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Original Article

The Effect of Salicylic Acid Antioxidant and Watering Intervals on Mitigating Drought Stress During the Vegetative Stage of Soybean (*Glycine max L. Merr.*)

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Abstract. Drought is one of thr abiotic factors that can hinder plant growth and productivity. The use of salicylic acid as an antioxidant is expected to enhance plant tolerance to water stress, while adjusting watering intervals serves as an effective water management strategy. This study aims to investigate the effect of salicylic acid application and watering intervals on the drought resistance of soybean plants (*Glycine max L.* Merr.) during the vegetative phase. This research employed an experimental method using a Randomize Complete Block Design (RCBD) with two treatment factors. The first factor was salicylic acid concentration at three levels: S0 (0 mM), S1 (1 mM) and S2 (2 mM). The second factor was watering interval with four levels: H0 (0-day interval), H1 (1-day interval), H2 (3-day interval) and H3 (5-day interval). Observed parameters included total leaf area, stomatal density, root dry weight, root volume, and canopy dry weight. The result showed that salylic acid concentration 1 mM treatment had a significant effect on total leaf area, stomatal density, root dry weight and canopy dry weight. Watering 5 day interval treatments significantly affected total leaf area and root volume. Meanwhile, the interaction between salicylic acid and watering treatment did not have a significant effect on any of the observed parameters.

Keywords: Soybean; Salicylic Acid; Antioxidant; Drought; Watering Interval

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INTRODUCTION

Soybeans (*Glycine max* L. Merr.) are one of Indonesia's major food crops. Soybeans rank third after rice and corn as a major food crop in Indonesia (Achmad Fatchul Aziez *et al*., 2021). The demand for soybeans in Indonesia is very high, but supply remains far from sufficient because production is still very low, so the country continues to rely on imports to meet that demand (Buulolo *et al*., 2024).

One of the main factors contributing to low soybean productivity is the increasing incidence of drought stress due to climate change. Climate anomalies such as rising temperatures, shifts in rainfall patterns, and longer dry seasons make crops more susceptible to water deficits. In soybean plants, drought stress can reduce vegetative growth, accelerate stomatal closure, disrupt photosynthesis, and significantly reduce pod formation (Ruminta *et al*., 2020). An imbalance between a plant's water requirements and the availability of water in the soil is the primary cause of stunted plant growth under dry conditions (Mandala *et al*., 2020). Efforts to improve soybean drought tolerance can be achieved through the use of tolerant varieties and the application of compounds that strengthen the plant's defense system. The Anjasmoro variety is known for its high yield potential, large seeds, and superior agronomic traits, making it widely cultivated by farmers. However, its drought tolerance still needs to be improved through agronomic practices (Jumiatun *et al*., 2022).

One potential physiological approach is the application of exogenous hormones or antioxidants, such as salicylic acid. This compound acts as a signaling molecule capable of regulating plant stress responses by increasing antioxidant enzyme activity, regulating stomatal function, promoting chlorophyll formation, enhancing photosynthetic efficiency, and maintaining cell growth. Various studies indicate that salicylic acid can mitigate the effects of abiotic stress, though its effectiveness is highly dependent on concentration, application method, and the plant's growth stage. Additionally, irrigation intervals which trigger drought stress play a crucial role in determining the plant's physiological adaptive capacity (Cahyani *et al*., 2022).

Although a number of studies have examined the role of salicylic acid in alleviating abiotic stress, few studies have specifically evaluated the response of the Anjasmoro variety under a combination of drought stress and the application of salicylic acid at different concentrations and with varying irrigation intervals. This gap forms the basis for the necessity of this study. Although a number of studies have examined the role of salicylic acid in alleviating abiotic stress, few studies have specifically evaluated the response of the Anjasmoro variety under a combination of drought stress and salicylic acid application at different concentrations and with varied irrigation intervals. This gap forms the basis for the necessity of this study.

This study is based on the hypothesis that the application of salicylic acid at certain concentrations can enhance the drought tolerance of the Anjasmoro soybean variety, and that the interaction between salicylic acid concentration and irrigation interval has a significant effect on plant growth. The approach involved varying salicylic acid concentrations and irrigation levels to measure physiological responses and plant growth under controlled drought conditions.

MATERIALS AND METHODS

Research Period and Location

The research was conducted from December 2024 to January 2025 at the UINSU Greenhouse on Jalan Lapangan Golf, Durin Jangak Village, Pancur Batu Subdistrict, Deli Serdang Regency, and the UINSU Laboratory.

