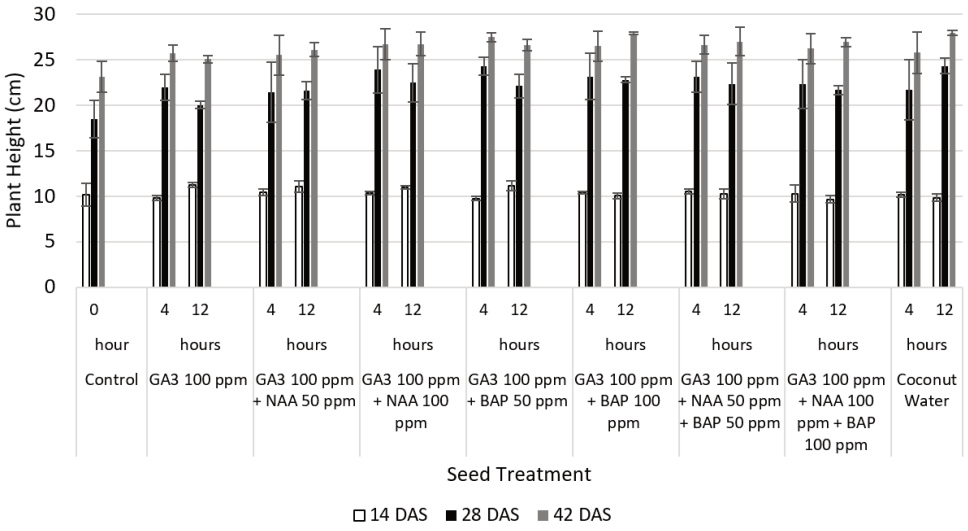

Figure 1. Plant height of 'Tuk Tuk' shallot planted from seed with PGRs seed treatment at seedling phase; DAS = days after sowing

such as soaking TSS with the combination of GA_3 100 ppm + NAA 50 ppm, GA_3 100 ppm + NAA 100 ppm, GA_3 100 ppm + BAP 100 ppm, GA_3 100 ppm+ BAP 50 ppm + NAA 50 ppm, GA_3 100 ppm+ BAP 100 ppm + NAA 1000 ppm, and the use of coconut water. In contrast, seed treatment did not significantly impact the leaf count until transplanting (42 DAS) compared to the control, sustaining a mean of 3 leaves for each plant (Figure 2).

Interestingly, the post-transplant phase exhibits a distinct pattern compared to the seedling phase. The PGRs combinations were ineffective in heightening plant growth at 84 DAS (Table 2). However, certain PGRs combinations successfully increased the number of leaves beyond that of control plants. These effective treatments included soaking TSS with GA_3 100 ppm + NAA 50 ppm, GA_3 100 ppm + BAP 100 ppm, GA_3 100 ppm + NAA 100 ppm + BAP 100 ppm, and the use of coconut water, resulting in an increase of 1–2 leaves (25–50%) per plant compared to the control (Table 2). Previous research by Wahyuni et al. (2021) demonstrated that soaking TSS seeds of 'Lokananta'

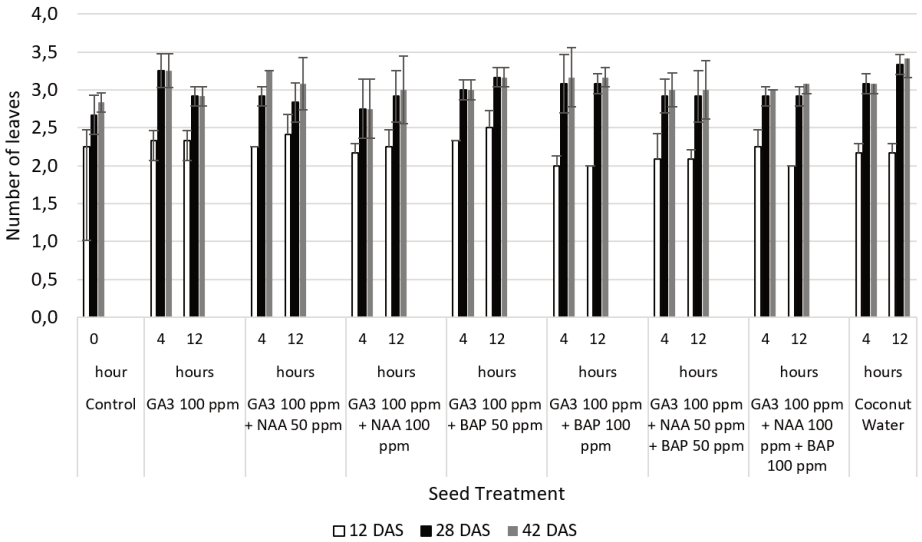

Figure 2. Number of leaves of 'Tuk Tuk' shallot planted from seed with PGRs seed treatment at seedling phase; DAS= days after sowing

Table 2. Plant height and number of leaves of 'Tuk Tuk' shallot planted from seed with PGRs seed treatment at 84 days after sowing

Treatment	Plant height (cm)	Number of leaves
Seed Treatment		
GA3 100 ppm	30.54 a	4.63 ab
GA3 100 ppm and NAA 50 ppm	34.79 a	5.71 a
GA3 100 ppm and NAA 100 ppm	33.04 a	5.38 ab
GA3 100 ppm and BAP 50 ppm	33.67 a	4.96 ab
GA3 100 ppm and BAP 100 ppm	32.69 a	5.50 a
GA3 100 ppm, NAA 50 ppm and BAP 50 ppm	33.13 a	4.92 ab
GA3 100 ppm, NAA 100 ppm and BAP 100 ppm	32.96 a	5.79 a
Coconut water	35.95 a	5.50 a
Control (without seed treatment)	30.75 a	4.33 b
Soaking time		
4 hours	36.26 a	5.00 ab
12 hours	30.43 b	5.59 a
Control (without soaking)	30.75 b	4.33 b
Interaction	(-)	(-)
Coefficient of variation (%)	12.8	15.61

Remarks: Means followed by the same letters in the column showed no significant different based on DMRT α=5%. The (-) sign indicates no interaction between the treatment factors investigated.

and 'Trisula' for 6 hours in a 100 ppm GA_3 solution alone could enhance plant height and leaf count. In our study, a sole application of 100 ppm GA_3 did not increase plant height or number of leaves compared to control plants at 84 DAS. This observation may be attributed to the necessity of synergistic interactions between PGRs to support the growth of 'Tuk Tuk' shallots, which exhibit a genetically low aggregation ability (Pangestuti, 2022).

In general, gene regulation that influences the plant growth process is regulated by more than one growth hormone (Miransari and Smith, 2014; Yang et al., 2004), so additional exogenous PGRs are needed to increase the performance of GA3 in stimulating plant growth. In this study, adding NAA and BAP in concentrations of 50–100 ppm synergized with gibberellin in increasing number of leaves but could not increase plant height compared to the control. The influence of genetic factors seems to be very dominant in plant height variables in 84 DAS, so it was not too affected by changes in environmental factors. This follows the research results of Degewione et al. (2011) and Waluyo et al. (2022), who found that genotype factors significantly affect shallot plant height.

There wasn't an interaction between the two treatment factors, namely seed treatment and long soaking time treatment, to significantly increase the amount of chlorophyll a, b, and total chlorophyll. (Table 3). This shows that applying PGRs through soaking seeds did not impact the chlorophyll content formed at 84 DAS. The application of nitrogen appears to be an exogenous element that has a more significant influence on the amount of chlorophyll compared to PGRs (Liu et al., 2015).

The combination of PGRs treatment that significantly increased the number of bulbs compared to control plants was GA_3 100 ppm + NAA 50 ppm with a soaking time of 12 hours (Table 4). The mechanism by which the synergistic interaction of gibberellin and auxin induces a rise in the number of bulbs in shallots from seeds has not been previously reported. It was suggested that the emergence of axillary shoots/lateral shoots that potentially form bulb aggregate in shallots from seeds resulted from the loss of apical dominance in the shoot meristem (shoot apical meristem/SAM). Following the formation of axillary buds, not all shoots can progress into bulbs due to the dominance of the main shoot; shoots lacking assimilation will subsequently become dormant shoots. This parallels findings in tulips, where apical dominance hinders some axillary meristems from developing into daughter bulbs, as they receive reduced assimilate/sucrose intake and become dormant buds (Pachon et al., 2018).

The optimal soaking duration for 'Tuk Tuk' shallots in this study was 12 hours. It is suspected that, at the concentration of the PGRs solution used (GA_3 100

Table 3. Chlorophyll content of 'Tuk Tuk' shallot planted from seed with PGRs seed treatment at 84 days after sowing

Treatment	Chlorophyll a (mg/g)*	Chlorophyll b (mg/g)*	Total Chlorophyll (mg/g)*
Seed Treatment			
GA3 100 ppm	17.77 ab	8.48 a	26.24 ab
GA3 100 ppm and NAA 50 ppm	17.94 ab	6.99 a	24.81 ab
GA3 100 ppm and NAA 100 ppm	16.62 b	6.94 a	23.55 b
GA3 100 ppm and BAP 50 ppm	16.02 b	6.26 a	22.28 b
GA3 100 ppm and BAP 100 ppm	18.78 ab	9.15 a	27.92 ab
GA3 100 ppm, NAA 50 ppm and BAP 50 ppm	18.91 ab	7.85 a	26.75 ab
GA3 100 ppm, NAA 100 ppm and BAP 100 ppm	19.37 ab	7.69 a	27.05 ab
Coconut water	22.57 a	9.01 a	31.57 a
Control (without seed treatment)	19.44 ab	8.30 a	27.74 ab
Soaking time			
4 hours	18.70 a	8.62 a	27.30 a
12 hours	18.27 a	6.97 a	25.28 a
Control (without soaking)	19.44 a	8.30 a	27.74 a
Interaction	(-)	(-)	(-)
Coefficient of variation (%)	10.26	13.13	10.62

Remarks: Means followed by the same letters in the column showed no significant different based on DMRT α=5%. The (-) sign indicates no interaction between the treatment factors investigated.

ppm + NAA 50 ppm), the imbibition and absorption process of PGRs yielded the most favorable outcomes after a 12-hour soaking period to loss apical dominant and increased bulbs aggregation. It is suspected that at the concentration of the PGR solution used (GA_3 100 ppm + NAA 50 ppm), the imbibition and absorption process of PGR yielded the most favorable outcomes after a 12-hour soaking period. These findings align with Sudaryono's (2018) report, wherein soaking TSS seeds for 12 hours with various PGRs compositions at a concentration of 200 ppm in 'Trisula' shallots resulted in similar effects. Singh et al. (2015) also noted that the optimum soaking time for seed treatment varied across plant types, ranging from 2

Table 4. Number of bulbs and bulb weight per plant of 'Tuk Tuk' shallot planted from seed with PGRs seed treatment

Treatment	Number of bulbs per plant	Bulb weight (g)
Seed Treatment		
GA3 100 ppm	1.25 b	12.71 ab
GA3 100 ppm and NAA 50 ppm	1.63 ab	14.50 ab
GA3 100 ppm and NAA 100 ppm	1.33 a	11.48 b
GA3 100 ppm and BAP 50 ppm	1.33 ab	15.60 a
GA3 100 ppm and BAP 100 ppm	1.38 ab	13.72 ab
GA3 100 ppm, NAA 50 ppm and BAP 50 ppm	1.25 b	12.97 ab
GA3 100 ppm, NAA 100 ppm and BAP 100 ppm	1.38 ab	12.81 ab
Coconut water	1.29 b	12.64 ab
Control (without seed treatment)	1.08 b	12.54 ab
Soaking time		
4 hours	1.26 ab	13.16 a
12 hours	1.45 a	13.45 a
Control (without soaking)	1.08 b	12.54 a
Interaction	(-)	(-)
Coefficient of variation (%)	16.90	1865

Remarks: Means followed by the same letters in the column showed no significant different based on DMRT α=5%. The (-) sign indicates no interaction between the treatment factors investigated.

Table 5. Bulb dimension of 'Tuk Tuk' shallot planting from seed with PGRs seed treatment

Treatment	Bulb height (mm)	Bulb diameter (mm)	Bulb Ratio (Height/Diameter)
Seed Treatment			
GA3 100 ppm	29.58 ab	27.68 ab	1.07 a
GA3 100 ppm and NAA 50 ppm	28.77 ab	28.52 ab	1.01 a
GA3 100 ppm and NAA 100 ppm	27.75 b	26.56 b	1.05 a
GA3 100 ppm and BAP 50 ppm	30.39 a	29.35 a	1.04 a
GA3 100 ppm and BAP 100 ppm	28.86 ab	28.41 ab	1.02 a
GA3 100 ppm, NAA 50 ppm and BAP 50 ppm	29.26 ab	28.39 ab	1.03 a
GA3 100 ppm, NAA 100 ppm and BAP 100 ppm	28.61 ab	28.32 ab	1.01 a
Coconut water	28.38 ab	27.32 ab	1.05 a
Control (without seed treatment)	28.51 ab	27.97 ab	1.02 a
Soaking time			
4 hours	29.34 a	27.90 a	1.06 a
12 hours	28.59 a	28.24 a	1.02 a
Control (without soaking)	28.51 a	27.97 a	1.02 a
Interaction	(-)	(-)	(-)
Coefficient of variation (%)	6.01	6.31	4.24

Remarks: Means followed by the same letters in the column showed no significant different based on DMRT α=5%. The (-) sign indicates no interaction between the treatment factors investigated.

to 48 hours, with the most effective average soaking time being 12 hours.

