Impact of salicylic acid and biosilica application on plant growth of shallot under water deficit

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Abstract. Shallot is a horticulture crop with a fibrous root system, which is susceptible to water deficit, particularly in the bulb formation stage. This study was carried out to examine the effects of salicylic acid (SA) and biosilica (Si) exogenous induction on plant growth of shallot grown under water deficit in plastic baskets. A factorial 4×2 experiment was laid out in a randomized complete block design with four blocks. The first factor of treatments was a four-level exogenous induction, i.e., 0.5mM SA, 6mM Si, combination (0.5mM SA and 6mM Si), and control (0 without SA and Si). The second factor was a level of interval irrigations i.e, one-day interval and three-day interval. The results showed that the treatment of combination 0.5mM SA and 6mM Si can maintained a plant height and the number of leaves was better than the control under water deficit. Avoidance was one of the mechanisms of shallot in dealing with water deficit, namely by reducing the stomatal density. Decreased stomatal density was negatively correlated with water use efficiency.

1. Introduction

Shallot is a crop with nutritional value that must be cultivated economically. The largest shallot production is in Asia at 54.7%, followed by Africa 28.3%, Europe 6.8%, America 5.5% and Oceania 4.7% [1]. Shallot production in Southeast Asia in 2017 is 137,949 tons and decreased to 131,381 tons in 2018 [1]. Climate change causes an increased temperature, and the average temperature has increased by 0.9 °C since the 19th century. The increase in temperature caused irregular rain patterns, increased droughts, floods, heatwaves and other extreme events affecting crop production [2]. Shallot will produce optimal bulbs if planted in suitable environmental conditions. The requirements for growing shallot are a minimum of 70% irradiation, 25-32 °C air temperature, 50-70% relative humidity, planted on crumb-structured soil with medium clay texture, and adequate drainage and aeration, with enough organic matter as well as a neutral soil pH (5.6-6.5). Shallot can be cultivated from lowlands to highlands (0-1000 mpdl) [3]. Shallot belongs to the Allium genus. More sensitive drought stress than onion and garlic, where they have a better mechanism in increasing moisture content in leaves and osmotic regulation than shallot [4]. The condition of water deficit is one of the constraints that affect the yields quality and quantity. Four mechanisms commonly occur in drought-stricken plants, i.e, drought avoidance, escape, tolerance and recovery mechanisms [5]. The avoidance mechanism is by increasing drought stress than onion and garlic, where they have a better mechanism in increasing moisture content in leaves and osmotic regulation than shallot [4]. The condition of water deficit is one of the constraints that affect the yields quality and quantity. Four mechanisms commonly occur in drought-stricken plants, i.e, drought avoidance, escape, tolerance and recovery mechanisms [5]. The avoidance mechanism is by increasing water absorption by increasing root elongation and depth of root
penetration and decreasing water loss by controlling the stomata's opening and closing and reducing leaf surface expansion, reducing the transpiration rate. The escape mechanism is by shortening the life cycle, while the tolerant mechanism is by a better osmoprotectant, antioxidant capacity and drying tolerance by accumulating biochemical compounds in osmotic adjustment [6]. The response of plants to drought is by changing the distribution of assimilates and to regulate the opening and closing of stomata [7]. Drought is oxidative stress that can increase the accumulation of reactive oxygen species (ROS). When the plants in water deficit condition, some of the stomata closed, which causes CO₂ to be inhibited from entering, reducing the photosynthetic activity [8][9]. The plant resistance mechanism by administering salicylic acid (SA) induced by abiotic stress is few to understand at the molecular level. However, recent research shows that the plant resistance mechanism caused by abiotic stress has similarities with the resistance mechanism to biotic stress, namely by accumulating Pathogenesis Related (PR) protein [10][11][12]. Exogenous salicylic acid is a synthetic organic chemical exogenous induction compound. The application of exogenous salicylic acid can reduce water pressure by maintaining leaves moisture content, modulating canopy temperature and more efficient water use to increase the yields [13]. One of the mechanisms for drought tolerance in SA-induced plants is the interaction with the other molecules such as ABA, JA, ethylene, Ca²⁺, NO, H₂O₂ etc. [14]. Salicylic acid can increase the closed stomata, and drought tolerance is also caused by ABA accumulation, so the possibility of SA and ABA in guard cells can synergistically regulating plant tolerance to drought stress by regulating the opening and closing of the stomata which is possible through Ca²⁺[15]. Silica (Si) is a beneficial element for plants considered to act as an essential nutrient for several plants. However, not all plants need it. Silica has various functions for different plant species [16]. Plants absorb Si in the form of monosilicic acid or orthosilicic acid (H₄SiO₄). Si accumulates in shoots varied by plant species, ranging from 0.1% -10% Si in dry weight [17]. The absorbed silica will accumulate in epidermis cell walls as polymerized monosilicate acid or silica amorph (SiO.nH₂O) [18]. Si is absorbed by plants and enters the xylem then transported together with water and translocated to the leaves. The water evaporated and the silica stored in the epidermis as monosilicate acid [19]. Si application produced a silica-cuticle double layer on the leaves epidermal tissue responsible for the higher leaves water potential during water deficit [20].

2. Methods
The research was conducted in Tri Dharma experimental station, Agriculture Faculty, Universitas Gadjah Mada, Yogyakarta, from November 2019 to April 2020. The experiment was arranged in factorial randomized complete block design. The first factor was application of salicylic acid, i.e., 0.5 mM salicylic acid, 6 mM biosilica, combination (0.5 mM salicylic acid and 6mM biosilica) and control (without salicylic acid and biosilica). The second factor was watering, i.e., one day watering with field capacity (moisture content 18.41%) and three days watering (moisture content 10.46%). The cultivar used Bima local Brebes was planted in a plastic basket with a dimension of 45 × 32 × 16 cm. There are 12 bulbs each basket. Shallot fertilizer consists of primary and supplementary fertilizer. The primary fertilizer was applied three days before planting by NPK fertilizer (16:16:16) at 250 kg/ha, KCL at 30 kg/ha, and SP-36 at 50 kg/ha. The first supplementary fertilizer was applied at 15 days after sowing (DAS) using ZA at 400 kg/ha. The second supplementary fertilizer was applied at 30 DAS using urea at 180 kg/ha [3]. Exogenous induction as salicylic acid, biosilica, or combination of salicylic acid and biosilica was applied at 14, 17, 20 and 23 DAS. Salicylic acid was applied in concentration 0.5 mM foliar at ± 1ml per plant. Biosilica was applied in a concentration of 6 mM on the soil at ± 2ml per plant and a combination treatment with salicylic acid 0.5 mM at 1ml per plant foliarly 6 mM biosilica 2ml per plant applied on the soil. Irrigation was carried out until the plants are 14 DAS, watering was carried out based on treatment in one-day and three-day. The data were statistically analyzed using the appropriate analysis of variance with the honestly significant difference value, calculated as Tukey's statistic at the 5% level. Observations was started at 49 DAS, including the anatomical
observation in epidermal cells of leaves, stomatal density, stomata width, relative water content (RWC) 
\[ \text{RWC} = \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Turgid Weight} - \text{Dry Weight}} \times 100\% \], water use efficiency (WUE) (g/plastic basket) = 
\[ \frac{\text{Dry weight per plastic basket}}{\text{Total water applied per basket}} \] , while the plant growth was observed at 2 weeks after planting (WAP) till 7WAP. As described in the Bima local Brebes cultivar description, harvesting was done on the 60th DAS based on the criteria of 60% leaves are withered.

