Akta Agrosia

The Growth Responses of Shallot (*Allium cepa* L.) Toward Different Levels of Salt Stress

Andi Kurniawan*[1], Deris Trian Rahmandhias[1] and Widi Dwi Noviandi[2]
[1]Department of Agronomy, Faculty of Agriculture, Brawijaya University, Indonesia
[2]Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Taiwan

Doi: 10.31186/aa.27.2.45-51

ARTICLE INFO

Keywords:
shallot,
abiotic stress,
salinity,
plant growth

Article history:
Received: Feb 5, 2024
Accepted: June 12, 2024

*Corresponding author:
E-mail:
kurniawan.andi@ub.ac.id

ABSTRACT

Shallot (*Allium cepa* L.) is an annual Liliaceae family plant and a major horticultural commodity globally. As a crucial horticultural product, market demand for shallots tends to increase. However, global climate change causes saline land areas to increase. Salt stress is one of the leading challenges limiting yield worldwide. This study aimed to understand the impact of salt stress on the growth of shallots and their stress tolerance limits. This research was conducted from August to October 2023 in Wonorejo Village, Poncokusumo District, Malang Regency. The plant material used in this study was the Tajuk variety of shallots. This study used a Randomized Block Design (RBD) with four salinity treatments (0, 100, 150, and 200 mM NaCl), each replicated three times. Variables observed in this study include plant length, leaf number hill^{-1}, tiller number hill^{-1}, and plant fresh weight hill^{-1} that were observed at 2, 4, 6, and 8 weeks after planting (WAP). Our results showed that plants treated with 100 mM NaCl decreased plant length, leaves number, tiller number, and plant fresh weight (shoots, roots, and bulbs) by 8%, 10%, 8%, and 12%, respectively. While at a concentration of 150 mM, the reduction was 14%, 30%, 18%, and 22%. Furthermore, the highest percentages of decrease of those growth variables, up to 27%, 42%, 31%, and 41%, respectively, were obtained at 200 mM treatment. These findings may help to understand the morphological and ecological aspects of salt stress in plants and how plants grow and develop under salinity conditions. This study also highlights the importance of further studies on the physiological of shallot on salt stress. This might result in innovative strategies and technology that crops' resilience to salinity, and ensure food security and the sustainability of farming in impacted areas

INTRODUCTION

The Shallot (*Allium cepa* L.) is an annual plant that belongs to the Liliaceae family and is a major horticultural commodity worldwide. Shallots are aromatic plants with high economic value frequently used as cooking ingredients (Bianchi, 2015) and medicinal purposes (Shahrajabian et al., 2020). Shallots are produced and consumed extensively in Indonesia, particularly in East Java, the center for shallot production. The population in East Java Province in 2022 increased to 41.15 million people

ISSN: 1410-3354 / e-ISSN:2615-7136

Cited this as: Kurniawan, A., D.T. Rahmandhias and W.D. Noviandi. 2024. The growth responses of shallot (*Allium cepa* L.) toward different levels of salt stress. Akta Agrosia 27(2):45–51 Doi: 10.31186/aa.27.2.45-51

Akta Agrosia, 2024, 27(2):45-51
https://ejournal.unib.ac.id/Agrosia

from the previous year, and shallot production in the same year reached 47398 tons (BPS, 2023). Expanding the population causes an increase in demand for shallots as the main horticulture product for cooking ingredients (Dasipah et al., 2023). Osmotic stress, ion toxicity, and nutritional imbalance are only a few of the negative consequences of salt stress on plant growth and yield that have been documented in earlier research (Yang and Guo, 2018; Syed et al., 2021). Nevertheless, there are limited comprehensive investigations on how these variables affect shallot production in Indonesia. According to Rahayu et al., (2019), salinity remains a major factor restricting plant growth in Indonesia's coastal regions. However, limited information has been discovered concerning the way shallots notably adapt to different salinity levels. Furthermore, there is a lack of research concerning the long-term impacts of salinity on shallot growth and development stages, although some studies have examined at the general effects of salinity on onions and allied species (Hanci and Cebeci, 2015; Solouki et al., 2023). Thus, increasing the evaluation of shallot morphological changes in salinity has become an important subject of focus.