Tools and Materials

The equipment used includes a hoe, bamboo, UV-resistant plastic, nails, a hammer, plastic rope, poly bags, clear plastic, a spray bottle, a digital scale, a measuring tape, an oven, tissues, a measuring cup, a petri dish, a cover slip, a utility knife, a microscope, a petri dish, a dropper, tweezers, label paper, a digital camera, paper envelopes, and writing utensils. The materials used were Anjasmoro soybean seeds, salicylic acid, distilled water, topsoil, and NPK fertilizer.

Experimental Design

This study employed a randomized block design (RBD) with two treatment factors: the first factor was salicylic acid concentration, and the second factor was watering interval. Factor 1, salicylic acid concentration, had 3 treatment levels: S0 = 0 mM salicylic acid, S1 = 1 mM salicylic acid, S2 = 2 mM salicylic acid. Factor 2, watering interval, had 4 treatment levels: H0 = no watering, H1 = daily watering, H2 = every 3 days, H3 = every 5 days.

Work Procedures

Construction of a Plastic House

The plastic house is 7 meters wide, 9 meters long, and 3 meters high. It uses bamboo as support posts and UV resistant plastic for the roof and walls.

Preparation of Growing Medium

The soil used has been dried and sifted beforehand to ensure it is fine and dry. The fine, dry soil is then placed into 30 x 30 cm poly bags lined with clear plastic, with each bag weighing approximately 5 kilograms.

Planting Seeds

Soybean seeds are planted in the growing medium after the best seeds have been selected; the seeds are then placed in holes in the growing medium at a depth of 2 cm. Each polybag is filled with 2 soybean seeds and labeled for identification.

Application of Salicylic Acid

Salicylic acid was applied by spraying the leaves at 2 MST and 3 MST. The spray volume administered was 25 ml per plant. The concentrations of salicylic acid used are 0 mM, 1 mM, and 2 mM. The salicylic acid solution is prepared by weighing 0.069 grams of salicylic acid for a 1 mM concentration and 0.138 grams for a 2 mM concentration. After weighing, the acid was dissolved in 500 milliliters of distilled water and then homogenized.

Plant Watering

Watering was conducted at 100% field capacity. Watering was carried out at predetermined intervals: the control group (no watering), daily watering, every three days, and every five days. Water was measured using a measuring cup and applied in the morning.

Plant Maintenance

Thinning is performed to prevent competition for soil nutrients. Thinning is carried out once a week by removing weeds growing around the plants.

Fertilization is performed when the plants reach 2 MST using NPK fertilizer.

Pest control is carried out by spraying pesticides, while disease control is carried out by completely removing the diseased parts of the plants.

Observation Parameters

Total Leaf Area (cm^2)

The total leaf area of the plants was measured at 6 MST. Total leaf area was calculated using the formula: $L \times W \times K$

L = Leaf length

W = Width

K = Leaf area constant; for soybean leaves, the central leaf area constant is 0.6531, and the left and right leaf area constants are 0.765.

Stomatal Density

Stomatal density was determined using a replica/cast. Stomatal density was calculated using the formula for the area of the field of view

$= \frac{1}{4} \pi d^2$

$= \frac{1}{4} \times 3.14 \times 0.52$

$= 0.19625\ mm^2$

$$Stomatal\ Density = \frac{Stomatal\ Density}{Field\ of\ View}$$

Dry Weight of Roots (g)

The measurement of root dry weight is conducted during the late vegetative phase.

Root Volume

To measure root volume, submerge the root in a measuring cup filled with water. Then observe the change in volume and measure the root's volume.

Dry Weight of Canopy (g)

The dry weight of the canopy was measured at the end of the vegetative period, specifically at 6 MST.

Data Analysis

The data obtained from the morphometric measurements and stomatal density were analyzed using an analysis of variance (ANOVA) test with SPSS software. Where significant differences were found ($P < 0.05$), the Duncan Multiple Range Test (DMRT) was conducted to compare differences among treatments.

RESULTS AND DISCUSSION

The Effect of Salicylic Acid Antioxidants and Watering Intervals on Mitigating Drought Stress During the Vegetative Phase of Soybeans (*Glycine max* L. Merr).

Total Leaf Area

Based on the results of the ANOVA test, it was found that the treatments involving salicylic acid application and irrigation intervals had a significant effect on the total leaf area of soybeans at 6 MST. Meanwhile, the interaction between salicylic acid and irrigation intervals had no significant effect on the total leaf area of soybeans at 6 MST. The average total leaf area of 6-month-old soybean plants under treatments involving salicylic acid application and different watering intervals is shown in Table 1.

