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Growth Response of Aromatic Grasses to Soil Salinity Stress

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Abstract. This research aims to determine the growth response of vetiver, lemongrass and citronella plants in terms most resistant to soil salinity stress levels. The study was carried out at the screen house of the Faculty of Agriculture, Santo Thomas Catholic University, Medan, which is 32 meters above sea level. This research was carried out in January to March 2024. The study used a factorial, Completely Randomized Design, which consisted of two factors. The first factor is the influence of several aromatic grasses (R), which consist of 3 types: lemongrass, citronella, and vetiver. The second factor is the salinity soil stress (S) level, which consists of five levels: without saline, 25% saline soil, 50% saline soil, 75% saline soil saline, and 100% saline soil. Based on these treatment combinations, there were 15 combinations, and each treatment was repeated 3 times so that 45 plants/pots measured 5L. Parameter observations were carried out during harvesting; the parameters observed were shoot fresh weight, shoot dry weight, root fresh weight, root volume, and root cross-section anatomy. The results of the study can be stated that the factor of several aromatic grasses has a significant effect on shoot fresh weight, shoot dry weight, roots fresh weight, and roots volume, where the best aromatic grasses is vetiver even though it was not significantly different from citronella which is resistant to growth at salinity stress levels of 50% to 100%. The salinity level also has a significant effect where salinity starting from 25% causes a decrease in the fresh weight and dry weight of the shoot of the three aromatic grasses.

Keywords: citronella; lemongrass; salinity; stress; vetiver

INTRODUCTION

Plants are facing many challenges due to the severe consequences of global climate change. Climate change caused huge problems in the agriculture sector by creating various abiotic stresses on plants. Salinity and drought are the two main abiotic stresses that interfere with plant development and productivity (Angon *et al.*, 2023). One of the significant consequences of climate change is the sea-level rise that causes both salinity and waterlogging stress in coastal regions. In recent years, the frequency of salinity-waterlogging stress has increased considerably in low-lying coastal lands (Tahjib-Ul-Arif *et al.*, 2023).

Salinity has been increasing in the soil by means of various factors, including climate change, industrialisation and entry of seawater into the groundwater. The intrusion of saltwater into agricultural land is one of the biggest environmental threats worldwide. About 20% of agriculture and 33% of salinity-affected lands are reported (Saranya *et al.*, 2022).

Salt stress is an ecological constraint that influences plant growth and development. It is a ruinous danger to worldwide agriculture. Outdated irrigational practices and inappropriate utilization of manures have mainly contributed to excess salt in agricultural lands. Excess salt in the soil causes the accumulation of Na^+ and Cl^- ions, causing hyperosmotic and hyperionic conditions, which obstruct plant retention of water and supplements from the soil (Rasool *et al.*, 2022).

Salt stress is an abiotic condition representing one of the most critical challenges in 21st-century agriculture worldwide (Silva *et al.*, 2024). Indonesia, as an archipelagic country, has enormous potential for saline soil. In Indonesia, salinity generally occurs in agricultural land near the coast, which is caused by rising sea levels due to climate change. It is estimated that coastal land susceptible to salinity is 12,020 million ha or 6.20% of Indonesia's total land area. The area of saline land is now certainly increasing due to world

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climate change, namely rising temperatures and sea level rise (Kusmiyati *et al*., 2014).

The land is increasingly fertile, and agricultural land decreases yearly. That matter causes agricultural development to shift to marginal lands, such as saline land. Ground causes becoming saline is the intrusion of seawater; irrigation water contains salt or high evaporation with low rainfall, so the salts will up to the root area (Kusmiyati *et al*., 2014; Sarvade *et al*., 2017).

Salt tolerance refers to the ability of a plant to withstand a concentration of soluble salts in the soil solution without hampering its normal growth. This level, known as the threshold salinity of the soil, is crop-specific. Usually, the concentration of soluble salts (salinity) in the soil is highly dynamic and requires frequent monitoring; therefore, it is complex to relate to plant growth throughout the crop cycle. The salinity of root zone soil, measured as ECe, has been commonly accepted as a representative and comparable measure of spatio-temporal root zone salinity (Minhas *et al*., 2020).

Aromatic grass, in this case, vetiver, citronella and lemongrass, is a medicinal plant. These plants showcase secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, polyphenols, and glycosides. These compounds possess therapeutic properties and are recognized as effective remedies for various ailments. Furthermore, the evolution of modern pharmaceuticals draws heavily from the insights gained from medicinal plants. Given their vast potential, the commercial cultivation of medicinal plants has gained traction across diverse geographical terrains. With many plant species celebrated for their medicinal virtues, each species manifests unique growth preferences and reacts distinctly to environmental shifts. Therefore, understanding these plants' specific growth requirements and stress responses becomes essential to ensure their sustainable cultivation and continued availability (Nazari *et al*., 2023).

Plants under salinity stress must find ways to obtain soil water with higher negative water potential and cope with potentially dangerous amounts of Na^+ and Cl^-. Drought and very saline soil will cause plants to become dehydrated. Aromatic grasses are plants that can withstand saline stress. Even though growth is not good, vetiver plants can tolerate moderate salt. According to research Novita *et al*., (2019), vetiver plants can survive saline stress conditions of up to 8 $dS.m^{-1}$, although their ability to grow during the vegetative phase is hampered.

Soil salinity, a major abiotic stress worldwide, reduces crop yield by lowering plant growth and development through osmotic, ionic, and nutritional imbalances. Excessive salt ions exposure, such as sodium (Na^+), magnesium (Mg^{2+}), potassium (K^+), chloride ($Cl^−$), carbonate ($CO3^{−2}$), and calcium (Ca^{2+}), will cause a negative impact on plant growth and development (Thakur *et al*., 2022; Alabdallah *et al*., 2024).

Salt stress, a phenomenon where soil salinity surpasses the tolerable threshold of plants, poses a significant threat to agricultural production. This stress profoundly impacts the physical attributes of soil. High salinity diminishes the porosity of the soil, effectively obstructing the flow of air and water, thereby hampering plant roots' respiratory functions and nutrient absorption capabilities. Plants struggle to maintain osmotic balance within saline environments, leading to water loss. This dehydration process causes cellular shrinkage and, in extreme cases, cell death. Furthermore, salt stress suppresses plant photosynthesis. Excessive salt concentrations reduce photosynthetic pigments, such as chlorophyll, ultimately limiting photosynthetic activity and compromising crop growth and yield (Yan *et al*., 2024).

In nature, plants are constantly exposed to various environmental stresses because of their immobile life. These adverse abiotic ecological conditions, such as drought, salt and extreme temperatures, negatively

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influence crop growth and inhibit agricultural productivity worldwide. In arid and semi-arid regions, evaporation and water uptake by plant roots result in higher salt concentrations in the soil, increasing the salinity level in the upper soil layers. Therefore, drought and salt stresses typically co-occur. Many morphological, physiological, and biochemical responses are common when plants are exposed to the two stresses; in some cases, their tolerance mechanisms overlap. However, the responses of plants to combined stress are unique and differ from those observed under individual stresses (Liang *et al*., 2024).

From several studies, many agricultural plants are tolerant to salt stress, but research on aromatic plants, especially aromatic grasses, is still very lacking. Aromatic grasses are chosen for study because aromatic grasses have unique characteristics that make them useful: they are annual plants with a long harvest period, fast growth rate, high biomass production capacity, easy and fast propagation, non-wasteful root systems, easy to harvest, tolerant to local stress conditions (pH changes, heavy metal poisoning, drought, and temperature), Aromatic plants are generally not tasty to eat and are tolerant to various stress conditions (Pandey *et al*., 2019).

METHODS

The research was carried out at the Screen House, Faculty of Agriculture, Santo Thomas Catholic University, Medan, North Sumatra, 32 meters above sea level. This research was conducted from January to March 2024 with a temperature range of 25 to 32 °C and humidity between 65 and 80%. The materials used in this research were vetiver (*Vetiveria zizanioides*), citronella (*Cymbopogon nardus*), lemongrass (*Cymbopogon citratus*), saline soil from Cermin coast, topsoil, 40 cm x 40 cm polybags, and sample paper. The tools used in this research are hoes, sacks, analytical scales, rulers, notebooks, cutter knives, ovens, microscope cameras, measuring cups and others that support research.

The research used a Randomized Block Design consisting of 2 treatment factors and three replications. The first treatment factor is the type of aromatic grass, which consists of 3 types, namely citronella (R_1), lemongrass (R_2), and vetiver (R_3). The second treatment is the comparison of soil salinity to topsoil which consists of 5 levels, namely S_0= saline soil: topsoil = 0%: 100%; S_1= saline soil: topsoil = 25%: 75%; S_2= saline soil: topsoil = 50%: 50%; S_3= saline soil: topsoil = 75%: 25%; S_4= saline soil: topsoil = 100% : 0%.

The research data were analyzed using the Analysis of Variance (ANOVA) method at 5% level. If a treatment factor has a significant effect, then a further Duncan test (DMRT) at 5% level is carried out.

Next, the coastal soil and topsoil are filtered to clean the soil of the garbage involved. Then, the saline soil and topsoil are mixed with a composition that has determined the level of salinity homogeneously and put into a polybag container. The soil put in the polybag to be planted is first measured using a pH meter.

