



VETIVER (*VETIVERIA ZIZANIOIDES* L.) ECOTYPES ASSESSMENT FOR SALINITY TOLERANCE

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SUMMARY

Vetiver is one of the essential oil-producing plants, commonly called vetiver oil. With a deep, broad, and thick root system, the species is characteristically well-adapted to various environmental stresses, including salinity. The presented study strived to evaluate several vetiver ecotypes under diverse salinity stress conditions and identify the best with enhanced salinity tolerance. The said study continued in a completely randomized design (CRD) with factorial arrangement and two factors. The first factor was salinity stress comprising six varying levels, i.e., control (without salinity) and saline soils with 4, 8, 12, 16, and 20 dsm^{-1} . The second factor consisted of three vetiver ecotypes: Bogor, Bojonegoro, and Padang. The results revealed that salinity stress levels, ecotypes, and their interactions significantly affected the growth, physiological, and oil yield traits, such as plant height, leaf area, number of tillers, chlorophyll a and b, root length and volume, and oil yield. Salinity stress at 16 dsm^{-1} significantly impacted plant growth but enhanced chlorophyll a and b content. The ecotype Bojonegoro had better canopy growth, while the ecotype Padang had better root growth, resulting in higher oil production compared with the ecotype Bojonegoro. The ecotype Bojonegoro with 16 dsm^{-1} salinity stress significantly increased chlorophyll a and b content, and the ecotype Padang showed the highest oil production without salinity stress compared with salinity stress conditions.

Keywords: Vetiver (*Vetiveria zizanioides* L.), ecotypes, salinity levels, genotype by environment interactions, growth, physiological traits, oil production

Key findings: Vetiver (*Vetiveria zizanioides* L.) ecotypes screening through different salinity levels are very useful for getting tolerant genotypes with enhanced growth and oil traits.

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INTRODUCTION

Vetiver (*Vetiveria zizanioides* L.) grass is a densely tufted bunch sod that spreads quickly in both tropics and temperate regions worldwide. Vetiver is a perennial grass of the family Poaceae, native to India and widely cultivated in the world's tropical and subtropical zones (Ghotbizadeh and Sepaskhah, 2015). The species is also distinguishable with its strong and extensive root system that can reach a depth of 5 m in tropical conditions. A broad, thick, and deep root system with tensile strength equivalent to 1/6 of mild steel gives plants a superior advantage to adapt to various environmental stresses like drought, flooding, temperature extremes, heavy metals, acidity and alkalinity, frost, heat, extreme soil pH, the toxicity of Al and Mn, and also salinity and various important metals, such as, Cd, Cu, Cr, and Ni (Truong and Hart, 2001; Truong *et al.*, 2002; Zhou and Yu, 2009). Vetiver has widely served to therapy saline soils (Datta *et al.*, 2011; Donjadee and Tingsanchali, 2012).

Vetiver plant grass and roots contain essential oils that have extensive use for perfumes, cosmetics, deodorants, lotions, soaps, medicines, insecticides and repellents, and aromatherapy applications (Rao *et al.*, 2015). Vetiver oil has a soft and subtle aroma due to the ester of vetinenic acid and vetivenol compounds. For essential oils, the world's prerequisite is increasing every year because of the significant development in modern industries of perfumes, cosmetics, foods, aromatherapy, and pharmaceuticals (Luu, 2007). The many benefits of essential oils have led to a high demand in domestic and foreign scenes, but Indonesia produces limited and middle-quality vetiver oils.

One of the efforts to increase production can be expanding the planting area in optimal and suboptimal lands, such as saline lands (Siregar *et al.*, 2021; Sakinah *et al.*, 2022; Smirnova *et al.*, 2023). The obstacle is that repeated drying and wetting cycles will enhance the soil salinity, leading to expectations of saline tolerance in the vetiver ecotypes. However, a detailed study on vetiver

has been inexistent on its physiological response under diverse environmental stresses. Understanding the vetiver stress physiology not only helps to describe its biological characteristics but also explores its potential for utilization in land management (Liu *et al.*, 2016).

Vetiver has a salinity threshold of 8.0 dsm^{-1} and, above the threshold for decreased growth, is 5.26% per unit dsm^{-1} . The vetiver wild ecotypes have responded to salinity at the same scale, and their relative growth rate (RGR) decreased by 10%, 37%, and 63%, respectively, at 100, 150, and 200 mM NaCl (Truong and Hart, 2001). Wild vetiver in Southern China has a salinity threshold of $\sim 10 \text{ dsm}^{-1}$ and is more tolerant to saline than most common vetiver cultivars (Liu *et al.*, 2016). The higher the salinity level in vetiver, the lower the relative growth.