The soaking treatment of shallot seeds with a solution containing 100 ppm GA_3 and 50 ppm NAA accelerated the imbibition process and enhanced the gibberellin content. This heightened gibberellin level is then conveyed to the aleurone layer (Mena et al., 2002). Consequently, this increased gibberellin prompts the production of hydrolytic enzymes, specifically α-amylase, glucanase, phosphatase and protease. These enzymes play a role in breaking down food reserve compounds into smaller molecules, such as converting starch into sucrose and providing the energy required to expedite germination and support the development of axillary shoots. Furthermore, the sucrose produced supports the growth of axillary buds into potential aggregate bulbs/daughter bulbs. The mechanism underlying the formation of more bulb aggregates as a result of exogenous gibberellin and auxin treatment is based on a similar mechanism observed in cherry plants (Elving et al., 2011) and woody plants (Liu and Sherif, 2019).

The results show that in 'Tuk Tuk,' the effect of soaking GA_3 100 ppm and NAA 50 ppm manifests as induction of bulb aggregation. This effect becomes apparent when examining the increased variation in leaves at 48 DAS (Table 2), which is believed to originate from the elevation of the axillary/lateral meristems and the reduced resistance of the formed lateral meristems to develop into lateral shoots.

Including 50 ppm NAA as an external auxin is associated with the role of auxin in averting potential abnormalities in seedlings. Auxin also indirectly triggers signals stimulating gibberellin accumulation during germination (Sun, 2010). Additionally, auxin is involved in the development of lateral roots during later germination stages and the initial growth of seedlings (Liu et al., 2007). The accumulation of auxin in cotyledons serves as the principal source to sustain the continued growth of germination (Miransari and Smith, 2014). Auxin is thought to induce the formation of lateral buds by making the cell wall softer and more elastic by releasing hydrogen bonds in the cell wall (Robinson et al., 2013). The softness of the cell walls makes it easier for potential shoots to emerge. The synergy between gibberellin and exogenous auxin can also reduce callose in plasmodesmata, thereby increasing intercellular communication and mobility of molecules, including sucrose (Liu and Sherif, 2019). This synergy will be seen in further growth after germination. In this study, it was seen that the synergy in the combination of GA_3 100 ppm and NAA 50 ppm treatment could produce a more significant number of leaves and bulbs compared to control plants (Table 2 and Table 4).

Concentration of 50 ppm NAA demonstrates a more effective synergy with GA3 100 ppm compared to NAA at 100 ppm in 'Tuk Tuk.' This discrepancy might arise because high concentrations of auxin can lead to an imbalance in signal integration or a lack of equilibrium with GA_3. The optimal ratio or balance between GA_3 and NAA varies among plant types. For instance, the combination of 20 ppm GA_3 and 100 ppm NAA in cucumber plants was considered the most effective, resulting in increased plant height, primary branches, and leaves (Dalai et al., 2015). The application of PGR in plants necessitates precise concentration and timing, including maintaining a balance with hormones or other growth regulators, as low doses often stimulate growth, while high doses inhibit plant growth (Rademacher, 2015).

The size of the aggregated bulbs did not differ significantly from that of the bulbs in control plants (Table 5). This is attributed to the relatively modest increase in the number of bulbs, with only 1.63 bulbs per plant compared to the control's 1.08 bulbs per plant (51%) (Table 4), which did not substantially impact the reduction of bulb size. Categorically, shallots are classified into two bulb shapes: slightly oval/ovate bulbs and broad oval shapes, with variations between the two (Perković et al., 2021). The 'Tuk Tuk' shallot bulbs shape tends to be round, with a height-to-diameter ratio ranging from 1 to 1.1 (Table 5).

CONCLUSIONS

The aggregation ability of 'Tuk Tuk' shallot bulbs from seeds, which has a genetic limitation to develop aggregations, could be enhanced by seed treatment with plant growth regulators. The optimal plant growth regulator treatment for increasing the aggregation ability of 'Tuk Tuk' was soaking the seeds in a solution of GA_3 100 ppm + NAA 50 ppm for 12 hours, which could raise the number of bulbs from one to an average of two bulbs per plant.

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