Abiotic stress, such as salinity, is currently the most significant challenge because it harms agricultural sustainability. Furthermore, the recent changes in global climate have caused an increase in salt levels, affecting soil deterioration and reducing crop production (Mukhopadhyay et al., 2021). Samson et al., (2016) reported that salt stress causes an enormous effect on plant growth and development traits by reducing the plants' relative photosynthetic rate. Moreover, salinity can trigger various negative impacts on plants, such as osmotic stress, ionic stress, oxidative stress (Yang and Guo, 2018), and limitation of water and nutrient availability (Syed et al., 2021). They are elevating the salt concentration in plant growth media results in a rise in the natural concentrations of sodium (Na^+) and chloride (Cl^-), as they could prevent the uptake of K^+ (Munns et al., 2020). The reduction of plant growth includes a reduction of plant height, root and shoot fresh weight, and leaf dry weight (Wan et al., 2020). Salinity stress (125 mM) negatively affects normal onion growth, which ultimately reduces its bulb yield (Hanci and Cebeci, 2015). Similar result, onion plants treated with salinity in range from 25, 50, 75, to 100 mM significantly reduce the root and leaves growth cause in reducing the yield (Solouki et al., 2023). Moroever, doses of NaCl (100, 125, 150, 175 and 200 mM) also reduce plant phtosynthetic rate (Chaudhry et al., 2020) and 50,100,150, and 200 mM affect seed germination, plumule and radicle growth (Sudha and Riazunnisa, 2015).

Salt stress dramatically suppresses the germination and growth of shallot bulb as well as bulb root meristem cell formation (ÇAvuşoğlu and ÇAvuşoğlu, 2021). As the secondary effect, salt stress causes the overgeneration of reactive oxygen species (ROS) (Hossain and Dietz, 2016). Whereas the production of ROS is a regular occurrence and an essential feature of plant cell metabolism, excessive generation of ROS due to environmental stress is hazardous and highly reactive. It harms DNA, lipids, proteins, and carbohydrates (Hasanuzzaman et al., 2019). Nevertheless, little investigation has been done on how different salt stress levels affect shallot growth. Thus, this study aims to understand salt stress's impact on shallots' growth and tolerance limits.

MATERIALS AND METHOD

Plant materials and experimental design

This study was conducted from August to October 2023 in Wonorejo Village, Malang Regency, Indonesia. Shallot bulbs of the Tajuk variety were used. A Randomized Block Design (RBD) with four salinity treatments (0, 100, 150, and 200 mM NaCl) was implemented, each replicated three times with 10 plants per replication. The following formula is used to determine the concentration of NaCl to dissolve:

$$M = \frac{g}{Mr} \times \frac{1000}{v}$$

Where:

M : Moles
g : The mass of NaCl needed
Mr : Molecular weight of NaCl
v : Volume needed

Medium preparation and planting

Loose soil was filled into polybags to reach 80% capacity. Then, the polybag is placed on a plastic plate. The purpose of using this plastic plate is to collect the salt solution that probably leaches due to watering. Thus, the salt concentration in each medium will not change. Furthermore, NaCl was dissolved into the water according to the concentration used. Then, the solution was poured onto the planting medium until saturated (1 l polybag-1) before planting the bulb followed by measuring the ion concentration of media using EC-meter. In the next step, the shallot bulbs' tip (growing point) was cut ±1/4 part to accelerate shoot emergence. The bulbs were then planted in the media (1 bulb polybag^{-1}). To maintain plant growth and development, the regular fertilizer which contains nitrogen, phosphorous, and potassium was added to the media. The soil conditions used in this are shown in Table 1.

Plant growth analysis

Plant length, leaf number, tiller number, and fresh weight were measured at 2, 4, 6, and 8 weeks after planting (WAP). Plant length was measured from the base to the tip, while tiller and leaf numbers were counted. Fresh weights of shoots, roots, and bulbs were recorded.

Data analysis

Data were analyzed using an F test of 5%. When significant differences were obtained, a Fisher's Least Significant Difference (LSD) test of 5% was carried out to determine the differences among treatments.

RESULTS AND DISCUSSION

Salt stress diminishes plant height

Our results confirmed that different saline conditions harm shallot plants. An increase in saline concentration leads to a decrease in plant height. The results showed that the plant treated with S3 (200 mM) significantly decreased in plant height compared to S0 (control) at 2 WAP. The decrease in plant height in S3 was 34% compared to S0, while in S2 and S1 was 21.67% and 9.58%, respectively (Figure 1). Moreover, plants treated with S2 (150 mM) and S3 (200 mM) significantly reduced plant height by 11.68% and 28.69% compared to control plants (S0), respectively.

In contrast, plants treated with S1 showed no significantly different results. S2 (150 mM) and S3 (200 mM) treatments at 4 WAP also showed similar results. In this age, salinity significantly reduced in plant height up to 11.68% and 28.69% compared to the untreated plant. However, significant results were not found in the S1 treatment. Furthermore, the decrease in plant height in S1 (100 mM), S2 (150 mM), and S3 (200 mM) at 6 WAP was 4.09%, 9.54%, and 24.43% compared to untreated plants (S0), while the decrease in plant height at 8 WAP was 12.15%, 15.73% and 24.01% respectively. These also indicated that the highest salt stress concentration caused a higher plant height reduction than other treatments.

The study by Syamsiyah (2020a) exhibited that shallot plants exposed to saline conditions did not change the plant height. On the contrary, another study by Syamsiyah (2020b) on shallot and Kumar (2021) on water dropwort (*Oenanthe javanica*). They reported that increasing salt concentrations caused a decrease in plant height. Saline conditions cause a reduction in plant height through several mecha-

Table 1. Soil Analysis

pH*H_2O	pH*KCl	Against dry samples 105˚C							
		Water content*	Kjedahl	P-Bray*	Cation Exchange Rates (NH_4OAc pH 7.0)				
			N-Total*	P_2O_5	K*	Na*	Ca*	Mg*	CEC*
1 : 5		%		ppm	Cmol$^{(+)}$/kg				
6.0	4.5	5.00	0.14	202	0.27	1.10	3.85	0.03	12.67

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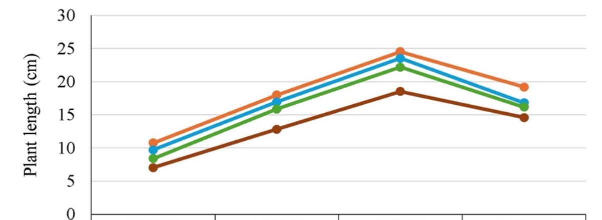
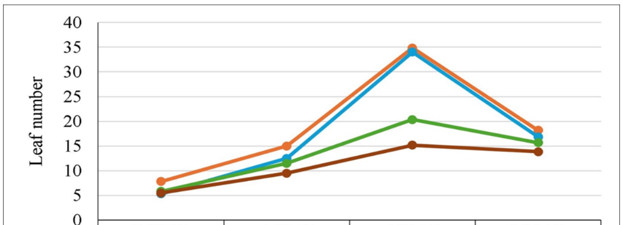


	2 WAP	4 WAP	6 WAP	8 WAP
S0	10.75 a	17.98 a	24.52 a	19.20 a
S1	9.72 ab	16.95 ab	23.52 a	16.80 ab
S2	8.42 ab	15.88 b	22.18 ab	16.18 b
S3	7.05 b	12.82 c	18.53 b	14.59 b

Figure 1. Effect of salinity on plant height at different growth ages. WAP: week after planting; S0, S1, S2, S3: level of salt stress treatments. The number followed by the same letter within a column is significantly different according to LSD at 5%.



	2 WAP	4 WAP	6 WAP	8 WAP
S0	7.83 a	15.00 a	34.83 a	18.17 a
S1	5.33 b	12.50 ab	34.00 a	16.83 ab
S2	5.83 b	11.50 bc	20.33 b	15.67 bc
S3	5.50 b	9.50 c	15.17 b	13.83 c

Figure 2. Effect of salinity on leaf number at different growth ages. WAP: week after planting; S0S1, S2, S3: level of salt stress treatments. The number followed by the same letter within a column is significantly different according to LSD at 5%.

nisms, such as excessive osmotic stress, nutrient deficiencies, and plant toxicity (Yang and Guo, 2018). Moreover, salinity reduces leaf area, chlorophyll content, and photosynthesis, decreasing plant growth (Puvanitha, 2017). Furthermore, salinity affects each phase of plant development, such as the germination phase, vegetative phase, and generative phase, where the absorption of water and nutrients from the soil is limited due to osmotic stress, toxicity, and oxidative stress in plants (Shrivastava, 2015).

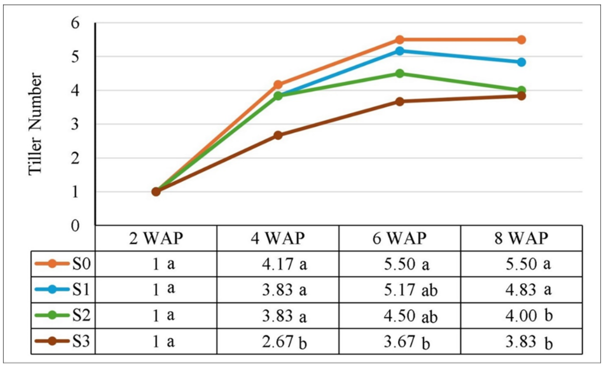
The effect of salt stress on leaf number

The statistical data showed that differences in saline levels significantly decreased leaf number compared to untreated plants (S0) at 2 WAP, 4 WAP, 6 WAP, and 8 WAP (Figure 2). The average leaf number hill-1 in plants treated with S1, S2, and S3 was significantly reduced compared to control plants (S0) at 2 WAP. The decreases were 31.93%, 25.54%, and 29.76%, respectively. Similar results were also observed at 4 WAP; the leaf number significantly decreased under S2 and S3 than in untreated plants (S0). Otherwise, plants treated with S1 showed no significant effect on the leaf number. Meanwhile, the decrease in S2 and S3 was 23.33% and 36.67%, respectively.

These results indicated that decreasing the leaf number was due to saline conditions. Moreover, the results demonstrated that plants treated with S2 (150 mM) and S3 (200 mM) also significantly reduced the leaf number at 6 WAP and 8 WAP. The decreasing percentage was 41.63% and 56.45% at 6 WAP and 13.76% and 23.89% at 8 WAP. Meanwhile, plants treated with S1 (100 mM) did not show a significant decrease in the leaf number. According to Budiono (2022), applying saline conditions causes an antagonistic effect on plants by reducing the bioavailability of soil nutrients, thereby reducing the number of plant leaves. Increasing the salt levels of entisol soil showed no significant reduction in the leaf number of shallot. Increasing salt concentrations dramatically reduces the leaf number due to osmotic stress. This condition indicates that the plant is exposed to high salt levels, accumulating ROS causing cell death, and the plant's growth rate decreases over time (Hossain and Dietz, 2016).

Salt stress reduced tiller number of shallots

Our results demonstrated that plants treated with S1, S2, and S3 did not significantly reduce tiller number at 2 WAP. Meanwhile, a significant reduction was obtained in plants treated with S3 (200 mM salt) compared to other treatments at 4 WAP, 6 WAP, and 8 WAP (Figure 3). At 4 WAP, the results showed that increasing salt levels in treatments S1, S2, and S3 caused a significant decrease in tillers compared to untreated plants (S0); these decreases were 8.15%, 8.15%, and 35.97%, respectively. The tiller number at 6 WAP also initially increased and then decreased at 8 WAP. Under saline conditions, the percentage of tiller numbers decreased by 18.18% and 33.27% in S2 and S3 (6 WAP). Otherwise, at 8 WAP, the decrease was 4.08% and 8.15% compared to the control plant (S0). The tiller



	2 WAP	4 WAP	6 WAP	8 WAP
S0	1 a	4.17 a	5.50 a	5.50 a
S1	1 a	3.83 a	5.17 ab	4.83 a
S2	1 a	3.83 a	4.50 ab	4.00 b
S3	1 a	2.67 b	3.67 b	3.83 b

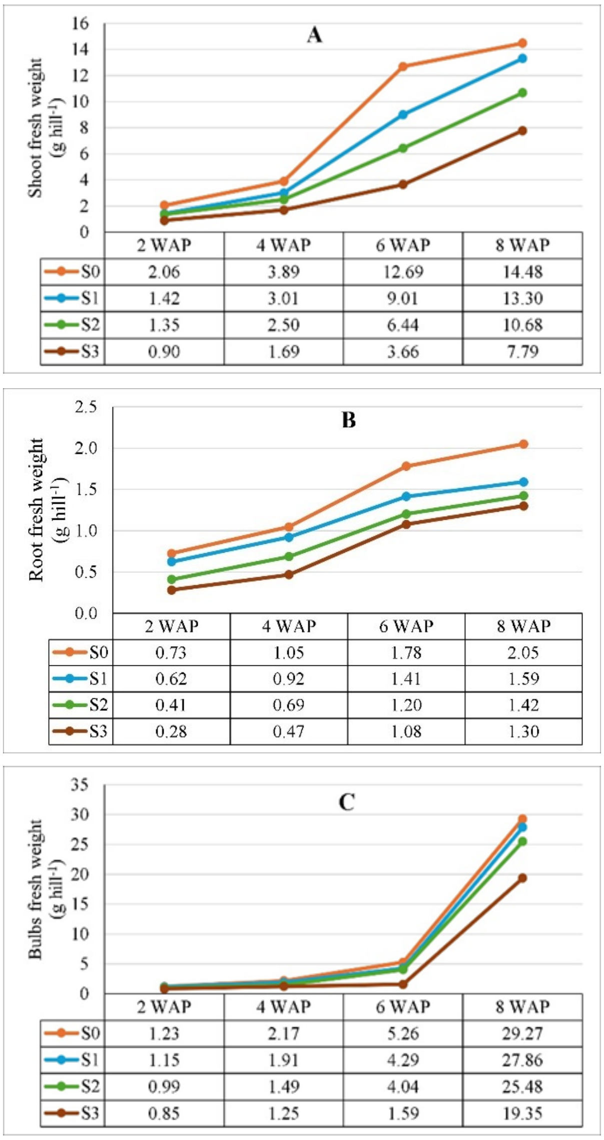
Figure 3. Effect of salinity on tiller number at different growth ages. WAP: week after planting; S0, S1, S2, S3: level of salt stress treatments. The number followed by the same letter within a column is significantly different according to LSD at 5%.