Table 1. Total Leaf Area at 6 MST

Watering Interval	Salicylic Acid			Average
	S0 (0 mM)	S1 (1 mM)	S2 (2 mM)	
	……………..(cm²)……………….			
H0 (0 day)	7,14	13,33	10,10	10,19^{b}
H1 (1 day)	7,80	13,68	10,15	10,54^{b}
H2 (3 day)	9,68	15,65	11,62	12,31^{a}
H3 (5 day)	9,81	16,13	12,45	12,80^{a}
Average	**8,61^{c}**	**14,70^{a}**	**11,08^{b}**	

Note: Numbers followed by different symbols in the same row or column indicate a statistically significant difference at the 5% DMRT level.

Table 1 shows that the 1 mM salicylic acid treatment (S1) produced the highest mean total leaf area, with a value of 14.70 cm², and showed a significant difference compared to the 2 mM treatment (S2), with a mean value of 11.08 cm², and the 0 mM treatment (S0), with a mean value of 8.61 cm². This is because the increase in total leaf area may be due to the role of salicylic acid in enhancing water use efficiency and activity in soybean plants. This is consistent with the statement Hayat *et al*., (2010) which states that salicylic acid plays an important role in improving water use efficiency in soybean plants, contributing to increased leaf area. However, salicylic acid must be used at an optimal concentration, as excessive concentrations can be toxic to the plants. In this study, it was observed that total leaf area decreased at a concentration of 2 mM compared to 1 mM, due to the phytotoxic effects of high salicylic acid concentrations.

The effect of watering intervals on soybean plants regarding the average total leaf area showed significant differences, with the highest average total leaf area observed in treatment H3 (every 5 days) at 12.80 cm², which was not significantly different from H2 (every 3 days) at 12.31 cm², while treatment H0 (no irrigation) showed no significant difference from H1 (every 1 day). Daily irrigation (H1), although providing sufficient water supply, did not yield better results compared to irrigation every three days (H2). Less frequent irrigation intervals can actually provide more optimal benefits for plant growth, particularly in terms of increased leaf area. Watering at 5-day intervals allows plants to experience drought stress, which actually stimulates active root growth into deeper soil layers in search of water. Deeper roots improve the efficiency of water and nutrient uptake, which is essential for the development of broad, healthy leaves (Mahmoud *et al.,* 2013).

Stomatal Density

Based on the results of the ANOVA test, it was found that the application of salicylic acid had a significant effect on the stomatal density of 6-week-old soybean plants, while the watering interval and the interaction between salicylic acid application and the watering interval had no significant effect on the stomatal density of 6-week-old soybean plants. The average stomatal density of 6-week-old soybean plants treated with salicylic acid and at different watering intervals is shown in Table 2.

Table 2. Stomatal Density on 6 MST

Watering Interval	**Salicylic Acid**			**Average**
	S0 (0 mM)	**S1 (1 mM)**	**S2 (2 mM)**	
	………………(mm^2)………………..			
H0 (0 day)	7,61	10,93	9,17	9,24
H1 (1 day)	8,20	11,71	10,15	10,02
H2 (3 day)	8,78	12,10	10,35	10,41
H3 (5 day)	8,78	13,47	10,74	11,00
Average	**8,34^c**	**12,05^a**	**10,10^b**	

Note: Numbers followed by different symbols in the same row indicate statistically significant differences at the 5% DMRT level.

Table 2 shows that salicylic acid has a significant effect on stomatal density. The salicylic acid treatments yielded significantly different results in terms of stomatal density; treatment S1 (1 mM) produced the highest average stomatal density of 12.05 mm², which was significantly different from the other treatments. Treatment S2 (2 mM) resulted in an average stomatal density of 10.10 mm². Meanwhile, treatment S0 (0 mM) resulted in the lowest stomatal density, namely 8.34 mm². A salicylic acid concentration of 1 mM (S1) yielded the most optimal results for increasing stomatal density compared to a concentration of 2 mM (S2) or the untreated control (S0). This is attributed to the physiological effects of the salicylic acid concentration being within the optimal range. Salicylic acid is known to stimulate antioxidant enzyme activity and maintain cell integrity during stress, including in leaf tissue cells that regulate stomatal opening. This is consistent with the statement Salsabila *et al.*, (2024) which states that the application of salicylic acid can improve the density and function of stomata in soybean plants subjected to drought stress, thereby enabling the plants to continue gas exchange and photosynthesis efficiently.

