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Screening sorghum varieties for salinity tolerance based on morpho-physiological and biochemical traits

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Abstract. *Tarigan DM, Lestami A, Nawar MK, Barus WA, Munar A. 2025. Screening sorghum varieties for salinity tolerance based on morpho-physiological and biochemical traits. Biodiversitas 26: 5226-5237.* The global food and energy crisis highlights the urgent need for alternative crops that can thrive on suboptimal land, including saline soils. Sorghum (*Sorghum bicolor*) is a promising candidate due to its wide varietal diversity and adaptive potential. Evaluating varietal responses under salinity is essential to identify the most tolerant and productive types. This study assessed the morphological, physiological, and biochemical responses of six sweet sorghum varieties (Suri 3, Super 1, Super 2, Soper 6, Soper 7, and Soper 9) under salinity stress with an electrical conductivity of 6.59 dS/m. A non-factorial Randomized Block Design (RBD) with three replications was employed. Plant performance was evaluated through key traits, including biomass, chlorophyll content, cuticle thickness, and seed biochemical composition. The results indicated that Suri 3 and Soper 6 demonstrated superior tolerance compared to the other varieties. These genotypes maintained higher dry weight, better chlorophyll retention, thicker cuticles, and enhanced protein content under saline conditions. Among the measured variables, dry weight, cuticle thickness, total chlorophyll, and protein concentration were the most influential in distinguishing varietal tolerance levels. The study highlights that a combination of morpho-physiological and biochemical traits can serve as reliable indicators for identifying salinity-tolerant sorghum genotypes. Such findings provide valuable guidance for sorghum breeding and improvement programs, particularly in the development of sustainable agriculture on saline-prone lands.

Keywords: Biochemical, morphological, physiological, salinity, sorghum

INTRODUCTION

Food security and energy crisis are currently one of the main problems in national development (Masriadi et al. 2024). Efforts to increase food production continue to be carried out to meet food needs in line with population growth rates (Hemathilake and Gunathilake 2022). Efforts made include diversifying food ingredients by developing alternative food crops (Widiastuti and Himawan 2021). One of the alternative food commodities that can be developed in Indonesia is sorghum, which has a lot of potential and good prospects. The potential of sweet sorghum includes its seeds have a high carbohydrates which can be used as a food ingredient rich in carbohydrates (Vanamala et al. 2018; Sulistyawati et al. 2019), and animal feed (Alane et al. 2020; Yusriani et al. 2023), its stems have a high sap content which can be used as a material for making sugar and raw materials for alternative energy in the form of biofuel (Ahmad Dar et al. 2018; Ariefin et al. 2021). As a food ingredient, sorghum has very good nutritional content with 70-80% carbohydrates, 8-18% protein, 6% fiber, 1-5% fat, 19% dietary fiber, and 3% crude fiber (Espitia-Hernández et al. 2022).

Sorghum plants have great potential to be developed in Indonesia because they are plants with wide adaptation. Sorghum plants can grow on less fertile soil or critical soil (saline soil), but to get maximum results, sorghum should be planted on fertile land (Siregar 2016). The total area of sorghum plants in Indonesia has only reached 8000 ha spread across various regions (Tarigan and Ismuhadi 2021). Based on the average national yield, sorghum productivity is still low, namely 2.68 tons ha^{-1}, with a potential yield that should reach 5-7 tons ha^{-1} (Yahfi et al. 2017).

The development of sorghum through extensification in North Sumatra is hampered by land conversion, so that its development is directed to suboptimal land, including saline soil. Saline soil contains dissolved neutral salts at concentrations that adversely affect plant growth and productivity. Plants that grow in saline soil will experience two different physiological stresses. First, the toxic effects of certain ions, such as sodium and chloride, which are commonly found in saline soil, will destroy the structure of enzymes and other macromolecules, damage cell organelles, interfere with photosynthesis and respiration, inhibit protein synthesis, and encourage ion deficiency. Second, plants exposed to low osmotic potential from saline solutions are susceptible to "physiological drought," as they must lower their internal osmotic potential to maintain water uptake, leading to ion accumulation that ultimately suppresses growth in some plants (Bartels and Sunkar 2005).

The evaluation of plant tolerance to salinity is not sufficient based solely on morphological parameters, as external characteristics often do not fully reflect the plant's internal response. Therefore, a multidisciplinary approach combining morphological, physiological, and biochemical parameters is necessary to gain a more comprehensive understanding of plant adaptation mechanisms (Kokebie et al. 2024). Physiological characteristics such as the number and density of stomata, as well as chlorophyll content, are important indicators for understanding photosynthetic efficiency and osmotic regulation (Yang et al. 2020; Thu et al. 2022), while biochemical characteristics such as protein and amylose content reflect metabolic stability under stress (Massange-Sánchez et al. 2022).

Recent studies have shown that sorghum has relatively good tolerance to salinity through various mechanisms, such as osmotic adjustment with organic substances (Wani et al. 2019) and inorganic ions (Wang et al. 2019), scavenging of Reactive Oxygen Species (ROS) through enzymatic antioxidants (Hasanuzzaman et al. 2020) and non-enzymatic antioxidants (Nadarajah et al. 2020), as well as other physiological regulation under salinity stress (Hao et al. 2021). Therefore, a deeper understanding of sorghum varieties' responses to salinity is crucial to support sorghum breeding and development programs on marginal lands.

The response of each sorghum variety may vary under salinity stress due to differences in physiological (Dewi et al. 2023) and genetic tolerance mechanisms (Jeon et al. 2023). The varieties used in this study, namely Suri 3, Super 1, