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Screening of Soybean Varieties against Salinity stress at an Early Vegetative Growth Stage under Hydroponic Conditions

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Abstract. Salinity is a principal environmental severe stress that significantly threatens crop production, including soybeans. It considerably affects various plant growth and physiological traits at different soybean growth stages, especially in coastal areas. However, the high soil pH in these regions presents a challenge. In this study, we screened two soybean varieties using an easy and rapid, and inexpensive screening technique. This study aimed to select soybean tolerance salinity stress in the early vegetative growth stage under hydroponic culture. The study was meticulously done with a randomized complete block design comprising two factors (varieties and NaCl) with three replications. Two soybean varieties, i.e., 'Gepak Kuning and Dering 1', were tested at three NaCl levels, i.e., 0, 60, and 120 mM. The results showed that each type of plant behaved differently to each stress level. The stress of 60 mM revealed that the Dering 1 variety was tolerant, whereas the Gepak Kuning variety was only somewhat tolerant. In conclusion, the Gepak Kuning variety was tolerant to 60 mM salinity stress, while the Dering 1 variety showed 60 mM and 120 mM salinity tolerance. These results will help select the most tolerant varieties to develop salinity-tolerant varieties in the future.

Keywords: coastal land, hydroponic, salt stress, soybean, vegetative stage

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1. Introduction

Soybean (*Glycine max* L.) is a legume commodity commonly consumed by Indonesians due to its substantial nutrient content and perceived health benefits. Moreover, soybeans are the third most significant food commodity, following rice and corn. Accordingly, they function in the food and feed sectors [1]. In addition, soybean plants are cost-effective protein sources, rendering them very suitable for meeting the nutritional needs of the population [2]. According to data from The Ministry of Agriculture [3], the per capita consumption of soybeans has risen from 9.76 kilograms per year in 2021 to 10.3 kilos per year in 2024.

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The increasing demand for soybeans in Indonesia cannot be met by indigenous supply. Hence, the country must import from other nations. In 2019, the national demand for soybeans reached 3.5 million tons annually, although local output only reached 700-800 thousand tons annually. Therefore 2019, soybean seed imports totaled 2.67 million tons, or 1.06 billion US dollars [4]. This circumstance forces Indonesia to rely further on its escalating soy imports [5]. A decrease in ideal land due to a change in land function is one of the causes contributing to Indonesia's low soybean yield. This justifies the need to boost soybean yield using several methods, including intensification and intensification.

Utilizing marginal land for soybean intensification is possible. Utilizing marginal land is one way for farmers to address the problem of diminishing fertile land. One of the marginal lands that can be utilized for soybean growth is soil with a somewhat high salt level [6]. The area of saline land in the world is 397 million ha, or 3.1% of the world's total land area [7]. In Indonesia, the total area of salinity land is estimated to be 440,300 ha, with slightly saline land comprising 304,000 ha and saline land comprising 140,300 ha [8], and the saline land area in Bengkulu is 525 Ha [9].

The soybean genotype can be altered by saline environments, which can induce plant stress. Saline environments can significantly change soybean genotypes by inducing stress, affecting growth and yield. Salinity causes osmotic stress, nutrient imbalance, and ion toxicity, impacting critical physiological processes [10]. Therefore, efforts are needed to overcome the salty conditions of the land so that soybeans can be cultivated on it. One way is to utilize the results of developing salt-tolerant soybean varieties or existing superior cultivars. Understanding the impact of salinity on plant growth is very important in its management. The process of plant tolerance to salt must be understood in terms of plant morphology, physiology, and biochemistry to design resistant cultivars [11,12].

According to Munns and James [13], adaptable soybean cultivars can utilize energy more efficiently to produce greater biomass under stressful conditions. However, K absorption remained elevated in saline-tolerant soybean genotypes [14]. According to Yadav et al. [15], the yield loss threshold for soybeans is 5 dS/m. Soybean variety Dering 1 is one of the high-yielding varieties with drought tolerance and a potential yield of 2.85 tons per hectare. However, its seed size is tiny and has low production potential. Similarly, the Gepak Kuning variety has a determinate type, adapts well to paddy fields and uplands, and has a yield potential of 2.86 t/ha [16]. These two kinds evaluate the resilience to saltwater conditions so soybeans can be successfully produced on saline soils. These results will help select the most tolerant varieties for breeding purposes to develop salinity-tolerant varieties in the future.

