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Physiological Response and Production of Soybean Plants (*Glycine max* (L.) Merril) as Affected by Salicylic Acid Application under Drought Stress Conditions

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ABSTRACT

Drought alters plant physiological systems, which has an additional influence on soybean yield. Salicylic acid is a biostimulant that can help plants cope with stress. The study's goal was to examine how salicylic acid foliar spray affected the physiological response and productivity of soybean plants in drought conditions. The study employed soybeans from the Grobogan variety, carried out from August to October 2022 at the Greenhouse and Laboratory of Plant Physiology and Breeding, Faculty of Animal and Agricultural Science, Universitas Diponegoro, Semarang City. The study employed a 3×3 Factorial Complete Randomized Design with three repetitions, yielding 27 experimental units. The first factor was drought stress, which had three levels (80%, 60%, and 40% field capacity). The second factor was the concentration of salicylic acid, which has three levels (0, 0.5, and 1 mM). The parameters measured included leaf chlorophyll, relative water content, stomatal density, number of flowers, fresh pod weight, dry pod weight, and number of seeds. The observation data was statistically analyzed using 5% ANOVA, followed by the Duncan multiple distance test (DMRT). A 40% field capacity drought stress treatment reduced chlorophyll levels a, b, as well as the total number of flowers, fresh pod weight, and dried pod weight. The application of salicylic acid at a dosage of 1 mM increased the fresh weight of the pods.

Keywords: drought, physiological response, salicylic acid, soybean

INTRODUCTION

Global climate change has had a severe impact on plant growth and productivity, especially in dryland areas. Drylands are abundant across Indonesia's agricultural regions. Agricultural drought is typically induced by low rainfall and excessive evapotranspiration, making it one of the most severe environmental pressures (Asyura *et al*. 2018). Indonesia's tropical monsoon climate frequently causes droughts due to El Niño Southern Oscillation (ENSO) abnormalities. Drought has become an annual issue, threatening agricultural land and frequently resulting in crop failure (Rahman *et al*. 2015).

Drought produces major physiological and biochemical dysfunctions in plants, affecting their growth and output. Soybean plant is an important food crop that is particularly vulnerable to drought stress. According to Apriyana *et al*. (2016), climate change is causing a 12.4% reduction in soybean yield in Southeast Asia. Drought disrupts plant physiological processes, which are frequently accompanied by morphological alterations. Previous research has shown that drought stress impacts a variety of physiological processes and biochemical regulators in plants, including photosynthetic rate, respiration, and assimilate translocation capacity. Furthermore, drought has a deleterious impact on ion absorption, plant temperature regulation, and nutrient metabolism (Osama *et al*. 2019). The deteriorating effects of drought are determined by the level of stress experienced by the plant. Depending on field capacity (FC), drought stress causes varied degrees of damage. Soybean plants at 60% of FC (moderate drought stress) are comparatively tolerant and capable of sustaining higher growth rates than plants exposed to 40% FC (severe drought stress) (Siregar *et al*. 2017).

Drought stress can be mitigated by using biostimulants, which directly boost plant physiological systems. Salicylic acid is a biostimulant and regulator that reduces drought stress by controlling plant osmotic capacity and nutrient utilization efficiency. It also contributes significantly to the development and activity of antioxidant enzymes and secondary metabolites such as carotenoids. Previous research has discovered that salicylic acid increases photosynthetic

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rates and proline production (Khan *et al.* 2015). Proline synthesis, which is regulated by gene expression after salicylic acid application, improves plant adaptability to drought stress conditions. Proline content in plants is strongly related to its role as an osmoprotectant molecule, which soybeans produce as part of their drought stress defense mechanism (Sutrisno *et al.* 2022).

The efficacy of exogenous salicylic acid application is determined by plant species, developmental stage, concentration, method of application, and environmental circumstances. However, its impact on increasing soybean drought tolerance needs to be investigated further. As a result, it is required to determine the optimal salicylic acid concentration that has a moderating effect at various levels of drought stress (mild, moderate, and severe). The goal of this study was to investigate the effects of salicylic acid treatment on soybean plant physiology and yield under drought stress circumstances. Salicylic acid spray is believed to be a practical approach for reducing drought stress in soybean cultivation.