Preparation of planting material is carried out in the following way. Citronella plants (R_1) and lemongrass (R_2) are used from cuttings taken from parents that are 6 months old, the leaves are cut to about 3-5 cm from the leaf midrib, as well as the roots, reduced by cutting to leave about 2.5 cm below the root collar. Likewise, vetiver grass (R_3) is taken from broodstock that is 6 months old, carried out by cutting them to a size of 20 cm and a root length of 8 cm. At the end of the research, observations were made, including shoot fresh weight, root fresh weight, shoot dry weight, root volume, and root cross-section anatomy.

RESULTS AND DISCUSSION

Shoot Fresh weight

Based on the analysis of variance results, it is known that the salinity soil stress level factor significantly influences

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shoot fresh weight. Some aromatic grasses did not have a significant effect on shoot fresh weight, so the interaction between the two treatment factors had no significant effect on shoot fresh weight. The impact of salinity soil stress levels on some aromatic grasses on the fresh weight of the shoots can be seen in Table 1.

Table 1. Effect of soil salinity stress on several aromatic grasses on plant shoot fresh weight (g)

Salinity Level	Shoot fresh weight (g)			Average
	Citronella	Lemongrass	Vetiver	
0%	95.23	92.72	90.82	92.93 e
25%	63.12	64.91	66.40	64.82 d
50%	58.14	64.77	54.07	58.99 cd
75%	49.59	54.13	48.78	50.83 bc
100%	46.16	58.54	45.54	50.08 a
Average	62.45	67.01	61.13	63.53

P value = 0.135

Note: Numbers followed by the same letter in the same column mean no difference in the 5% level DMRT test

Table 1 shows that 100% salinity greatly suppressed shoot fresh weight (50.08 g), significantly different from other treatments, including the control. While the weight of 75% salinity was significantly different from the control and 25% salinity, it was not significantly different from the 50% salinity treatment. Meanwhile, the weight of 25% salinity was significantly different from the control but not significantly different from 50% salinity. The effect of soil salinity stress on shoot fresh weight can be seen in Figure 1.

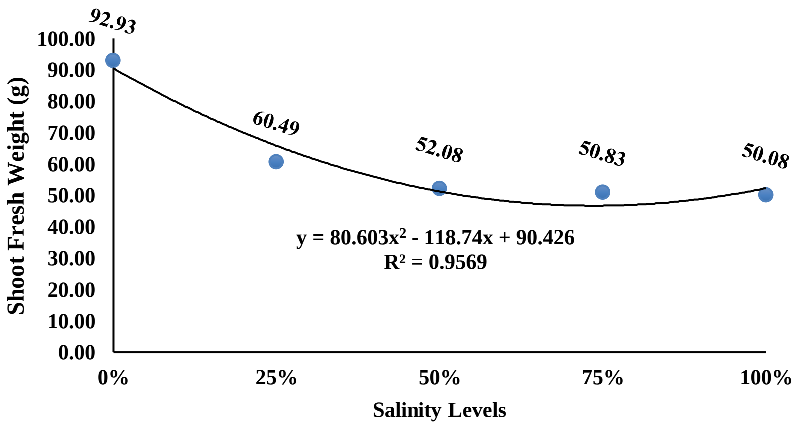

Figure 1. The curve of the influence of salinity level on the fresh weight of aromatic plant shoots.

The level of soil salinity stress significantly affects the fresh weight of the shoot of some aromatic grasses; plants that are stressed by salinity can inhibit the process of absorbing nutrients. This is because the absorption of excess Na elements causes a decrease in the absorption of water and potassium. Inhibited water

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absorption will disrupt the process of photosynthesis, namely closing the stomata so that the supply of CO_2 to the chloroplasts will decrease. This indicates that salinity stress hurts the fresh weight of the shoot, resulting in obstruction of the nutrient absorption process. According to Suryaman *et al*., (2021) salinity stress causes detrimental negative impacts on the growth process, including the generative phase. Saline stress causes osmotic stress, disrupts nutrient balance and ion poisoning, increases the production of reactive oxygen species (ROS) and reduces photosynthesis.

Shoot Dry Weight

Based on the results of the variance analysis, it is known that the level of soil salinity stress factor has a significant effect on the dry weight of the shoot. The type of aromatic grass did not significantly affect shoot dry weight, while the interaction between the two treatment factors had no significant effect on shoot dry weight. The influence of salinity soil stress levels and aromatic grass types on shoot fresh weight can be seen in Table 2.

In Table 2, it can be explained that in treating salinity stress levels on shoot dry weight, 100% salinity greatly suppressed shoot dry weight (19.73 g), which was significantly different from other treatments, including the control. While the weight of 75% salinity was significantly different from the control and 25% salinity, but it was not significantly different from the 50% salinity treatment. Meanwhile, the weight of 25% salinity was not significantly different from the control and from 50% salinity. The effect of soil salinity stress on shoot dry weight can be seen in Figure 2.

Table 2.. Effect of salinity soil stress level on some aromatic grasses on shoot dry weight (g)

Salinity Level	Shoot dry weight (g)			Average
	Citronella	Lemongrass	Vetiver	
0%	37.07	33.19	28.37	32.88 e
25%	27.89	28.61	23.65	26.72 de
50%	26.36	28.14	22.38	25.63 cd
75%	20.92	23.81	21.68	22.14 bc
100%	19.98	19.98	19.22	19.73 a
Average	26.44	26.75	23.06	25.42
P value = 0.159				

Note: Numbers followed by the same letter in the same column mean no difference in the 5% level DMRT test

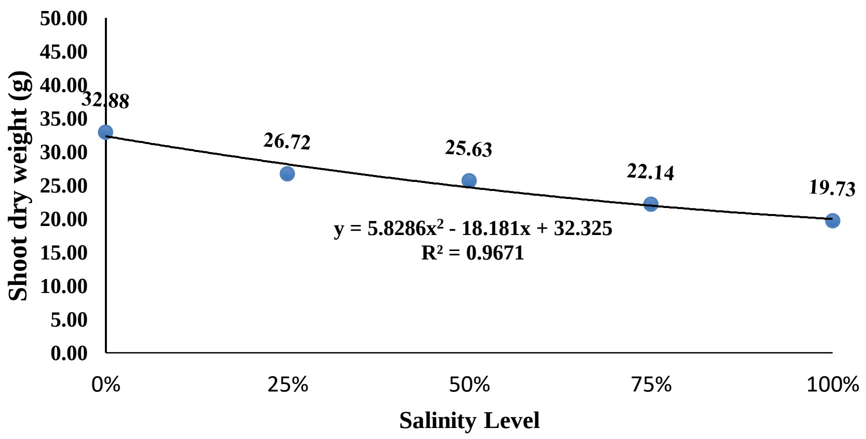

Figure 2. Influence curve of salinity stress level on shoot dry weight

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Figure 2 shows that the increasing level of salinity causes the dry weight of the canopy to decrease (in line with the wet weight of the canopy). According to research results of Liang et al. (2024), salinity causes a decrease in leaf area and dry weight of tomato plants. In agreement with earlier studies of Xue *et al*., (2021), both individual (salt stress) or combined stresses decreased leaf water potential (Ψl) and root water potential (Ψr), but the combined stress had more significant effects than that of individual stress. Increasing salt concentrations in the soil will cause plants to experience osmotic stress, nutrient imbalance, ion toxicity and oxidative stress, apart from that, it will reduce the plant's ability to absorb water and reduce the ability to photosynthesis, thereby affecting metabolic processes (Kristianto *et al*., 2013).

Root Fresh Weight

Based on the results of the variance analysis, it can be seen that several aromatic plants have a very significant effect on the fresh weight of aromatic grassroots. The treatment of salinity soil stress levels and the interaction between the two factors did not significantly affect the fresh weight of roots. The effect of several aromatic grasses and different levels of salinity soil stress on the fresh weight of roots can be seen in Table 3.

Table 3. The effect of several aromatic grass factors on root fresh weight (g) at different levels of salinity soil stress.

Salinity Level	Root fresh weight (g)			Average
	Citronella	Lemongrass	Vetiver	
0%	11.50	5.87	11.03	9.49
25%	6.65	5.45	10.21	7.44
50%	11.08	5.60	8.57	8.42
75%	8.30	6.01	11.02	8.44
100%	8.31	5.13	17.06	10.17
Average	9.18 bc	5.61 a	11.58 c	8.79
P value = 0.003				

Note: Numbers followed by the same letter in the same column mean no difference in the 5% level DMRT test

Table 3 shows that in the treatment of aromatic grass species, the largest fresh root weight was obtained in the vetiver grass (11.58 g), which was significantly different from lemongrass (5.61 g) but not significantly different from citronella grass (9.18 g). This means vetiver grass and citronella grass are plants resistant to saline soil. The effect of several different aromatic grasses on the fresh weight of roots can be seen in Figure 3.

The three aromatic grass species tested (Figure 3 and Table 3) above show that vetiver grass has resistance to salinity, although the citronella is not significantly different. This is following the results of research conducted by Novita et al. (2019); vetiver grass is able to survive soil stress conditions of salinity 8 dsm-2, but in the vegetative phase, vetiver roots become hampered. This causes the wet root weight of aromatic lemongrass grass to be hampered under salinity conditions, but it does not rule out the possibility that plant root formation will continue to increase, but not under normal conditions. From the results of research conducted by Edelstein *et al*., (2009) on vetiver with salinity treatment, it was concluded that the vetiver root system excludes Na absorption under high Na concentrations in the irrigation water.

In addition to activating specific physiological and molecular responses to stress, plants undergo adjustments in multiple metabolic pathways to alleviate the impact of stress on their growth and development. These adaptations enable

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them to cope with the increased energy requirements imposed by changing environmental constraints. Among the metabolites produced by plants in response to different developmental or environmental stimuli, primary metabolites, such as carbohydrates, amino acids, polyols or polyamines, are known to be directly required for plant growth, whereas specialized metabolites, such as phenolics, terpenes, and nitrogen-containing compounds are involved in regulating plant–environment interaction (Segarra-Medina *et al*., 2023).

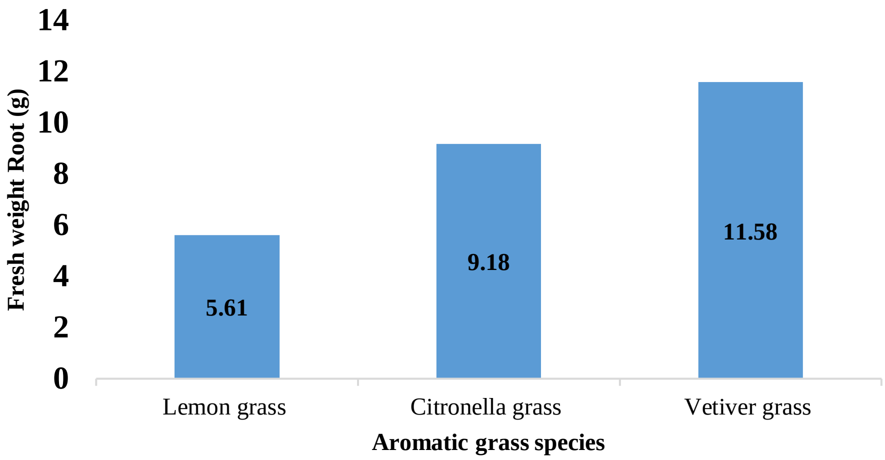

Figure 3. Histogram of the influence of aromatic grass species on root fresh weight.

Table 4. The effect of several aromatic grass factors on root volume (ml) at different levels of salinity soil stress.

Salinity Level	Root volume (ml)			Average
	Citronella	Lemongrass	Vetiver	
0%	10.67	4.00	11.33	8.67
25%	7.00	6.00	9.67	7.56
50%	11.33	5.00	8.67	8.33
75%	7.67	5.00	11.67	8.11
100%	7.33	5.33	11.00	7.89
Average	8.80 bc	5.07 a	10.47 c	8.11
P value = 0.002				

Note: Numbers followed by the same letter in the same column mean no difference in the 5% level DMRT test

Root Volume

Based on the results of the variance analysis, it is known that the influence of several aromatic grass factors has a very significant effect on root volume. While different levels of salinity stress and the interaction between the two treatments have no significant effect on root volume. The impact of several aromatic plants on root volume (ml) at different levels of saline soil stress can be seen in Table 4.

Following the fresh weight of the roots of the three aromatic grass species studied, the root volume showed the same (Table 4), from the table above, it can be seen that the salinity level causes a decrease in the root volume of the three aromatic grasses, but it is not statistically significantly different. The root volume of the highest vetiver plant (10.47 ml) was significantly different from lemongrass (5.07 ml) but not significantly different from citronella (8.80 ml). This

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means that vetiver and citronella are plants that are resistant to salinity. The influence of several aromatic grasses and different levels of soil salinity stress on root volume can be seen in Figure 4.