2. Materials and Methods, or Methods

This study was conducted from March to July 2022 at the University of Bengkulu, Agronomy Laboratory Greenhouse, Department of Agricultural Cultivation, Faculty of Agriculture. This research employed a completely randomized design (CRD) with two factors: soybean varieties (V) and salinity stress (N). The initial consideration is to utilize two variations (V), including the following: V1: Gepak Kuning variety; and V2: Dering 1 Variety. The second element is Salinity Stress (N), which utilizes a solution (NaCl) with two levels: N0: 0 mM (Control); N1: 60 mM (4-5 dS/m); and N2: 120 mM (9-10 dS/m). Based on the information provided, 18 treatment combinations are possible. There were 15 plants in each experimental unit, for a total of 270 plants.

2.1. Research Stage

Soybean seeds are first sown in a tray containing a mixture of roasted husks and compost in a ratio of 1:1 (v/v) for 7 days. Then the nutrient culture media consists of 0.24 mM NH4NO3, 0.03mM (NH4)2SO4, 0.088 mM K2SO4, 0.38 mM KNO3, 1.27 mM Ca(NO3)2.4H2O, 0.27 mM Mg(NO3)2.4H2O, 0.14 mM NaCl, 6.6 µM H3BO3, 5.1 µM MnSO4.4H2O, 0.61 µM ZnSO4.7H2O, 0.16 µM CuSO4.5H2O, 0.1 µM Na2Mo7.7H2O, 45 µM FeSO4.7H2O-EDTA [17]. The nutrient solution has previously been calculated according to needs. Nutrient culture solutions are made by dissolving them in water. Transplanting is carried out after sowing the soybean seeds for 7 days and immediately transferred to nutrient culture. The soybean plants that will be moved are soybean plants that have uniform growth, that is, they have the same number of leaves. After the soybean plants were transferred to nutrient culture for 5 days, the nutrient solution was replaced and treated with salinity stress by adding NaCl with concentrations of 60 mM and 120 mM. The pH of the growth medium was adjusted to a neutral level, namely pH 7 for the control treatment and pH 8-9 for the solution with the addition of salinity stress.

2.2. Observation Variable

The observed variables were the plant height, number of leaves, primary root length, lateral root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, chlorophyll index, and stomatal density.

2.3. Data Analysis

The collected data were analyzed with the Variance Analysis F test. To assess the actual differences among all observed variables, the Duncan Multiple Range Test (DMRT) was conducted at a 5% significance level.

3. Results and Discussion

3.1. Plant Growth of Three Soybean Varieties

3.1.1. Plant Height

The Gepak Kuning variety, ranging from 0 to 15 DAP under salinity stress (60 mM NaCl), had a plant height of 21.65 cm to 114.82 cm. Meanwhile, under salinity stress (120 mM NaCl), the plant height was 23.66 cm to 97.89 cm. Not much different from the Dering 1 variety, where from 0 to 15 DAP under salinity stress (60 mM NaCl), the plant height was 15.57 cm to 106.02 cm. Under salinity stress (120 mM NaCl), the plant height was 16.30 cm to 97.08 cm. This shows that the plant height decreased along with the increased concentration of NaCl (Figure 1). Differences in the genetic characteristics of the varieties used also cause this difference in soybean plant height. Genetic factors determine differences in varietal growth power in adapting, where plants experience physiological and morphological changes in a direction following the environment [18].

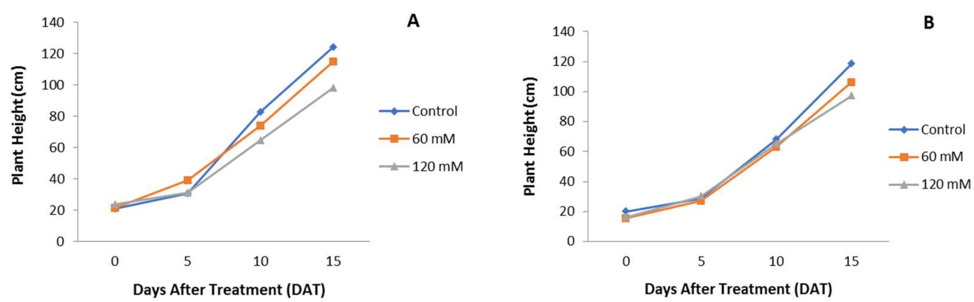

Figure 1. Soybean Plant Height Growth Based on the Level of NaCl Treatment on Varieties (A = Gepak Kuning; B = Dering 1)

Salinity stress (60 mM and 120 mM NaCl) caused a decrease in plant height starting at 5 DAT, while the inhibition of new plant height was significant at 15 days after treatment (DAT) (Figure 2). Salinity stress due to 60 mM NaCl caused significant growth inhibition compared to 0 mM NaCl. This shows that the NaCl concentration of 60 mM in the nutrient culture system can stimulate salinity stress conditions. Simulations of salinity stress using NaCl have been carried out to screen for salinity-tolerant genotypes in soybean [19], sorghum [20], and wheat [13], as well as to study the mechanism of tolerance of barley to stress—salinity [21].

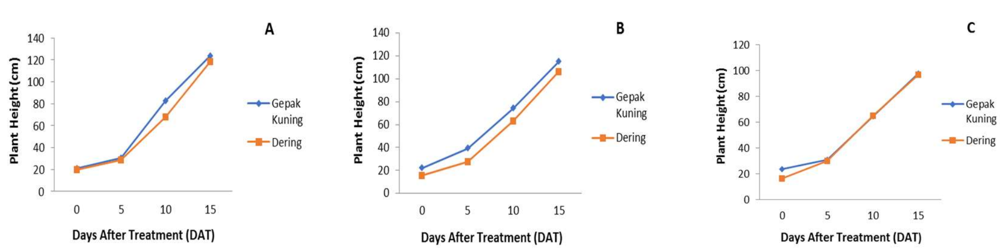

Figure 2. Soybean Plant Height Growth is Based on Varieties at the Treatment Level. A = 0 mM NaCl; B = 60mM NaCl; and C=120 mM NaCl

3.1.2. Number of Leaves

The variable number of leaves under control conditions (0 mM NaCl) to salinity stress (120 mM NaCl) showed a significant difference. The Gepak Kuning variety had the highest number of leaves compared to the Dering 1 variety. The Gepak Kuning variety started from 0 to 15 DAP under stress. Salinity (60 mM NaCl) increased the number of leaves, ranging from 1.67 to 3.89. Meanwhile, under salinity stress (120 mM NaCl), the number of leaves increased from 2.45 to 2.89. Not much different from the Dering 1 variety, where from 0 to 15 DAT under salinity stress (60 mM NaCl), the number of leaves increased from 1.00 to 2.89. Under salinity stress (120 mM NaCl), leaves increased from 2.55 to 2.67 (Figure 3).

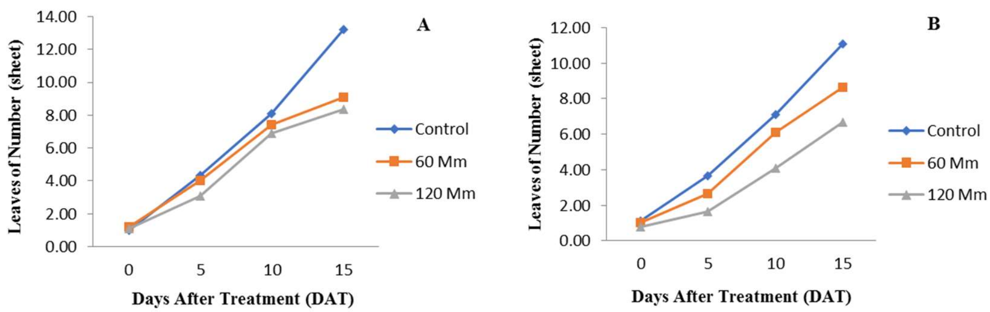

Figure 3. Soybean Number of Leaves is Based on the Level of NaCl Treatment on Varieties (A = Gepak Kuning; B = Dering 1)

Plant resistance to salinity is a complex trait involving many genes. Each gene contributes a small amount, and the effect is not known. Identifying genes that determine salinity tolerance is one of the requirements for assembling salinity-tolerant varieties [22].

The number of leaves of the Gepak Kuning variety under salinity stress (60 mM and 120 mM NaCl) 15 DAT decreased by 31% and 29%, respectively, when compared to control conditions (0 mM NaCl), while the Dering 1 variety under salinity stress (60 mM). and 120 mM NaCl) decreased by 30% and 22%, respectively, compared to the control conditions (0 mM NaCl) (Figure 4).

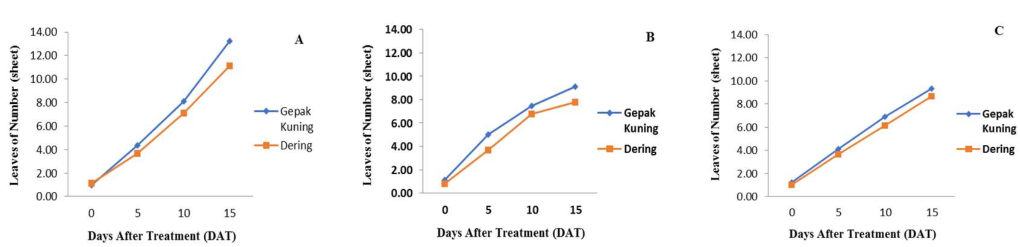

Figure 4. Soybean Number of Leaves Based on Varieties at Treatment Level. A = 0 mM NaCl; B = 60mM NaCl; and C=120 mM NaCl

At 15 days after treatment (DAT), the number of leaves in the Gepak Kuning variety decreased by 31% and 29% under salinity stress (60 mM and 120 mM NaCl, respectively) compared to control conditions (0 mM NaCl). Similarly, the Dering 1 variety showed a reduction in leaf number by 30% and 22% under 60 mM and 120 mM NaCl stress, respectively, when compared to the control (0 mM NaCl) (Figure 4).

This indicates that the number of leaves decreases as NaCl concentration increases. Additionally, leaves, which are the primary organs for photosynthesis, suffer chlorophyll damage when exposed to high NaCl concentrations for extended periods (Figure 5) [23]. This chlorophyll damage disrupts the photosynthesis process in soybean plants, leading to a significant reduction in leaf number [24]. This finding aligns with the research of Dolatabadian et al. [25], which showed that salinity stress causes morphological changes in soybean plants, including a notable reduction in plant height and leaf number at 100 mM NaCl concentration.

Figure 5. Condition of the leaves of the Gepak Kuning variety at a concentration of 120 mM NaCl at 10 DAT.

3.2. Analysis of Variance

Analysis of variance carries out on a few soybean growth variables such as plant height, crown fresh weight, shoot dry weight, primary root length, primary root length, fresh root weight, root dry weight, number of leaves, chlorophyll index, stomata density to salinity stress (Table 1). The analysis of variance revealed highly significant interactions between varieties and stress factors for plant height, shoot fresh weight, and shoot dry weight five days after treatment (DAT). Additionally, the results indicated a significant difference in the effect of varieties on plant height (0 and 5 DAT), shoot fresh weight (10 DAT), shoot dry weight, primary root length (0 DAT), number of leaves (5 and 10 DAT), and stomatal density (5 DAT). Moreover, stress had a significant impact on plant height (15 DAT), shoot fresh weight, shoot dry weight, and the number of leaves (10 and 15 DAT) (Table 1).

Table 1. Analysis of Variance

Variable	F Value			DC (%)
	Variety (V)	**Salinity (S)**	**Interaction (V x S)**	
Plant Height (PH)				
0 DAT	8.69^{*}	0.40^{ns}	1.30^{ns}	17.84
5 DAT	20.20^{**}	3.77^{ns}	9.68^{**}	7.45
10 DAT	4.38^{ns}	2.29^{ns}	1.37^{ns}	12.26
15 DAT	0.94^{ns}	6.65^{*}	0.19^{ns}	19.26
Number of Leaves (NL)				
0 DAT	4.00^{ns}	1.75^{ns}	3.25^{ns}	15.01
5 DAT	19.31^{**}	2.11^{ns}	2.08^{ns}	9.65
10 DAT	6.68^{*}	4.17^{*}	0.10^{ns}	9.42
15 DAT	3.35^{ns}	9.66^{**}	0.31^{ns}	16.04
Primary Root Length (PRL)				
0 DAT	6.28^{*}	0.27^{ns}	3.01^{ns}	14.41^{T}
5 DAT	2.70^{ns}	0.63^{ns}	3.03^{ns}	23.06
10 DAT	0.67^{ns}	2.78^{ns}	0.21^{ns}	14.86^{T}
15 DAT	1.91^{ns}	1.30^{ns}	1.27^{ns}	17.38^{T}
Lateral Root Length (LRL)				
0 DAT	0.34^{ns}	0.14^{ns}	1.85^{ns}	12.29^{T}
5 DAT	2.28^{ns}	0.11^{ns}	0.20^{ns}	22.28
10 DAT	0.71^{ns}	0.82^{ns}	0.70^{ns}	15.54^{T}
15 DAT	0.22^{ns}	0.51^{ns}	0.45^{ns}	12.86^{T}
Shoot Fresh Weight (SFW)				
0 DAT	1.08^{ns}	2.10^{ns}	1.43^{ns}	10.41
5 DAT	0.10^{ns}	0.40^{ns}	5.36^{*}	14.88
10 DAT	5.10^{*}	19.06^{**}	2.01^{ns}	11.91
15 DAT	1.20^{ns}	15.52^{**}	0.19^{ns}	20.07
Shoot Dry Weight (SDW)				
0 DAT	9.97^{**}	0.03^{ns}	1.43^{ns}	11.42
5 DAT	0.32^{ns}	1.00^{ns}	7.81^{**}	15.07
10 DAT	2.02^{ns}	9.85^{**}	1.37^{ns}	14.98
15 DAT	2.85^{ns}	12.51^{**}	0.38^{ns}	21.04
Root Fresh Weight (RFW)				
0 DAT	0.33^{ns}	0.53^{ns}	0.43^{ns}	23.52
5 DAT	0.08^{ns}	0.62^{ns}	0.93^{ns}	23.06
10 DAT	0.86^{ns}	0.40^{ns}	1.51^{ns}	17.16^{T}
15 DAT	1.35^{ns}	0.44^{ns}	0.54^{ns}	25.79
Root Dry Weight (RDW)				
0 DAT	0.60^{ns}	0.07^{ns}	0.60^{ns}	1.26^{T}
5 DAT	0.36^{ns}	3.62^{ns}	4.42^{ns}	19.23
10 DAT	$0,33^{ns}$	0.30^{ns}	1.73^{ns}	5.27^{T}
15 DAT	10.15^{**}	1.38^{ns}	0.18^{ns}	16.98
Chlorophyll Index (CI)				
0 DAT	0.05^{ns}	0.06^{ns}	0.35^{ns}	4.11
5 DAT	0.004^{ns}	0.41^{ns}	0.09^{ns}	10.02
10 DAT	2.52^{ns}	1.25^{ns}	0.38^{ns}	7.83
15 DAT	0.68^{ns}	0.32^{ns}	1.09^{ns}	12.61
Stomatal Density				
0 DAT	0.16^{ns}	$1,.42^{ns}$	1.49^{ns}	21.32
5 DAT	10.65^{**}	1.53^{ns}	0.09^{ns}	16.25
10 DAT	1.13^{ns}	0.62^{ns}	0.17^{ns}	16.47^{T}
15 DAT	3.55^{ns}	0.05^{ns}	1.87^{ns}	19.12

Noted: * = Significant effect based on F Table 0.05, ** = very significant effect based on F Table 0.01, ns = Not significant effect, T = Transformation.

3.3. Interaction between Soybean Varieties and Salinity Stress

The research results showed that there was an interaction between soybean variety and salinity stress on the variables of plant height (Table 2), shoot fresh weight (Table 3), and shoot dry weight (Table 4).

Table 2. Interaction Between Soybean Varieties and Salinity Stress on Plant Height

Concentration NaCl	Soybean Varieties	
	Gepak Kuning	Dering 1
0 mM	30.78 B b	28.48 B B
60 mM	39.01 A a	27.35 B B
120 mM	30.99 B b	30.22 B B

Noted: Numbers followed by lowercase letters are read horizontally and numbers followed by uppercase letters are read vertically

Under control conditions (0 mM NaCl), there was no significant difference in plant height between the Gepak Kuning and Dering 1 varieties, with 30.78 cm and 28.48 cm, respectively. Similarly, under salinity stress (120 mM NaCl), the plant heights were 30.99 cm for Gepak Kuning and 30.22 cm for Dering 1, showing no notable difference. However, there was a significant difference under moderate salinity stress (60 mM NaCl), with Gepak Kuning reaching 39.01 cm and Dering 1 only 27.35 cm. This indicates that the Gepak Kuning variety is more adaptable to salinity stress at 60 mM NaCl (Table 2). As noted by Hussein et al. [26], stress can impact plant growth, including height, which may be attributed to the effects of salt stress conditions.

Table 3. Interaction Between Soybean Varieties and Salinity Stress on Shoot Fresh Weight

Concertation NaCl	Soybean Varieties	
	Gepak Kuning	Dering 1
0 mM	5.14 B b	5.85 B b
60 mM	6.33 A a	4.72 B b
120 mM	4.52 B b	5.77 B b

Noted: Numbers followed by lowercase letters are read horizontally and numbers followed by uppercase letters are read vertically

It is important to note that, in addition to plant height, the shoot fresh weight variable showed significantly different results under salinity stress conditions (60 mM NaCl) between the Gepak Kuning and Dering 1 varieties, 6.33 g, and 4.72 g, respectively. However, under salinity stress conditions (0 mM and 120 mM), it was not significantly different between the Gepak Kuning variety (5.14 g and 4.52 g) and the Dering 1 variety (5.85 g and 5.77 g), respectively (Table 3). This reaffirms the resilience and adaptability of the Gepak Kuning variety to salinity stress (60 mM NaCl).

Table 4. Interaction Between Soybean Varieties and Salinity Stress on Shoot Dry Weight

Concentration NaCl	Soybean Varieties	
	Gepak Kuning	Dering 1
0 mM	0.79 B b	0.82 B b
60 mM	0.96 A a	0.64 B b
120 mM	0.62 B b	0.82 B b

Noted: Numbers followed by lowercase letters are read horizontally and numbers followed by uppercase letters are read vertically.

The following variable that showed significantly different results was the shoot dry weight under salinity stress conditions (60 mM NaCl) between the Gepak Kunig and Dering 1 varieties, namely 0.96 g and 0.64 g, respectively. However, salinity stress conditions (0 mM and 120 mM) showed no significant difference between the Gepak Kuning (0.79 g and 0.62 g) and Dering 1 (0.82 g and 0.82 g) varieties.

Each variety will show a specific response to salinity, but this response is influenced by variations in the characteristics of the variety [27]. So, resistant varieties will show a significant response when the salinity conditions on the land are high, and it is thought that they will show better results than susceptible varieties.

3.4. Stress Tolerance Index (STI)

Determination of the stress tolerance level can be done using the stress tolerance index. Stress index analysis determines the level of stress given to plants for each observed variable. The average stress sensitivity index results can be seen in Tables 5 and 6.

Table 5. The Average Stress Sensitivity Index (STI) Results 60 mM NaCl

Varieties	SFW	SDW	RFW	RDW	Average	Results
V1 (Gepak Kuning)	1,64	1,51	-0,30	0,54	0,85	M
V2 (Dering 1)	2,58	3,16	-6,56	-3,81	-1,16	T

Noted: SFW = Shoot Fresh Weight; SDW = Shoot Dry Weight; RFW = Root Fresh Weight; RDW = Root Dry Weight; S= Sensitive; M = Mediun Tolerance; and T= Tolerance

According to Savitri [28], the criteria for determining the level of tolerance to salinity stress are if the SI value < 0.5 is included in the tolerant genotype category, 0.5 < SI < 1.0 is included in the medium tolerant genotype category, and SI > 1.0 is included in the sensitive genotype category. In Tables 5 and 6, the average values of the two varieties tested are obtained. The variety that is tolerant to NaCl stress with concentrations of 60 mM and 120 mM is Dering 1, while Gepak Kuning is a variety that is medium tolerant at a concentration of 60 mM and sensitive at a concentration of 120 mM (Table 6). This is in line with research by Yunita et al. [29], which stated that under salinity conditions of 9 dS/m (120 mM), the growth and production of the Dering 1 soybean variety had better resistance than Demas 1 and Devon1. According to research by Muzaiyana et al. [30], each variety responded differently to the high saline treatment of ± 10 dS/m

(120 mM). This response shows that each variety has different defenses against saline conditions, a novel and intriguing finding that adds to our understanding of soybean varieties' responses to salinity stress.

Table 6. Average Stress Sensitivity Index (STI) Results 120 mM NaCl

Varieties	SFW	SDW	RFW	RDW	Average	Results
V1 (Gepak Kuning)	7,43	4,31	-1,76	0,02	2,50	P
V2 (Dering 1)	-8,71	-5,60	-0,87	-0,87	-3,29	T

Noted: SFW = Shoot Fresh Weight; SDW= Shoot Dry Weight; RFW= Root Fresh Weight; RDW= Root Dry Weight; S= Sensitive; M= Mediun Tolerance; and T= Tolerance

4. Conclusion

Our study has identified several soybean varieties that show the potential to resist salinity stress at an early vegetative stage. The findings are particularly significant as they shed light on the tolerance of Dering 1 soybean to salinity stress (60 mM and 120 mM NaCl). Conversely, the study also highlights the sensitivity of Gepak Kuning Varieties to salinity stress (120 mM NaCl) based on stress tolerance index (STI) conditions for root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), and shoot dry weight (SDW).

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