METHODS

The research was carried out at the Greenhouse and Laboratory of Plant Physiology and Breeding, Faculty of Animal and Agricultural Sciences, Diponegoro University in Semarang. from August to October 2022. The plant material employed was the Grobogan soybean variety (BSIP Aneka Kacang). Other materials included pure salicylic acid, planting media (6 kg/pot) made up of soil, rice husk charcoal, and goat manure, urea, SP-36, KCl, 80% acetone, 1 L distilled water, 10 mL propylene glycol, pesticide Curacron 500 EC (active ingredient: Profenofos 500 g/L), and water. Equipment used included 35 cm × 35 cm pots, PVC pipes, spectrophotometer, optical microscope, analytical balance, digital balance, dark tubes, Erlenmeyer flasks, oven, measuring cylinder, pestle and mortar, cuvettes, stationery, and a camera.

The experiment utilized a 3 × 3 factorial randomized full design with three replications. The first factor was the drought stress level: 80% FC, 60% FC, and 40% FC. The second element was the concentration of salicylic acid (S0), 0.5 mM (S1), and 1 mM (S2). ANOVA was used to examine the data at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT) as a post-hoc test. The parameters measured were chlorophyll content, relative leaf water content, stomatal density, number of flowers, fresh pod weight, dried pod weight, and number of seeds.

RESULTS AND DISCUSSION

Chlorophyll Content

Chlorophyll is a photosynthetic pigment that captures the light energy needed for photosynthesis. The buildup of total chlorophyll represents plants' photosynthetic rate during dry circumstances. The analysis of variance (ANOVA) revealed that drought stress had a significant influence on soybean leaf chlorophyll content, whereas salicylic acid application and the interaction of drought stress and salicylic acid concentration did not (Table 1). Drought stress levels had a considerable effect on soybean leaf chlorophyll concentration. Plants grown at 80% and 60% FC had significantly higher chlorophyll a, b, and total content

Table 1 Soybean leaf chlorophyll content under drought stress and salicylic acid application

Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
	Chlorophyll a (mg/g)			
80% FC	1.140	1.207	1.172	1.173 a
60% FC	0.892	0.978	0.903	0.924 a
40% FC	0.963	0.578	0.415	0.652 b
Mean S	0.998	0.921	0.830	
Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
	Chlorophyll b (mg/g)			
80% FC	1.140	1.207	1.172	0.476 a
60% FC	0.892	0.978	0.903	0.439 a
40% FC	0.963	0.578	0.415	0.260 b
Mean S	0.418	0.375	0.381	
Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
	Total chlorophyll (mg/g)			
80% FC	1.595	1.695	1.655	1.648 a
60% FC	1.306	1.389	1.392	1.362 a
40% FC	1.346	0.804	0.584	0.911 b
Mean S	1.416	1.296	1.210	

Remarks: FC = field capacity. Values followed by the same letter in the same column are not significantly different according to DMRT at the 5% level.

than those cultivated at 40% FC. The decline of chlorophyll a, b, and total concentration at 40% FC suggests that chlorophyll biosynthesis is dependent on appropriate water supply in plant cells. Under 40% FC, water supply was insufficient for chlorophyll synthesis. According to Hariandi *et al*. (2019), a lack of water disturbs chlorophyll synthesis, which impairs physiological functions such as photosynthesis.

Water shortage also causes chlorophyll breakdown due to elevated leaf temperature and transpiration. Chlorophyll concentration can be used to assess plant resistance to drought stress. According to Rosawanti (2016), one of plants' physiological responses to water deficit is a drop in chlorophyll concentration. This decline is produced by the activation of stress-related genes, which then reduces the activity of enzymes involved in chlorophyll synthesis. According to Ahmadikhah and Marufinia (2016), drought stress causes the generation of reactive oxygen species (ROS) such O_2 and H_2O_2, resulting in lipid peroxidation and damage to chlorophyll pigments. The drop in chlorophyll accumulation has also been linked to pigment molecule degradation caused by ROS buildup. Severe drought stress causes oxidative stress, which happens when excessive ROS generation destroys numerous biomolecules such as cell membranes, lipids, proteins, and chlorophyll. As a result, plant biomass buildup reduces, resulting in lower yields (Rosawanti 2016).