number of shallots is affected by several factors, such as the fertilizer, the quality of seeds, and the growth medium conditions (Wahyuni, 2020). According to Zhang (2023), tiller numbers in rice plants significantly decrease in response to saline conditions. Still, the study concluded that the effects of salt stress on changes in tiller number and the specific mechanism need to be further studied.

Reducing plant fresh weight caused by salt stress

Plant fresh weight data is obtained by weighing all plant organs, including shoots, roots, and bulbs. Our results showed that salt stress treatment significantly reduced shoot fresh weight (Figure 4). A decrease in shoot fresh weight was found in S1 (100 mM), with an average reduction of 22% at all ages of observation compared to the untreated plant. Increasing salinity levels harm changes in shoot fresh weight. This result was evidenced that in S2 (150 mM) treatment, the reduction of shoot fresh weight at 2, 4, 6, and 8 WAP were 34%, 35%, 49%, and 26%, respectively. The highest reduction in leaf fresh weight was obtained in the salt stress treatment at S3 (200 mM). The decrease of leaf fresh weight in S3 was observed at 2, 4, 6, and 8 WAP by 56%, 56%, 72%, and 46% respectively (Figure 4A).

Furthermore, salt stress treatments decreased root fresh weight (Figure 4B) and bulb fresh weight (Figure 4C). For these two variables, the highest reduction in fresh weight was also obtained from the S3 treatment. In root fresh weight, the average reduction was 48% at all ages of observation. Meanwhile, the average decrease in the bulb's fresh weight was 44%. Salt stress triggers ion toxicity, limitation of water availability, and nutrient absorption (Sheldon et al., 2017). These conditions cause a reduction in plant growth rates and biomass (Berhanu and Berhane, 2014). According to Swaefy et al., (2021), shoot fresh weight, root fresh weight, and overall plant growth of fenugreek plants were significantly decreased by salt stress treatment.



A)

	2 WAP	4 WAP	6 WAP	8 WAP
S0	2.06	3.89	12.69	14.48
S1	1.42	3.01	9.01	13.30
S2	1.35	2.50	6.44	10.68
S3	0.90	1.69	3.66	7.79

B)

	2 WAP	4 WAP	6 WAP	8 WAP
S0	0.73	1.05	1.78	2.05
S1	0.62	0.92	1.41	1.59
S2	0.41	0.69	1.20	1.42
S3	0.28	0.47	1.08	1.30

C)

	2 WAP	4 WAP	6 WAP	8 WAP
S0	1.23	2.17	5.26	29.27
S1	1.15	1.91	4.29	27.86
S2	0.99	1.49	4.04	25.48
S3	0.85	1.25	1.59	19.35

Figure 4. Effect of salinity on plant fresh weight at different growth ages. A) Shoot fresh weight, B) Root fresh weight, C) Bulbs fresh weight. WAP: week after planting; S0, S1, S2, S3: level of salt stress treatments. The number followed by the same letter within a column is significantly different according to LSD at 5%.

CONCLUSIONS

Shallot plants performed slower growth rates when subjected to salt-stressed conditions. Increasing the NaCl concentration up to

200 mM decrease the growth performance of shallot, including plant length, leaves number, tiller number, and plant fresh weight (shoots, roots, and bulbs) starting from 2 WAP. Our results suggest that shallot growth tolerances to salt stress may range from 0 to 100 mM. Further research regarding the physiological mechanisms of shallot plants adaptation to salt stress needs to be investigated. This might result in innovative strategies and technology that crops' resilience to salinity and ensure food security and the sustainability of farming in salinity-impacted areas.

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