Planting with high salinity stress may produce higher essential oil compounds by boosting the plant's secondary metabolites but affecting the growth and decreasing at high salinity levels. Salinity mainly results in higher concentrations of Na^+ and Cl^- in the soil, producing hyperosmotic and hypertonic solutions that stop water and nutrient uptake by crop plants (Ismail *et al.*, 2014). However, appropriate salinity stress will increase plant secondary metabolites of vetiver, and growth remains good.

The ability to grow and acquire a real genetic potential of crop plants primarily depends on the interaction between genotypes and the environment spread, including vetiver. According to Reed (2008), plant morphological characterization is critical for detecting the desirable traits, identifying duplicated accessions, and structuring further the populations for conservation purposes. Morphological variations due to environmental conditions indicate that a plant is passing through an adaptation process, and the plant population that is adaptive to specific ecological situations is called an ecotype. Different ecotypes of a plant population will form a pattern based on the variations in environmental conditions in the geographical distribution area of the species. Therefore, the

presented study underlies several vetiver ecotypes screening with various salinity levels to identify the best tolerant vetiver ecotypes at a particular salinity level.

MATERIALS AND METHODS

The contemporary research on vetiver ecotypes in September 2021 transpired at the Greenhouse, Medan Agricultural Development Polytechnic, Indonesia. The material used in this study comprised six-month vetiver seedlings obtained from three different ecotypes, i.e., Bogor, Bojonegoro, and Padang, saline soil, and other supporting materials.

This study proceeded in a completely randomized design (CRD) with factorial arrangement and two factors. The first factor was salinity stress (S), comprising six different levels, i.e., S0 = no salinity (control) and saline soil with S1 = 4 dsm^{-1} , S2 = 8 dsm^{-1} , S3 = 12 dsm^{-1} , S4 = 16 dsm^{-1} , and S5 = 20 dsm^{-1} . The second factor consisted of three vetiver ecotypes (E), i.e., E1 = Bogor, E2 = Bojonegoro, and E3 = Padang.

Crop husbandry

The greenhouse soil and seedbed at the Medan Agricultural Development Polytechnic, Indonesia, became the study's experimental plot. First, providing an extensive cleaning from all plant stubble and residuals, the soil's salinity content measurement followed according to each treatment. Saline soil came from the Percut Sei Tuan Sub-District, Deli Serdang Regency, Medan, Indonesia. According to concentrations, the polybags (50 cm \times 50 cm) filled with saline soil (4, 8, 12, 16, and 20 dsm^{-1}) had the level of salinity measured using a refractometer. Then, the polybags filled with desired soil brought to the study site gained stacking and labeling according to each treatment.

Seed preparation

The genotype seeds ordered uniformly were according to each ecotype, i.e., Bogor, Bojonegoro, and Padang. The sows used in this

study came from the vetiver plantations. Selecting vetiver seeds ensued with uniform growth according to each ecotype. Those selected seeds had no pests or disease infestation. After planting, all plot preparation was according to treatments for easier identification.

Salinity treatments

Before applying the salinity treatment, the salinity level measurement used a digital refractometer in the soil, adjusted to a predetermined salinity level to obtain a salinity level of 4, 8, 12, 16, and 20 dsm^{-1} .

Seed planting

Polybags containing soil according to the different salinity treatments acquired planting with vetiver ecotype seedlings according to each ecotype (Bogor, Bojonegoro, and Padang).

Data recording

The data recorded on the traits included plant height at 10 weeks after planting (WAP), leaf area and number of tillers at 10 WAP, chlorophyll a and b, root length, root volume, and essential oil, all at 12 WAP.

Statistical analysis

All the recorded data underwent analysis of variance (ANOVA) with the means further compared and separated with a Duncan's Multiple Range Test (DMRT) (Hanafiah, 2016).

RESULTS AND DISCUSSION

Plant height

Based on analysis of variance, differences in salinity stress levels, vetiver ecotypes, and their interactions have significant impacts, revealing differences in the plant height of vetiver ecotypes (Table 1). The highest plant height came from vetiver ecotype Bojonegoro with control (non-salinity treatment) and by

Table 1. Salinity levels' effect on the plant height of vetiver ecotypes.

Salinity levels	Ecotypes			Means
	E ₁	E ₂	E ₃	
cm.....			
S ₀	159.33 abc	178.00 a	173.50 ab	170.28
S ₁	150.50 bcde	172.00 abc	161.83 abc	161.44
S ₂	138.33 cde	152.67 abcd	140.33 cde	143.78
S ₃	123.83 fg	136.17 def	127.67 ef	129.22
S ₄	99.67 gh	127.17 ef	114.50 fg	113.78
S ₅	74.50 h	109.17 fgh	94.67 gh	92.78
Means	124.36	145.86	135.42	

Note: S₀: no salinity, S₁: 4 dsm⁻¹, S₂: 8 dsm⁻¹, S₃: 12 dsm⁻¹, S₄: 16 dsm⁻¹, S₅: 20 dsm⁻¹.