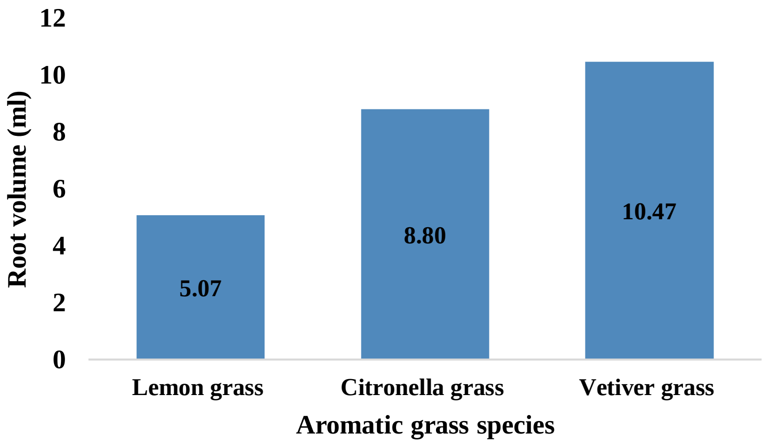

Figure 4. Histogram of the influence of species aromatic grasses on root volume

Figure 4 shows that vetiver (10.47 ml) is an aromatic grass that is tolerant of salinity stress levels in terms of the highest root volume, but it is not significantly different from citronella (8.80 ml). High root volume is due to increased root length and increased root branch growth. Root elongation functions to find water deeper into the soil. Vetiver grass has short rhizomes and a massive, finely structured root system that grows very quickly. It has been reported to grow to a depth of as much as 4 m in the first year of growth. This deep root system makes the vetiver plant extremely drought tolerant and very difficult to dislodge when exposed to a strong water flow (Truong, 2023). Edelstein *et al*., (2009) research results show that the vetiver root system excludes Na absorption under high Na concentrations in the irrigation water. The same thing was expressed by Othman *et al*., (2023), that the overall ability of citrus rootstocks to tolerate salt stress depends on their capacity to exclude or sequester excess toxic ions.

Cross-section anatomy of root

When observing cross-sections using a microscope with 10x magnification, cut to a size of 0.5 mm and placed in an object glass that had been dripped with water, the plant roots observed included vetiver, citronella and lemongrass. Salinity has an effect on the roots of several aromatic grasses, which shows differences in physiology. The impact of salinity levels on the roots of several aromatic grasses can be seen in Figures 5, 6, and 7.

Figure 5 shows the anatomy of a cross-section of citronella roots affected by saline soil, the epidermis, stele and cortex are still normal (A), the epidermis begins to show thickening, the stele starts to decrease in diameter, and the cortex begins to experience plasmolysis (cell wrinkling) due to saline soil (B-E), epidermis

This strongly suggests that this thickening of the walls is an attempt by the cells to protect themselves from high osmotic stress caused by saline soil, the middle part of the root stele which contains xylem and The phloem experiences a decrease in diameter following salinity and the cortex which is located under the epidermis experiences damage/wrinkling due to high salinity (D-E). (Ep Epidermis, St Stele, Kt Cortex).

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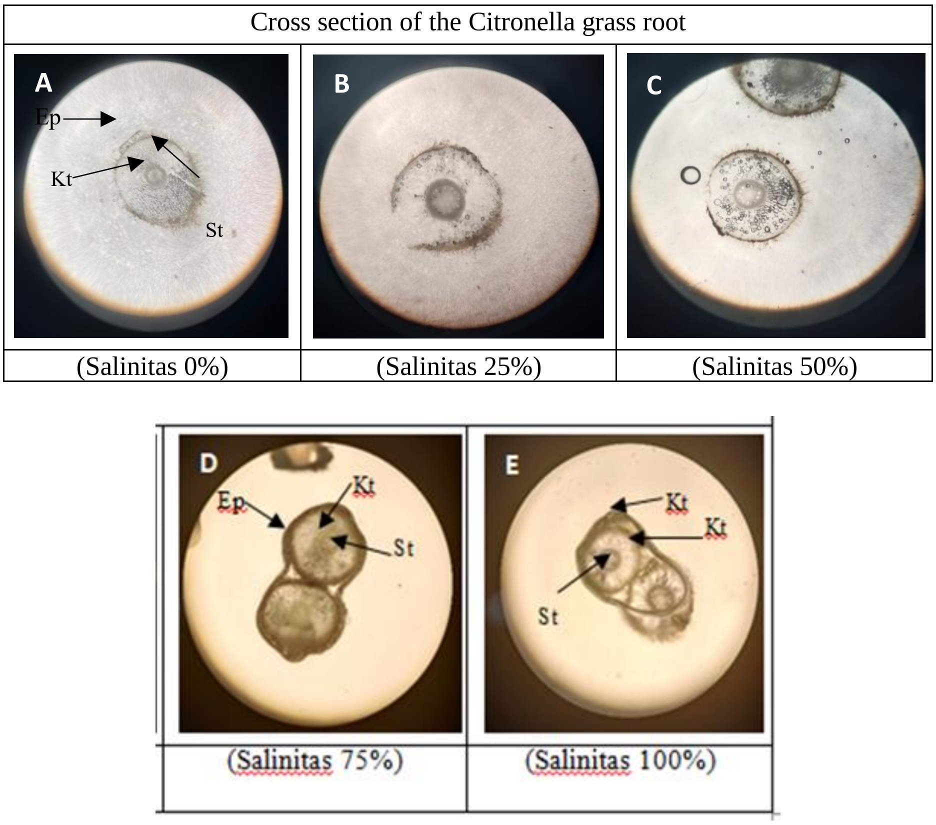

Figure 5. Cross section of citronella roots

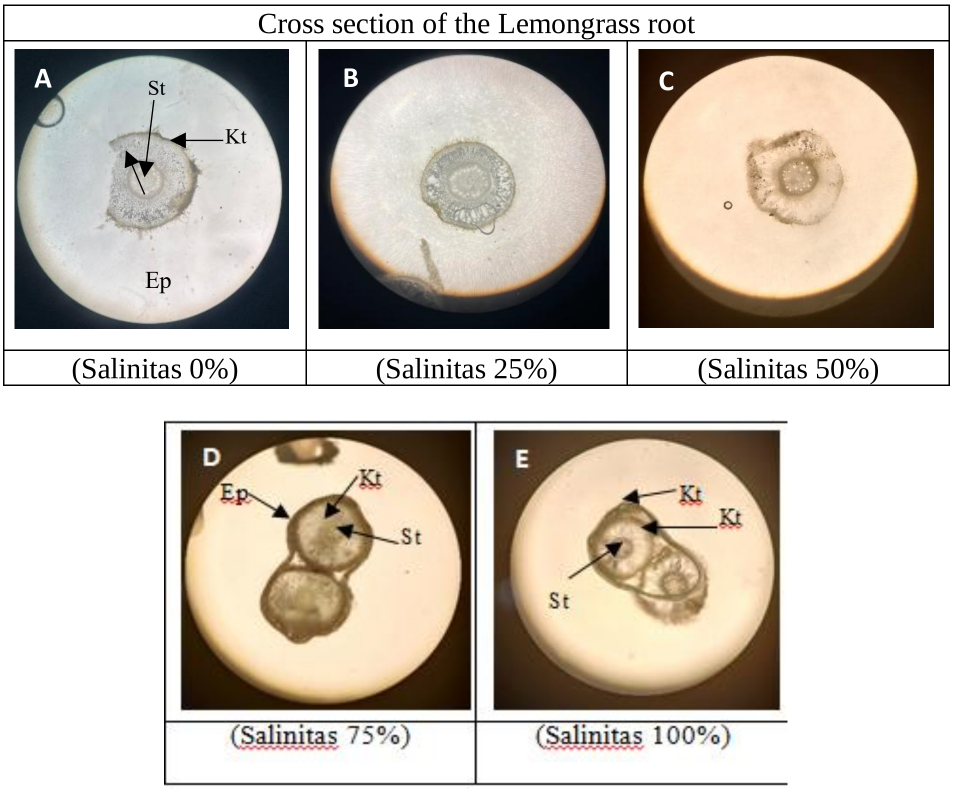

Figure 6. Cross section of Lemongrass roots

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Next in Figure 6 shows the anatomy of a cross-section of kitchen lemongrass roots affected by saline soil. The epidermis, stele and cortex are still normal (A-B), the epidermis begins to show thickening, the stele starts to decrease in diameter and the cortex begins to experience plasmolysis (cell wrinkling) due to saline soil (C-E), epidermis This strongly suggests that this thickening of the walls is an attempt by the cells to protect themselves from high osmotic stress caused by saline soil, the middle part of the root stele which contains xylem and The phloem experienced a decrease in diameter following salinity and the cortex located below the epidermis experienced severe damage/wrinkling due to high salinity (D-E). (Ep Epidermis, St Stele, Kt Cortex).

Figure 6 shows the anatomy of a cross-section of lemongrass roots affected by saline soil, the epidermis, stele and cortex are still normal (A-B), the epidermis begins to show thickening, the stele starts to decrease in diameter, and the cortex begins to experience plasmolysis (cell wrinkling) due to saline soil (C-E), epidermis This strongly suggests that this thickening of the walls is an attempt by the cells to protect themselves from high osmotic stress caused by saline soil, the middle part of the root stele which contains xylem and The phloem experienced a decrease in diameter following salinity and the cortex located below the epidermis experienced severe damage/wrinkling due to high salinity (D-E). (Ep Epidermis, St Stele, Kt Cortex).