The application of salicylic acid had no significant effect on photosynthetic components such as chlorophyll a, b, or total content. The quantities used were only effective in alleviating water stress and did not restore leaf chlorophyll levels. Jaiswal *et al*. (2014) discovered that salicylic acid influences some physiological processes while suppressing others, depending on concentration, plant species, development stage, and ambient factors. However, Vazirimehr and Rigi (2014) found that using salicylic acid in soybeans boosted photosynthesis and pigment content.

Relative Leaf Water Content (RWC)

Drought stress reduces water content in plant cells, which can be measured using relative leaf water content (RWC). Under stress conditions, RWC is one of the most sensitive indicators of plant physiological responses and tolerance to dehydration. Several factors drive the drop in RWC during drought stress: (a) limited water availability for root uptake, (b) reduced root activity and water absorption, and (c) compromised membrane stability, which causes electrolyte leakage and water loss (Rahmianna & Purnomo 2018).

The ANOVA revealed that drought stress and salicylic acid administration had no significant influence on RWC, and no interaction effect was seen between the two treatments (Table 2). RWC reflects the plant's endurance to drought stress. Although the effect was not statistically significant, rising drought stress levels (from 60% to 40% FC) appeared to diminish RWC. Rahmianna and Purnomo (2018) indicated that the drop in RWC is linked to roots' ability to absorb water during drought conditions. At 60% and 40% FCs, water supply dropped, reducing root water uptake capability. The absence of a substantial effect could be attributed to the fact that the RWC of leaves in each treatment had not reached the crucial drought threshold. This suggests that plants were still able to maintain turgor pressure and thus metabolic processes.

Salicylic acid application had no discernible effect on RWC. Ghassemi-Golezani and Farhangi-Abriz (2018) found that applying 1 mM salicylic acid to soybeans boosted root H^+-ATPase activity, root development, root water content, plant biomass, and seed yield. Salicylic acid's effect is determined by a variety of factors, including concentration, plant species, growth stage, and environmental circumstances (Jaiswal *et al*. 2014).

Table 2 Relative leaf water content of soybean under drought stress and salicylic acid concentrations

Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
		%/leaf		
80% FC	0.70	0.68	0.69	0.69
60% FC	0.67	0.69	0.69	0.68
40% FC	0.65	0.63	0.63	0.64
Mean S	0.67	0.67	0.67	

Remarks: FC = field capacity.

Table 3 Stomatal density of soybean leaves under drought stress and salicylic acid concentrations

Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
		(mm^2/leaf)		
80% FC	2.90	2.98	3.14	3.00
60% FC	2.60	3.46	2.22	2.76
40% FC	2.64	2.29	2.57	2.50
Mean S	2.71	2.91	2.64	

Remarks: FC = field capacity.

Stomatal Density

Stomatal density is one of the indicators used to assess the effects of drought stress on plants. The analysis of variance revealed that neither drought stress nor salicylic acid application had a significant influence on stomatal density, and there was no interaction between treatments (Table 3). Stomatal density was calculated as the ratio of stomata number to observed leaf area. A larger number of stomata suggests greater stomatal density. In this study, soybean leaf stomatal density decreased as drought stress levels increased, however the difference was not statistically significant.

Salicylic acid did not have a significant effect on stomatal density in soybean leaves. This finding implies that salicylic acid largely helps plants cope with water stress via enhancing photosynthesis rather than directly regulating stomatal growth. According to Barus *et al*. (2021), salicylic acid increases chlorophyll and carotenoid concentration, both of which are required for photosynthesis to function properly. Furthermore, salicylic acid stimulates protein and peroxidase enzyme activity, which contributes to enhanced plant growth by lowering respiration losses under abiotic stress conditions.

Number of Flowers

One of the plant's defense strategies against drought stress is rapid blooming. Drought escape (DE) is a natural adaptation technique in which plants reduce their developmental stages to finish their life cycle before a severe drought hits. Ardian *et al*. (2024) found that under dry conditions, plants produce more abscisic acid (ABA), which increases the production of florigen, the flowering hormone, resulting in earlier blooming. Early blooming and a shorter vegetative phase can be beneficial in extreme drought conditions because they limit exposure to dehydration throughout the flowering and seed-filling stages. According to Maimunah *et al*. (2018), dryness during the vegetative phase has an impact on the reproductive system, increasing floral sterility. As a result, blossoming may occur earlier, but fertilization is generally unsuccessful if water scarcity persists.

The ANOVA revealed an interaction between drought stress and salicylic acid application on the quantity of soybean flowers (Table 4). At 80% FC, 0 mM salicylic acid produced considerably more flowers (17) than 0.5 mM (12) or 1 mM (13). Under 60% FC, 0.5 mM salicylic acid produced considerably more flowers (15) than 1 mM (11), but not significantly higher than 0 mM (12). Meanwhile, at 40% FC, 0.5 mM salicylic acid produced more flowers (15) than 1 mM (13) but had no significant difference from 0 mM (12).

Flower development is frequently enhanced in response to water stress as a molecular adaptation mechanism. In this study, drought stress was applied 4 weeks after planting, during the generative period, resulting in flower abortion. According to Kesuma *et al*. (2019), a lack of water affects photosynthesis by reducing assimilation and water availability to the leaves, resulting in flower drop. Number of flowers is highly associated with pod formation. A higher number of flowers often results in more pods, which increases yield potential. Salicylic acid application at higher doses (1 mM) assisted plants under both mild and severe water stress in producing blossom counts comparable to ideal conditions (80% and 60% FCs). However, even with excellent water availability, salicylic acid application did not significantly boost flower count. According to Arif *et al*. (2020), exogenous salicylic acid can improve flowering by increasing flower number and

Table 4 Number of soybean flowers under drought stress and salicylic acid concentrations

Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
		(flower/plant)		
80% FC	17 a	12 bc	13 bc	14
60% FC	12 bc	15 ab	11 c	13
40% FC	12 bc	15 ab	13 bc	13
Mean S	14	14	12	

Remark: Values followed by the same letter in the same row/column are not significantly different according to DMRT at the 5% level.

Table 5 Fresh pod weight of soybean under drought stress and salicylic acid concentrations

Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
		(g/plant)		
80% FC	10.72 b	9.67 bc	14.47 a	11.62 a
60% FC	8.59 bc	8.84 bc	7.92 c	8.45 b
40% FC	7.58 cd	4.82 e	5.30 de	5.90 c
Mean S	8.96	7.77	9.23	

Remark: Values followed by the same letter in the same row/column are not significantly different according to DMRT at the 5% level.

minimizing stress effects, although its efficacy varies depending on environmental conditions.

Fresh Pod Weight

Drought stress and the application of salicylic acid as a mitigating agent affected soybean yield components. The ANOVA revealed a significant relationship of drought stress and salicylic acid treatment on fresh pod weight (Table 5). The number of pods generated influences the fresh pod weight. Drought stress treatment and salicylic acid concentration combine to influence fresh pod weight. At 80% FC, 1 mM salicylic acid resulted in considerably higher fresh pod weight (14.47 g) than 0 mM (10.72 g) and 0.5 mM (9.67 g). At 60% FC, 0 mM and 0.5 mM treatments produced higher fresh pod weights than 1 mM, although the differences were not as significant. At 40% FC, 0 mM salicylic acid produced considerably more fresh pod weight than 0.5 mM, but no significant difference from 1 mM.

The number of pods generated influences the fresh pod weight. Drought stress treatment and salicylic acid concentration combine to influence fresh pod weight. At 80% FC, 1 mM salicylic acid resulted in considerably higher fresh pod weight (14.47 g) than 0 mM (10.72 g) and 0.5 mM (9.67 g). At 60% FC, 0 mM and 0.5 mM treatments produced larger fresh pod weights than 1 mM, although the differences were not as significant. At 40% FC, 0 mM salicylic acid produced considerably more fresh pod weight than 0.5 mM, but no significant difference from 1 mM.

The decrease in pod weight is positively connected with the level of drought stress experienced by the plants because water stress affects photosynthesis, resulting in lower assimilate production, which is insufficient to meet sink demands. As a result, plants redistribute nutrients obtained from other organs, such as seeds. Hidayati *et al.* (2017) observed that when soybean plants are subjected to drought and heat stress, their stomata close, limiting photosynthesis. Under optimal water conditions (80% FC), applying 1 mM salicylic acid enhanced fresh pod weight. This improvement is most likely owing to the role of salicylic acid in increasing photosynthetic efficiency under water stress. Razmi *et al*. (2017) discovered that salicylic acid improves soybean genotypes' resistance to water deficits by minimizing lipid peroxidation, enhancing antioxidant enzyme activity, and improving yield components. Salicylic acid increases the activity of antioxidant enzymes, which reduces oxidative stress, protects cells, and modulates gene expression by neutralizing reactive oxygen species (ROS) produced by environmental stress. As a result, this technique improves fresh pod weight, pod quality, and overall plant development. Wang *et al*. (2018) also found that salicylic acid spray protects plants from oxidative stress and enhances soybean fresh weight by increasing antioxidant enzyme activity.