E₁: Bogor Ecotype, E₂: Bojonegoro Ecotype, and E₃: Padang Ecotype.

Numbers followed by different letters in the same column are significantly different according to DMRT at 5%.

the ecotypes Padang and Bogor. In all the ecotypes with different salinity levels, the plant height ranged from 74.50–159.33 cm in Bogor, 109.17–178.00 cm in Bojonegoro, and 94.67–173.50 cm in the ecotype Padang. Variations in plant height of the different vetiver ecotypes might be due to their ability to adapt to salinity stress conditions caused by their genetic makeup. According to Dos-Santos *et al.* (2022), plant adaptation to salinity depends on various factors like the stress level experienced, stress duration, the growth phase of plants when experiencing stress, and crop cultivar. The degree of salt tolerance varies with plant species and varieties within a species based on the genetic variations in the genotypes (Barus *et al.*, 2023). Genetic variations and differential responses of the genotypes to salinity stress enable plant biologists to identify physiological mechanisms and involved genes in enhancing stress tolerance to incorporate those genes in other commercial cultivars for salt tolerance (Gupta and Huang, 2014).

Salinity stress also reduced all the vetiver ecotypes' general growth and plant height. The decreased growth and plant peak might be due to osmotic pressure, which makes it difficult for plants to absorb moisture and nutrients, and the influence of excessive Na and Cl ions inhibited cell division and enlargement. James *et al.* (2011) also stated that one of the most detrimental effects of salinity stress was the accumulation of Na⁺ and Cl⁻ ions in plant tissues exposed to high NaCl concentrations in the soil. The entry of Na⁺ and

Cl⁻ in the cells causes severe ion imbalance, and excess uptake might cause significant physiological disorders. High Na⁺ concentration inhibits the uptake of K⁺ ions, an essential element for growth and development that results in lower productivity and even death.

Leaf area

Based on the analysis of variance, salinity stress levels, vetiver ecotypes, and their interactions showed significant differences for leaf area (Table 2). Leaf area with different salinity treatments and vetiver ecotypes gave the highest leaf area resulted in the ecotype Bojonegoro with control treatment (8653.35 cm²), whereas the lowest value for the said trait (3027.82 cm²) from ecotype Padang with salinity of 20 dsm⁻¹. These results confirmed that the higher salinity level affects and reduces growth, leaf morphology, level of transpiration, and the total chlorophyll content in vetiver ecotypes. The findings of Novita *et al.* (2019) detailed that salinity stress conditions increase chlorophyll a and b and cause morphological changes in leaf size and area. Purwaningrahayu and Taufiq (2017) explained that salinity stress causes plants to suffer from physiological drought, with the plants unable to absorb moisture optimally, eventually decreasing the relative water content in the leaves. A decline in the relative water content of the leaves is due to a decrease in turgor pressure, resulting in disruption of the cell expansion process

Table 2. Salinity levels' effect on the leaf area of vetiver ecotypes.

Salinity levels	Ecotypes			Means
	E ₁	E ₂	E ₃	
cm ²			
S ₀	7193.02 bcd	8653.35 a	7951.52 ab	7932.63
S ₁	6411.67 de	7778.32 abc	7060.80 bcd	7083.59
S ₂	5752.07 ef	6950.43 cd	6778.47 d	6493.66
S ₃	4958.75 fgh	5791.80 ef	5391.37 fg	5380.64
S ₄	4057.02 hi	4496.47 ghi	3976.10 ij	4176.53
S ₅	2882.42 k	3111.73 jk	3027.82 k	3007.32
Means	5209.16	6130.35	5697.68	

Note: S₀: no salinity, S₁: 4 dsm⁻¹, S₂: 8 dsm⁻¹, S₃: 12 dsm⁻¹, S₄: 16 dsm⁻¹, S₅: 20 dsm⁻¹.

E₁: Bogor Ecotype, E₂: Bojonegoro Ecotype, and E₃: Padang Ecotype.

Numbers followed by different letters in the same column are significantly different according to DMRT at 5%.

Table 3. Salinity levels' effect on the number of tillers of vetiver ecotypes.

Salinity levels	Ecotypes			Means
	E ₁	E ₂	E ₃	
tiller.....			
S ₀	4.67 cdef	4.83 cde	7.33 a	5.61
S ₁	5.17 c	5.17 c	6.33 b	5.56
S ₂	4.17 fgh	4.67 cdef	5.00 cd	4.61
S ₃	4.17 fgh	4.33 efg	4.50 def	4.33
S ₄	3.83 ghi	3.67 hi	4.17 fgh	3.89
S ₅	2.33 k	3.00 j	3.50 ij	2.94
Means	4.06	4.28	5.14	

Note: S₀: no salinity, S₁: 4 dsm⁻¹, S₂: 8 dsm⁻¹, S₃: 12 dsm⁻¹, S₄: 16 dsm⁻¹, S₅: 20 dsm⁻¹.