3. Results and discussion
Water is an important molecule that supports different physiology and metabolism processes in plant cells and tissues. Water deficit in plants can be reduced the water potential of leaves, loss of cell turgor and stomata closure, thereby reducing transpiration and photosynthesis can inhibit plant growth. In conditions of severe water deficit, it is possible plants withered and deceased [21]. The study conducted a significant interaction at P<0.05 between the treatment of salicylic acid and the irrigations interval factors in stomatal density (Table 2). However, there was no significant interaction in stomata width (Table 1).

| Table 1. Anatomical and physiological data |
|-------------------------------------------|
| Treatment | Stomata width (μm) | Relative water content (%) |
| Salisylic acid | | |
| 0,5 mM salicylic acid | 5,55 a | 54,89 a |
| 6 Mm biosilica | 6,03 a | 50,78 a |
| Combination | 6,05 a | 51,96 a |
| Control | 4,98 a | 52,57 a |
| Watering | | |
| One day interval | 5,67 p | 57,26 p |
| Three day interval | 5,63 p | 47,84 q |
| CV (%) | 18,24 | 15,16 |
| Interaction | (−) | (−) |

Noted: a number followed by the same letter in the same column shows no significant difference based on Tukey's adjustment at the 5% level.

The highest average value of stomatal density was control treatment (without salicylic acid and biosilica) with one-day watering at 61.50 cells/mm², while the lowest average value of stomatal density was the combination treatment (0.5mM salicylic acid and 6mM biosilica) with a three-day watering at 48.50 cells/mm² (Table 2). Stomata have a role as a controller for exchanging gases and CO₂ that enter and leave the leaves [22]. Transpiration can be through stomata, cuticles and lenticels. However, the highest transpiration occurred in stomata [23]. Plants that have a water deficit would decrease the stomatal density, thereby reduced transpiration. Meanwhile, plants with adequate irrigation have tighter stomata (Table 2). Naturally, plants would reduce stomatal density and activated the ABA (abscisic acid) pathway to closed stomata in water deficit conditions [24]. On the other hand, the closure of stomata could also reduced CO₂ fixation which caused disruption of the photosynthesis process. However, based on the analysis shows that stomata width was significantly no difference, so the CO₂ concentration that entered the stomata might have been the same (Table 2). Shallot with combination application (0.5 mM salicylic acid and 6 mM biosilica) with a three-day watering could reduce transpiration by reducing stomatal density. So that plants can avoid water deficit conditions and can still perform better growth and development than the control.
Table 2. Interaction of salicylic acid treatment and watering at variable stomatal density (cells/mm²) 7 MST

| Watering  | Salicylic acid | 0,5 mM Salicylic acid | 6 mM Biosilica | Combination | Control | Mean (±) |
|-----------|----------------|----------------------|----------------|-------------|---------|---------|
| One day   | 56,25 ab       | 55,00 ab             | 52,50 b        | 61,50 a     | 56,31   |
| Three day | 60,00 a        | 49,50 b              | 48,50 b        | 55,75 ab    | 53,44   |
| Mean      | 58,13          | 52,25                | 50,50          | 58,63       |
| CV (%)    | 5,91           |                      |                |             |

Noted: a number followed by the same letter in the same column shows no significant difference based on Tukey's adjustment at the 5% level.

There is a significant interaction between salicylic acid treatment and watering on the water use efficiency variable (Table 3). On one day watering, salicylic acid application did not have a significant effect on WUE. Meanwhile on three days watering, salicylic acid application gave a significant difference on WUE. Plants with combination of SA 0,5 mM and Si 6 mM had a better water use efficiency than on three days watering control. The highest mean WUE value in combination treatment of SA 0,5 mM and Si 6 mM with the three days watering was 112,38 g/basket. While the lowest mean WUE value was 50,34g/basket on three days watering control treatment (Table 3). Increased plants tolerance through the increased water use efficiency possibly caused by manipulating sugar metabolism in guard cells. The sucrose was functioned as osmoticum guard cells and acted as a substrate on guard cells. The decreased sucrolytic activity was associated with the decreased stomata conductance which it could increased WUE, and conversely with the increased sucrolytic activity, it could decreased WUE. This is a metabolic manipulation on guard cells that improves stomata turgor regulation to increase the plants tolerance of water deficit [25].