The irrigation interval treatment had no significant effect on the stomatal density of soybean plants. The mean value for irrigation interval H3 (every 5 days) yielded the highest stomatal density, namely 11.00 mm², followed by H2 (every 3 days) at 10.41 mm², H1 (daily) at 10.02 mm², and the lowest was H0 (no irrigation) at 9.24 mm². This is due to the plant's ability to physiologically respond to mild water stress by activating defense mechanisms, one of which is an increase in stomatal number. Water stress encourages plants to use water and absorb carbon dioxide more efficiently, thereby increasing stomatal density to support the photosynthesis process (Nabilah *et al*., 2022).

Root Dry Weight

Neither the irrigation interval treatments nor the interaction between salicylic acid and irrigation interval had a significant effect on the dry weight of the roots of 6 month old soybean plants, while the application of salicylic acid had a significant effect on the dry weight of the roots of 6 month old soybean plants. The mean dry weight of 6-month-old soybean roots treated with salicylic acid and at different irrigation intervals is shown in Table 3.

Table 3. Root Dry Weight 6 MST

Watering Interval	**Salicylic Acid**			**Average**
	S0 (0 mM)	**S1 (1 mM)**	**S2 (2 mM)**	
	………………(g)………………...			
H0 (0 day)	0,07	0,13	0,10	0,10
H1 (1 day)	0,09	0,15	0,12	0,12
H2 (3 day)	0,11	0,16	0,13	0,13
H3 (5 day)	0,11	0,20	0,14	0,15
Average	**0,10^b**	**0,16^a**	**0,12^b**	

Note: Numbers followed by different symbols in the same row indicate statistically significant differences at the 5% DMRT level.

Based on Table 3, the average dry weight of roots at various concentrations of salicylic acid shows that treatment S1 (1 mM) yielded the highest result, namely 0.16 g. Meanwhile, the 2 mM concentration (S2) produced a root dry weight of 0.12 g, and the treatment without salicylic acid (S0) reached only 0.10 g. The effectiveness of the 1 mM concentration is due to it being within the optimal physiological range, where salicylic acid can enhance drought tolerance without causing additional stress. The effectiveness of salicylic acid is highly dependent on the correct dosage, as excessive concentrations not only reduce its benefits but also have the potential to harm the plants (El-Shafey, 2017).

The 5 day watering interval (H3) yielded the highest root dry weight at 0.15 g, followed by the 3 day interval (H2) at 0.13 g, daily watering (H1) at 0.12 g, and the lowest value in the no-watering treatment (H0) at 0.10 g. The drought stress occurring in the 5 day watering interval (H3) prompted soybean plants to develop a root system as an adaptive response to limited water availability. The plants responded to these conditions by elongating and strengthening their roots to seek water from deeper soil layers. This increased root biomass and resulted in high root dry weight. This is consistent with the statement Kunert *et al*., (2016). which states that soybean plants subjected to drought stress alter their root architecture, including root depth, root angle, and root branching density. Plants with deeper roots and more branches have better survival rates under drought conditions.

Root Volume

Neither the application of salicylic acid nor the interaction between salicylic acid and watering intervals had a significant effect on the root volume of 6 month old soybean plants; however, watering intervals did have a significant effect on the root volume of 6 month old soybean plants. The average root volume of 6 month old soybean seedlings treated with salicylic acid and at different watering intervals is shown in Table 4.

Table 4. Root Volume 6 MST

Watering Interval	Salicylic Acid			Average
	S0 (0 mM)	**S1 (1 mM)**	**S2 (2 mM)**	
		……………..(ml)……………..		
H0 (0 day)	0,50	0,67	0,50	0,56^{b}
H1 (1 day)	1,17	1,50	1,33	1,33^{a}
H2 (3 day)	1,17	1,83	1,00	1,33^{a}
H3 (5 day)	1,17	1,50	1,50	1,39^{a}
Average	**1,00**	**1,38**	**1,08**	

Note: Numbers followed by different symbols in the same row indicate statistically significant differences at the 5% DMRT level.

Based on Table 4, the 1 mM salicylic acid concentration treatment (S1) produced the highest average root volume of 1.38 compared to the treatment without salicylic acid (S0), which had an average value of 1.00, and the 2 mM concentration treatment (S2), which had an average value of 1.08. The 1 mM concentration (S1) was the most effective, as at this level, salicylic acid was able to trigger a physiological response that supported root growth without causing toxic effects. One of the main mechanisms supported by salicylic acid application is the enhancement of the plant's antioxidant defense system. Thus, oxidative damage to root tissues can be minimized, allowing the roots to grow healthier and actively absorb water and nutrients (Khan *et al*., 2015).