E₁: Bogor Ecotype, E₂: Bojonegoro Ecotype, and E₃: Padang Ecotype.

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because of moisture shortage in the tissues (Katerji *et al.*, 1997). Disruption of cell expansion can be visible due to a decreased leaf area with exposure to salinity stress conditions.

Number of tillers

Analysis of variance revealed that salinity stress, vetiver ecotypes, and their interactions significantly impacted plant tillers with varied differences for the said trait (Table 3). Vetiver ecotype Padang without saline soil had the highest number of tillers while growth and the number of tillers declined with salinity stress. Exposure to salinity stress conditions also inhibited plant growth in other ecotypes. Niu *et al.* (2012) revealed that after exposing the sorghum and maize plants to salinity stress (at 8 dsm⁻¹ for 40 days), a reduced number of

tillers and dry weight of leaves and stems occurred.

Tillering is an influential growth and development parameter, and with saline stress conditions, the number of vetiver tillers was less than the control treatment. The salinity level of 4 dsm⁻¹ recorded the maximum number of tillers, with the minimum tiller number observed at the highest salinity level (20 dsm⁻¹) (Table 3). Salinity stress conditions reduced the nutrient and water absorption by the roots, which showed a decline in tillers (Ashraf *et al.*, 2004).

Chlorophyll a and b

Based on the analysis of variance, it was evident that different salinity stress levels, vetiver ecotypes, and interactions between them exhibited significant differences for

Table 4. Salinity levels' effect on chlorophyll a content of vetiver ecotypes.

Salinity levels	Ecotypes			Means
	E ₁	E ₂	E ₃	
mg/g.....			
S ₀	0.61 c	0.73 c	0.70 c	0.68
S ₁	1.02 c	1.43 bc	0.97 c	1.14
S ₂	1.05 c	1.29 c	1.72 bc	1.35
S ₃	0.88 c	0.87 c	1.50 bc	1.08
S ₄	1.54 bc	2.91 a	1.21 c	1.89
S ₅	1.71 bc	2.54 ab	1.76 abc	2.00
Means	1.14	1.63	1.31	

Note: S₀: no salinity, S₁: 4 dsm⁻¹, S₂: 8 dsm⁻¹, S₃: 12 dsm⁻¹, S₄: 16 dsm⁻¹, S₅: 20 dsm⁻¹.

E₁: Bogor Ecotype, E₂: Bojonegoro Ecotype, and E₃: Padang Ecotype.

Numbers followed by different letters in the same column are significantly different according to DMRT at 5%.

Table 5. Salinity levels' effect on the chlorophyll b content of vetiver ecotypes.

Salinity levels	Ecotypes			Means
	E ₁	E ₂	E ₃	
mg/g.....			
S ₀	0.33 c	0.39 c	0.38 c	0.37
S ₁	0.54 c	0.94 abc	0.67 bc	0.72
S ₂	0.99 abc	1.34 ab	1.64 a	1.32
S ₃	0.68 bc	0.64 bc	0.81 bc	0.71
S ₄	0.50 c	1.59 a	0.65 bc	0.91
S ₅	0.74 bc	1.58 a	0.99 abc	1.10
Means	0.63	1.08	0.85	

Note: S₀: no salinity, S₁: 4 dsm⁻¹, S₂: 8 dsm⁻¹, S₃: 12 dsm⁻¹, S₄: 16 dsm⁻¹, S₅: 20 dsm⁻¹.

E₁: Bogor Ecotype, E₂: Bojonegoro Ecotype, and E₃: Padang Ecotype.

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chlorophyll a and b (Tables 4 and 5). The vetiver ecotype Bojonegoro experienced increased chlorophyll due to salinity stress and showed higher chlorophyll content with enhanced salinity stress. It suggested that the vetiver ecotype Bojonegoro better responded to salinity stress and was physiologically capable of increasing chlorophyll levels after adapting to saline conditions. These results also aligned with the past findings of Shah *et al.* (2017), who mentioned chlorophyll decreased with reduced salinity from tolerant wheat genotypes.

Plants exposed to higher concentrations of salts for a long time will experience adverse impacts on the chlorophyll content in the leaves. It is also evident from the lower leaf chlorophyll index with higher soil salinity levels. The abiotic stress factors can

influence photosynthetic efficiency; thus, concentration of chlorophyll pigments is crucial to knowing the tolerance of the genotypes to various stressors. Recent studies also showed that salinity stress leads to halophytes' increased chloroplasts (Bose *et al.*, 2017). However, with low salt concentrations, an increased concentration of chlorophyll a and b emerged; however, with higher salt concentrations, the chlorophyll pigment concentrations decreased in purslane (*Portulaca oleracea* L.) (Amirul-Alam *et al.*, 2015) and Iranian licorice (*Glycyrrhiza glabra* L.) (Mousavi *et al.*, 2022).

Root length

The results on the root length of the three vetiver ecotypes under varied salinity stress

conditions appear in Table 6. Based on analysis of variance, salinity stress conditions, vetiver ecotypes, and their interactions have influenced significant effects on root length. The outcomes showed that the control treatment (without salinity) emerged with the longest vetiver roots, but the root length gradually decreased as salinity levels increased. These results were also analogous to Du and Truong (2006), with the vetiver genotypes' roots piercing to 70 cm while penetrating the saline soil and reaching subsurface moisture, while other plants cannot go deep where the mostly reduced dissolved salt content point. Zhao *et al.* (2020) stated that salinity stress inhibits plant growth and development with osmotic pressure as the main obstacle, which decreases the external water potential and interferes with the ability of plants to absorb water. This process triggers several vital events in plant tissues, such as the expansion of root and shoot cells immediately stopping due to a turgor pressure decrease. Plants have to make osmotic adjustments, and the cell turgor in the roots returns within 40–60 minutes with increased uptake of inorganic ions, and growth resumes.

In addition to environmental conditions, the difference in root length growth might refer to genetic factors, in which some ecotypes have a better adaptation mechanism under salinity conditions. Plants will try to survive by maximizing root growth to increase water absorption to balance turgor pressure. The NaCl stress can cause plants to distribute more photosynthate into the roots to maximize nutrient and water absorption. According to Negrão *et al.* (2017), the plant's response to salinity comprises two main steps: the first is rapid, described as the osmotic phase, wherein as the salt reaches, the roots decrease the osmotic potential of the soil–plant relationship, triggering a reduction in shoot growth due to reduced water potential, and the second is described as slow, comprising absorption and signaling of toxic ions, such as Na⁺.

Root volume

The analysis of variance on salinity stress levels, vetiver ecotypes, and their interactions

provided significant differences for root volume (Table 7). Observations expressed that, without saline stress, the ecotype Padang showed the highest root volume, whereas, with salt treatment (12 dsm⁻¹), the ecotype Bojonegoro showed the lowest root volume. Without salinity, the plant roots were undisturbed, and the root growth significantly increased; however, at the highest salinity (20 dsm⁻¹), a significant effect appeared on decreasing the root volume. Kafi and Rahimi's (2011) findings indicated that salinity caused a reduction in root growth (volume, area, diameter, total and core length, and root dry weight) and shoot biomass in purslane (*Portulaca oleracea* L.). Furthermore, Bidalia *et al.* (2019) explained that salinity can induce elemental nutrient deficiencies and imbalances in crop plants. The presented study also revealed that low-salinity-induced growth may be due to salinity-induced acceleration of N, K, and Cl uptake by the vetiver ecotypes. With the competition between nutrients and major salt species, the plant's uptake and accumulation of nutrients often weaken under saline conditions.

Essential oil

Observations on the essential oil production of three vetiver ecotypes under different salinity stress conditions are available in Table 8. Based on the analysis of variance, a significant effect on essential oil production occurred with salinity stress levels, vetiver ecotypes, and their interactions. The highest essential oil production surfaced in the ecotype Padang without salinity stress, whereas the lowest was in ecotype Bogor, with salinity stress at 20 dsm⁻¹. In all three ecotypes, essential oil production decreased with increased salinity levels. Results further revealed that salinity stress can affect the plant growth parameters (plant height, leaf area, number of tillers, root length, and root volume), causing decreased oil production. Mbark *et al.* (2018) stated that salinity affects the physiological activities of the leaves in crop plants, particularly photosynthesis, which is the chief cause of reduced plant productivity. According to Charles *et al.* (1990), the stimulation of

Table 6. Salinity levels' effect on the root length of vetiver ecotypes.

Salinity levels	Ecotypes			Means
	E ₁	E ₂	E ₃	
cm.....			
S ₀	62.90 cd	88.67 ab	97.63 a	83.07
S ₁	63.83 cd	77.45 bc	85.43 ab	75.57
S ₂	62.72 cd	64.63 cd	76.50 bc	67.95
S ₃	49.27 def	54.38 de	61.43 cd	55.03
S ₄	39.58 efg	40.02 efg	47.58 def	42.39
S ₅	28.93 g	32.13 fg	35.70 fg	32.26
Means	51.21	59.55	67.38	

Note: S₀: no salinity, S₁: 4 dsm⁻¹, S₂: 8 dsm⁻¹, S₃: 12 dsm⁻¹, S₄: 16 dsm⁻¹, S₅: 20 dsm⁻¹.

E₁: Bogor Ecotype, E₂: Bojonegoro Ecotype, and E₃: Padang Ecotype.

Numbers followed by different letters in the same column are significantly different according to DMRT at 5%.

Table 7. Salinity levels' effect on root volume of vetiver ecotypes.

Salinity levels	Ecotypes			Means
	E ₁	E ₂	E ₃	
cm ³			
S ₀	200.33 c	213.67 b	265.33 a	226.44
S ₁	160.56 d	170.28 d	210.39 bc	180.41
S ₂	112.72 f	131.17 e	169.11 d	137.67
S ₃	74.94 h	84.72 gh	95.28 g	84.98
S ₄	55.33 i	60.83 i	78.39 h	64.85
S ₅	35.89 k	52.56 ij	42.28 jk	43.57
Means	106.63	118.87	143.46	

Note: S₀: no salinity, S₁: 4 dsm⁻¹, S₂: 8 dsm⁻¹, S₃: 12 dsm⁻¹, S₄: 16 dsm⁻¹, S₅: 20 dsm⁻¹.

E₁: Bogor Ecotype, E₂: Bojonegoro Ecotype, and E₃: Padang Ecotype.

Numbers followed by different letters in the same column are significantly different according to DMRT at 5%.

Table 8. Salinity levels' effect on essential oil production of vetiver ecotypes.

Salinity levels	Ecotypes			Means
	E ₁	E ₂	E ₃	
ml.....			
S ₀	5.77 bc	6.27 b	8.03 a	6.69
S ₁	4.57 de	5.03 cd	6.43 b	5.34
S ₂	2.78 ghi	3.53 fg	5.23 cd	3.85
S ₃	2.32 hij	2.88 gh	4.18 ef	3.13
S ₄	1.97 ijk	2.48 hij	3.42 fg	2.62
S ₅	1.18 k	1.67 jk	2.75 gh	1.87
Means	3.10	3.64	5.01	

Note: S₀: no salinity, S₁: 4 dsm⁻¹, S₂: 8 dsm⁻¹, S₃: 12 dsm⁻¹, S₄: 16 dsm⁻¹, S₅: 20 dsm⁻¹.

E₁: Bogor Ecotype, E₂: Bojonegoro Ecotype, and E₃: Padang Ecotype.

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essential oil production under a moderate degree of salinity could be due to a higher oil gland density and an increase in the absolute number of glands produced before leaf emergence in peppermint.

Besides, salt stress may also affect the essential oil accumulation indirectly through its effect on the net assimilation or the partitioning of assimilates among growth and development processes in lemon grass (*Cymbopogon schoenanthus* L.) (Khadhri et al., 2011). In plants, the formation and accumulation of essential oil were also attributable to environmental factors. Further claims may be that the essential oil formation and accumulation directly depend on the perfect growth and development of the plant's producing oil (Mostajeran et al., 2014).

CONCLUSIONS

Salinity stress levels, vetiver ecotypes, and their interactions had significantly affected plant height, leaf area, number of tillers, chlorophyll a and b, root length and volume, and essential oil production. Salinity stress of 16 dsm⁻¹ significantly reduced plant growth and development but increased the chlorophyll a and b content. The vetiver ecotype Bojonegoro had better shoot growth, though the ecotype Padang had better root growth, resulting in higher essential oil production versus the ecotype Bojonegoro. Ecotype Bojonegoro at 16 dsm⁻¹ showed a significantly increased chlorophyll a and b content. However, ecotype Padang with control showed the highest essential oil compared with the salinity stress treatments.