Table 3. Interaction of salicylic acid treatment and watering at variable water use efficiency (g/plastic basket)

| Irrigation interval | Salicylic acid | 0,5 mM Salicylic acid | 6 mM Biosilica | Combination | Control | Mean (±) |
|---------------------|----------------|----------------------|----------------|-------------|---------|---------|
| One day             | 58,41 a        | 70,04 a              | 75,97 a        | 55,24 a     | 64,92   |
| Three day           | 88,47 ab       | 70,00 b              | 112,38 a       | 50,34 b     | 80,30   |
| Mean                | 73,44          | 70,02                | 94,18          | 52,79       |
| CV (%)              | 26,77          |                      |                |             |

Noted: a number followed by the same letter in the same column shows no significant difference based on Tukey's adjustment at the 5% level.

The stomatal density negatively correlates to water use efficiency with a Pearson correlation at -0.37 (weak correlation). So, the higher the stomatal density value, the lower water use efficiency by plants. Conversely, the lower the stomatal density value, the higher water use efficiency by plants. Water deficit could inhibit growth and significantly lead to decreased crop yields [26]. There was no significant interaction between the salicylic acid treatment and irrigations interval on relative water content variable, but there was a significant on main effect of irrigation interval. The highest relative water content in the one-day interval and the lowest relative water content in the three-day interval (Table 1). This is supported by the previous study, that plants grown under water deficit conditions showed lower relative water content values than plants grown under normal conditions (no water deficit) [27].
Figure 1. A-C: Longitudinal cross section of shallot leaves 100 × (μm) magnification. (A) Biosilica 6mM with three-day interval, (B) Combination (Salicylic acid 0.5mM and biosilica 6mM) with three days watering, (C) Control (without salicylic acid and biosilica application) with three days watering. Ctl (Cuticle); Up Ep (Upper Epidermis).

Anatomical observation showed that the shallot leaves given the 6mM biosilica with three days watering of the thickest cuticle layer at 7.8 μm. This is followed by a combination (0.5mM salicylic acid and 6mM biosilica) with a three-day watering with a cuticle thickness at 7.1 μm. The control treatment (without salicylic acid and biosilica) with three days watering is the lowest cuticle layer thickness at 3.8 μm (Figure 1. A-C) and (Table 4). The combination (0.5Mm salicylic acid and 6mM biosilica) with three days watering had a smaller cuticle thickness than the 6mM biosilica with three days watering. However, the combination treatment (0.5Mm salicylic acid and 6mM biosilica) with three days watering has a higher water use efficiency value. This occurred because the main plant transpiration happen in stomata, where is the combination treatment (0.5 mM salicylic acid and 6 mM biosilica) with three days watering has a low stomatal density, so it is better at preserving the water content in the leaves and water use efficiency compared with 6 mM biosilica under water deficit (three days watering). Silica does not have an essential role for plants grown under controlled conditions. However, under stress condition, silica is known to affect
plant health by strengthening the cell walls through sedimented silica which is translocated from the roots as silica acid from xylem until it settles under the cuticle and intercellular space [28]. Application of Na$_2$SiO$_3$ in wheat under water deficit can increase the relative water content of leaves, the leaves water potential, make a wider leaf area which may be useful for maintaining high assimilation ability, as well as leaves thickening which is useful to reduce loss transpiration compared with plants without Si application [29][30].