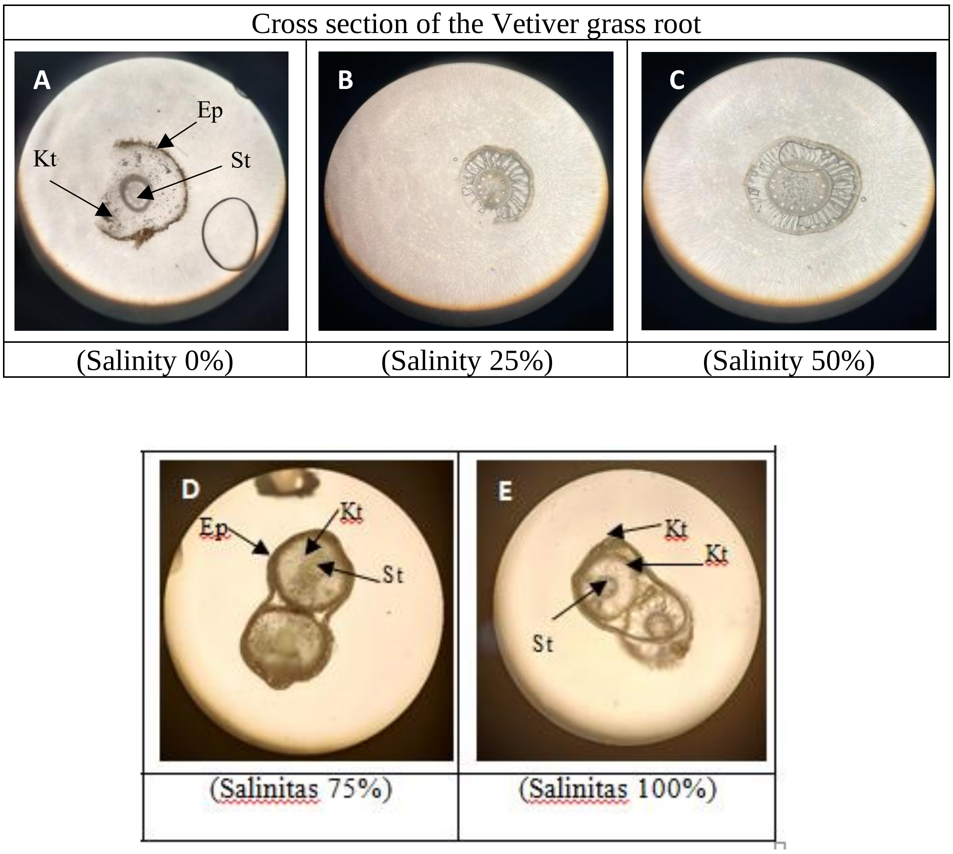

Figure 7. Cross section of Vetiver roots

Figure 7, shows the anatomy of a cross-section of citronella roots affected by saline soil; the epidermis, stele and cortex are still normal (A-C); the epidermis shows the

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thickening of the walls. This thickening is an effort by the cells to protect themselves from the high osmotic stress caused by saline soil. The stele in the middle part of the root, which contains the xylem and phloem, experienced a decrease in diameter following salinity and the cortex located below the epidermis experienced damage/wrinkling due to high salinity (D-E). (Ep Epidermis, St Stele, Kt Cortex).

The picture shows the anatomical differences in different parts of the root. The epidermis, the outermost part of the root, shows the thickening of the root wall. This thickening is an effort by the cells to protect themselves from high osmotic stress due to stress levels in soil salinity. The cortex, which is located under the epidermis, experiences plasmolysis (wrinkling cells) due to saline soil at a salinity level of 75%, the cortex has not shown wrinkles, while at 100%, the cortex on the roots shows wrinkles, and in the stele, the middle part of the root which contains xylem and phloem decreases in diameter. The salinity stress environment has a strong influence on physiological function, especially on the stele, which contains xylem and phloem. Plant survival is very dependent on the ability of the xylem to maintain water sunlight to the plant canopy. The work of the xylem and phloem is fundamentally interconnected because these two channels intersect at the stomata. The most apparent influence between the xylem and phloem is during the hydraulic process and photosynthetic assimilation when exchanging water and CO_2 on the leaf surface (Achmad et al., 2018; Kim et al., 2014). Dannoura et al. (2018) stated that stress on plant roots, which causes reduced water supply, will cause changes in the phloem tissue by shrinking and thinning the phloem diameter. In addition, Loudari et al. (2022) stated that wheat plants experienced irregularities observed in the root cross-section under salt stress in both fertilized and unfertilized plants, indicating disturbances in the production of components, especially secondary wall components, which cause modifications in their mechanical characteristics and thus make them sensitive to negative pressure.

CONCLUSION

Salinity stress beginning at 25% negatively impacted both the fresh and dry weight of shoots across all three aromatic grasses under study. Among them, vetiver exhibited superior root growth, particularly in fresh root weight and volume. However, its performance was not significantly different from citronella, which demonstrated resilience even at salinity levels ranging from 50% to 100%. Anatomical observations revealed a typical response among all three species, thickening of the outer epidermal walls of the roots. At the same time, cortical cells underwent plasmolysis (cell shrinkage) as salinity intensified from 75% to 100%. Further research must be done on vetiver and citronella to determine their resistance to higher salt levels.

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