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REFERENCES

- Amirul-Alam M, Juraimi AS, Rafii MY, Hamid AA, Aslani F, Alam MZ (2015). Effects of salinity and salinity-induced augmented bioactive compounds in purslane (*Portulaca oleracea* L.) for possible economical use. *Food Chem.* 169: 439-447. <https://doi.org/10.1016/j.foodchem.2014.08.019>.
- Ashraf M, Mukhtar N, Rehman S, Rha ES (2004). Salt-induced changes in photosynthetic activity and growth in a potential plant Bishop's weed (*Ammolei majus* L.). *Photosynthetica* 42: 543-550. <https://doi.org/10.1007/S11099-005-0011-4>.
- Barus WA, Sulistiani R, Utami S, Tarigan DM, Lestami A (2023). Black soybean response to antioxidant application for growth and yield under salinity stress conditions. *SABRAO J. Breed. Genet.* 55(2): 417-426. <http://doi.org/10.54910/sabrao2023.55.2.14>.
- Bidalia A, Vikram K, Yamal G, Rao KS (2019). Effect of salinity on soil nutrients and plant health. In: Salt Stress, Microbes, and Plant Interactions: Causes and Solution. M.S. Akhtar (ed.). Springer: Singapore, pp. 273-297. https://doi.org/10.1007/978-981-13-8801-9_13.
- Bose J, Munns R, Shabala S, Gilligham M, Pogson B, Tyerman SD (2017). Chloroplast function and ion regulation in plants growing on saline soils: Lessons from halophytes. *J. Exp. Bot.* 68: 3129-3143. <https://doi.org/10.1093/jxb/erx142>.
- Charles DJ, Joly RJ, Simon JE (1990). Effect of osmotic stress on the essential oil content and composition of peppermint. *Phytochemistry* 29: 2837-2840. [https://doi.org/10.1016/0031-9422\(90\)87087-B](https://doi.org/10.1016/0031-9422(90)87087-B).
- Datta R, Quispe MA, Sarkar D (2011). Greenhouse study on the phytoremediation potential of vetiver grass, *Chrysopogon zizanioides* L., in arsenic-contaminated soils. *Bull. Environ. Contam. Toxicol.* 86:124-128. <https://doi.org/10.1007/s00128-010-0185-8>.
- Donjatee S, Tingsanchali T (2012). Reduction of runoff and soil loss over steep slopes by using vetiver hedgerow systems. *Paddy Water Environ.* 11: 573-581. <https://doi.org/10.1007/s10333-012-0350-2>.
- Dos-Santos TB, Ribas AF, de Souza SGH, Budzinski IGF, Domingues DS (2022). Physiological responses to drought, salinity, and heat stress in plants: A review. *Stresses* 2(1): 113-135. <https://doi.org/10.3390/stresses2010009>.

- Du LV, Truong P (2006). Vetiver grass for sustainable agriculture on adverse soils and climate in South Viet Nam. In The Fourth International Conference on Vetiver (ICV4): Vetiver and People. Caracas, Venezuela. pp. DAS06.
- Ghotbizadeh M, Sepaskhah AR (2015). Effect of irrigation interval and water salinity on growth of vetiver (*Vetiveria zizanioides*). *Int. J. Plant Prod.* 9: 17-38. <https://doi.org/10.22069/ijpp.2015.1864>.
- Gupta B, Huang B (2014). Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization. *Int. J. Genomics* 701596. <https://doi.org/10.1155/2014/701596>.
- Hanafiah KA (2016). Experimental Design: Theory and Applications. Sriwijaya University Press, Palembang.
- Ismail A, Takeda S, Nick P (2014). Life and death under salt stress: Same players, different timing? *J. Exp. Bot.* 65: 2963-2979. <https://doi.org/10.1093/jxb/eru159>.
- James RA, Blake C, Byrt CS, Munns R (2011). Major genes for Na⁺ exclusion, Nax1 and Nax2 (wheat HKT1;4 and HKT1;5), decrease Na⁺ accumulation in bread wheat leaves under saline and waterlogged conditions. *J. Exp. Bot.* 62(8): 2939-2947. <https://doi.org/10.1093/jxb/err003>.
- Kafi M, Rahimi Z (2011). Effect of salinity and silicon on root characteristics, growth, water status, proline content and ion accumulation of purslane (*Portulaca oleracea* L.). *Soil Sci. Plant Nut.* 57(2): 341-347. <https://doi.org/10.1080/00380768.2011.567398>.
- Katerji N, Van-Hoorn JW, Hamdy A, Mastrorilli M, Mou Karzel E (1997). Osmotic adjustment of sugar beets in response to soil salinity and its influence on stomatal conductance, growth and yield. *Agric. Water Manag.* 34: 57-69. [https://doi.org/10.1016/S0378-3774\(96\)01294-2](https://doi.org/10.1016/S0378-3774(96)01294-2).
- Khadhri A, Neffati M, Smiti S, Manuel J, Nogueira F, Eduarda M, Araujo M (2011). Influence of salt stress on essential oil yield and composition of lemon grass (*Cymbopogon schoenanthus* L. Spreng. Ssp. Laniger (Hook) Maire et Weil). *Nat. Product. Res.* 25(2): 108-117. <https://doi.org/10.1080/14786419.2010.505169>.
- Liu WG, Liu JX, Yao MI, Ma QF (2016). Salt tolerance of a wild ecotype of vetiver grass (*Vetiveria zizanioides* L.) in Southern China. *Bot. Studies* pp. 1-8. <https://doi.org/10.1186/s40529-016-0142-x>.
- Luu TD (2007). Development of process for purification of α and β vetivone from vetiver essential oil & investigation of effect of heavy metals on quality and quantity of extracted vetiver oil. Dissertation. University of New South Wales. Sydney, Australia.
- Mbarki S, Sytar O, Cerda A, Zivcak M, Rastogi A, He X, Zoghalmi A, Abdelly C, Brestic M (2018). Strategies to mitigate the salt stress effects on photosynthetic apparatus and productivity of crop plants. In: V. Kumar, S. Wani, P. Suprasanna, L.S. Tran (eds.). Salinity Responses and Tolerance in Plants, Volume 1. Springer, Cham. pp. 85-136. https://doi.org/10.1007/978-3-319-75671-4_4.
- Mostajeran A, Gholaminejad A, Asghari G (2014). Salinity alters curcumin, essential oil, and chlorophyll of turmeric (*Curcuma longa* L.). *Res. Pharm. Sci.* 9(1): 49-57. PMID: 25598799; PMCID: PMC4292181.
- Mousavi SS, Karami A, Maggi F (2022). Photosynthesis and chlorophyll fluorescence of Iranian licorice (*Glycyrrhiza glabra* L.) accessions under salinity stress. *Fron. Plant Sci.* 13: 984944. <https://doi.org/10.3389/fpls.2022.984944>.
- Negrão S, Schmöckel SM, Tester M (2017). Evaluating physiological responses of plants to salinity stress. *Ann. Bot.* 119: 1-11. <https://doi.org/10.1093/aob/mcw191>.
- Niu G, Xu W, Rodriguez D, Sun Y (2012). Growth and physiological responses of maize and sorghum genotypes to salt stress. *International Scholarly Research Network.* <https://doi.org/10.5402/2012/145072>.
- Novita A, Julia H, Rahmawati N (2019). Salinity response to the growth of vetiver (*Vetiveria zizanioides* L.) seedlings. *Agrica Ekstensi* 13(2): 55-58. <https://doi.org/10.55127/ae.v13i2.15>.
- Purwaningrahayu RD, Taufiq A (2017). Morphological responses of four soybean genotypes to salinity stress. *J. Biol. Indonesia* 13(2): 175-188. <https://doi.org/10.47349/jbi/13022017/175>.
- Rao EVSP, Akshata S, Gopinath CT, Ravindra NS, Hebbar A, Prasad N (2015). Vetiver production for small farmers in India. In: E. Lichtfouse (ed.). *Sustainable Agriculture Reviews Volume 17*. Springer International Publishing, Switzerland. https://doi.org/10.1007/978-3-319-16742-8_10.
- Reed MS (2008). Stakeholder participation for environmental management. Literature Review. *Biol. Conserv.* 141: 2417-2431. <https://doi.org/10.1016/j.biocon.2008.07.014>.