The no-irrigation treatment (H0) produced the lowest mean root volume of 0.56 ml, which was statistically significantly different from the other treatments. However, treatment H3 (1.39) was not significantly different from treatments H2 (1.33) and H1 (1.33). The data indicate that watering at 5-day intervals (H3) tends to have a more favorable effect on root volume growth compared to the other treatments. Plants under drought stress adapt their root morphology by increasing root length and volume as part of their drought tolerance mechanism. Less frequent but regular watering intervals can stimulate root growth toward deeper layers. This is a form of morphological adaptation by plants to pursue decreasing soil moisture at the surface. Deeper and more extensive roots help plants survive better under drought stress because they can access water reserves in soil layers that do not easily evaporate (Daniel & Hartman, 2024).

Canopy Dry Weight

The application of salicylic acid, irrigation intervals, and the interaction between salicylic acid and irrigation intervals had no significant effect on the dry weight of the canopy of 6 month old soybean plants. The average dry weight of the soybean canopy at 6 MST, with treatments involving salicylic acid application and different irrigation intervals, is shown in Table 5.

Table 5 . Dry weight of the canopy at 6 months after planting

Watering Interval	Salicylic acid			Average
	S0 (0 mM)	S1 (1 mM)	S2 (2 mM)	
	………………..(g)………………			
H0 (0 day)	1,38	1,77	1,73	1,63
H1 (1 day)	1,77	2,63	2,09	2,16
H2 (3 day)	1,52	2,68	2,06	2,09
H3 (5 day)	1,44	2,54	2,08	2,02
Average	**1,53^{b}**	**2,40^{a}**	**1,99^{ab}**	

Note: Numbers followed by different symbols in the same row indicate statistically significant differences at the 5% DMRT level.

Based on Table 5, the treatment with a 1 mM salicylic acid concentration (S1) produced the highest canopy dry weight of 2.40 g, compared to the treatment with a 2 mM salicylic acid concentration (S2), which was only 1.99 g, and the treatment without salicylic acid (S0), which was 1.53 g. This indicates that the application of salicylic acid at a concentration of 1 mM is the most optimal in helping soybean plants cope with drought stress during the vegetative phase. The 1 mM concentration (S1) is capable of providing an appropriate stimulatory effect on the plant's defense mechanisms against drought stress. It is this combination of enhanced metabolism and cellular protection that allows the plants to maintain their vegetative growth and produce high canopy biomass. This suggests that without exogenous treatment, the plants lack additional protection against drought stress (Salsabila *et al*., 2024).

The H1 irrigation interval (daily irrigation) produced the highest canopy dry weight, at 2.16 g. This was followed by H2 (irrigation every 3 days) at 2.09 g, H3 (irrigation every 5 days) at 2.02 g, and the lowest was H0 (no irrigation) at 1.63 g. These differences reflect that watering frequency significantly affects soil moisture availability, which directly impacts the vegetative growth of soybean plants. The H1 watering interval was the most optimal because soil moisture was consistently maintained, preventing the plants from experiencing prolonged water deficits. This aligns with the statement Parveen *et al*., (2021) which states that without water, vital processes such as nutrient transport, protein synthesis, and cell expansion cannot function properly, resulting in the lowest dry weight of the canopy.

CONCLUSION

Based on the results of the study The Effect of Salicylic Acid Antioxidants and Watering Intervals on Alleviating Drought Stress in the Vegetative Phase of Soybeans (*Glycine max* L. Merr.), it can be concluded that a 1 mM concentration of salicylic acid is optimal for alleviating drought stress in soybean plants and yields the highest results for the parameters of total leaf area, stomatal density, root dry weight, and canopy dry weight. A watering interval of once every 5 days (H3) showed the best results for the parameters of total leaf area and root volume. The interaction between 1 mM salicylic acid and a 5-day irrigation interval yielded the highest means for total leaf area, stomatal density, and root dry weight. Meanwhile, the interaction between 1 mM salicylic acid and a 3-day irrigation interval yielded the highest means for root volume and shoot dry weight. Contains clear statements about the main conclusions of the research results. g

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CONFLICT OF INTEREST

The authors declare no conflict of interest to report study.

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