Dry Pod Weight

Soybean yield components are impacted by both environmental factors and mitigating agents used during vegetative growth. The ANOVA revealed that drought stress had a significant influence on dry pod weight, whereas salicylic acid application and the interaction of drought stress and salicylic acid concentrations had no significant effect (Table 6). Drought stress significantly affects the dry weight parameters of pods. At 80% FC, soybean plants generated considerably more dry pod weight than plants at 60% and 40% FCs. Increasing drought stress (from 60% to 40% FCs) lowered dry pod weight, most likely because water stress impeded photosynthesis. This resulted in decreased assimilate production, assimilation rates, and, eventually, dry pod weight and growth rate. Aziez *et al*. (2021) found that water shortage reduces plant growth rate due to reduced evapotranspiration, resulting in a poorer total yield.

Salicylic acid treatment had no significant effect on dry pod weight. In contrast, Ghassemi-Golezani and Farhangi-Abriz (2018) found that applying 1 mM salicylic acid to soybeans boosted root H^+-ATPase activity, root development, relative water content, plant biomass, and seed yield. The discrepancy could be attributed to insufficient concentration or environmental conditions in the current investigation, which reduced the effects of salicylic acid on dry pod weight. According to Jaiswal *et al*. (2014), salicylic acid can either stimulate or inhibit several physiological processes depending on its concentration, plant type, developmental stage, and environmental conditions.

Number of Seeds

The number of seeds is a major factor in influencing soybean production. The ANOVA showed that drought stress and salicylic acid administration had no

Table 6 Dry pod weight of soybean under drought stress and salicylic acid concentrations

Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
		(g/plant)		
80% FC	6.8	5.9	7.1	6.6 a
60% FC	5.4	5.2	5.2	5.3 b
40% FC	5.1	4.2	4.1	4.5 b
Mean S	5.8	5.1	5.5	

Remarks: Values followed by the same letter in the same row/column are not significantly different according to DMRT at the 5% level.

Table 7 Number of soybean seeds under drought stress and salicylic acid concentrations

Drought stress	Salicylic acid concentration			
	0 mM	0.5 mM	1 mM	Mean K
		Seed		
80% FC	22	21	25	23
60% FC	22	20	19	20
40% FC	21	17	18	19
Mean S	22	19	21	

Remarks: FC = field capacity.

significant effect on the number of soybean seeds (Table 7). Although not statistically significant, increasing drought stress levels (from 60% to 40% FC) reduced the number of seeds. This could be because under drought conditions, plants devote more resources to root growth than shoot development. This root prioritizing enables soybean plants to continue nutrient intake from fertilizers (urea, KCl, and SP-36). Potassium and phosphorus elements, which are essential for seed development, could still be adequately absorbed. Wijayanti *et al*. (2021) observed that potassium and phosphorus are required throughout soybean growth, with phosphorus being particularly necessary for flower, fruit, and seed development.

Salicylic acid application had no significant effect on seed number, most likely because its major function was to improve plant physiological quality under water stress rather than to directly promote reproductive development. According to Vazirimehr and Rigi (2014), salicylic acid promotes photosynthesis by increasing CO_2 assimilation and nutritional absorption. Through these improvements, salicylic acid indirectly contributes to higher yielding components.

CONCLUSION

At 80% field capacity (FC), applying 1 mM salicylic acid enhanced pod weight and total leaf chlorophyll content during drought stress. Salicylic acid application at concentrations ranging from 0.5 mM to 1 mM under drought stress at 40% FC produced flowers comparable to those at 60% and 80% FCs. Thus, applying salicylic acid at 0.5−1 mM is efficient in preserving plant physiology and yield during mild (80% FC) and moderate (60% FC) drought stress situations.

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