- Sakinah AI, Musa Y, Farid M, Hairmansis A, Anshori MF, Nasaruddin N (2022). Rice selection criteria based on morphological and image-based phenotyping under drought- and salinity-stress conditions. *SABRAO J. Breed. Genet.* 54(4) 686-699. <http://doi.org/10.54910/sabrao2022.54.4.1>.
- Shah SH, Houborg R, McCabe MF (2017). Response of chlorophyll, carotenoid and SPAD-502 measurement to salinity and nutrient stress in wheat (*Triticum aestivum* L.). *Agronomy* 7(3): 61. <https://doi.org/10.3390/agronomy7030061>.
- Siregar MPA, Hanum C, Siregar LAM, Tistama R (2021). Morphological and physiological performance of brown rice (*Oryza nivara* L.) under salinity stress. *SABRAO J. Breed. Genet.* 53(2): 228-238.
- Smirnova I, Sadanov A, Baimakhanova G, Faizulina E, Tatarkina L (2023). Using salt tolerant rhizobia to improve the soybean (*Glycine max*) Resilience to salinity. *SABRAO J. Breed. Genet.* 55(3): 810-824. <http://doi.org/10.54910/sabrao2023.55.3.17>.
- Truong P, Gordon I, Armstrong F, Shepherdson J (2002). Vetiver grass for saline land rehabilitation under tropical and Mediterranean climate. In: Eighth National Conference Productive Use of Saline Lands. Perth, Australia.
- Truong P, Hart B (2001). Vetiver system for wastewater treatment. *Pacific Rim Vetiver Network Tech. Bull.* No. 2001/2. Bangkok, Thailand. pp. 1-26.
- Zhao C, Zhang H, Song C, Zhu J, Shabala S (2020). Mechanisms of plant responses and adaptation to soil salinity. *The Innovation* 1(1): 100017. <https://doi.org/10.1016/j.xinn.2020.100017>.
- Zhou Q, Yu BJ (2009). Accumulation of inorganic and organic osmolytes and their role in osmotic adjustment in NaCl stressed vetiver grass seedlings. *Russ. J. Plant Physiol.* 56: 678-685. <https://doi.org/10.1134/s1021443709050148>.