Table 4. Anatomy data of shallot leaves.

| Exogenous induction                      | Watering       | Cuticle width (μm) |
|------------------------------------------|----------------|-------------------|
| 0,5Mm salicylic acid                     | One day        | 4.4               |
| 6mM biosilica                            | One day        | 6.1               |
| Combination (0,5mM salicylic acid + 6mM biosilica) | One day        | 6.1               |
| Control (without salicylic acid and biosilica) | One day        | 4.0               |
| Salicylic acid 0,5Mm                     | Three days     | 5.7               |
| Biosilica 6mM                            | Three days     | 7.8               |
| Combination (0,5mM salicylic acid + 6mM biosilica) | Three days     | 7.1               |
| Control (without salicylic acid and biosilica) | Three days     | 3.8               |

Note: Numbers in bold indicate the highest and lowest value.

The water deficit condition in shallot plants could reduced plant height (Figure 2. A-B) and number of leaves (Figure 3. A-B). Shallot plants were experienced a rapid increase in height, namely at 2 to 3 week after planting (WAP), while at 4 to 7 WAP the height of shallot plants is relatively constant. Generally, the plants experience three phases in their life, namely the lag phase, the log phase and the stationary/fixed phase. The lag phase of plants was experienced slow growth, on log phase the plant growth was fast while the stationary phase the plants was no longer doing vegetative growth [31] [32]. The height pattern of shallot plants was formed a sigmoid curve, where the application of 6 mM Si could maintain the plant height with a higher trend compared to other treatments under one day watering conditions at the last observation (7 WAP) with a height of 32.81 cm (Figure 2.A ). When the watering application is carried out every three days, it had been seen that the combination treatment of 0.5 mM salicylic acid and 6 mM biosilica has a plant height with a higher trend of 32.47 cm, while the control treatment has a plant height with the lowest trend of 29.04 cm at the last observation (7 WAP) (Figure 2.B). This shows that the application of salicylic acid from a combination of 0.5 mM salicylic acid and 6 mM biosilica can reduce the negative effects of drought so it can maintain plant height better than control under three-days watering conditions. The graph on the leaves number observation parameter shown that in one day watering, the combination treatment of 0.5mM salicylic acid and 6 mM biosilica had the lowest number of leaves with an average of 8.95 leaves, while the highest number of leaves was the control treatment with the average of leaves was 10.22 leaves (Figure 3.A). The number of leaves in each treatment were formed a sigmoid curve with a fast rapid increased number of leaves, namely at 2 to 3WAP while the average number of leaves from 4 to 7WAP had a relatively fixed number of leaves (Figure 3. A-B). The number of shallots leaves with the lowest trend was in water deficit conditions (three days of watering), namely the 6 mM biosilica treatment with an average number of leaves of 8.16 leaves. While the number of shallots leaves with the highest trend was in the combination of 0.5 mM salicylic acid and 6 mM biosilica with control treatment which had relatively the same number of leaves since 4WAP to 7WAP (Figure 3.B).
In general, the plant growth was decreased when there is a water deficit (three days of watering), compared to normal condition (one day watering). The decreased growth includes a decrease in plant height and number of leaves. However, the shallot plants with a combination treatment of 0.5 mM salicylic acid and 6 mM biosilica were able to maintain higher growth than shallot plants with other treatments under water deficit conditions (three days of watering). The results showed that the application of a combination of 0.5 mM salicylic acid and 6 mM biosilica was able to maintain the growth of shallot plants with a higher trend compared to the control treatment under water deficit conditions. This was supported by the previous studies, that the effect of simultaneous application of salicylic acid and biosilica was greater than that of salicylic acid or biosilica separately under drought stress conditions in wheat [33].

4. Conclusions
The growth and development of shallot plants under water deficit (three days watering) had a higher trend with the combination of 0.5 mM salicylic acid and 6 mM biosilica compared to the control. The plant
resistance to water deficit can be done through an avoidance mechanism, namely by reducing the stomatal density, so the plants can increase water use efficiency. Stomatal density has a weak negative correlation (-0.37) on water use efficiency of shallot under water deficit (three days watering).

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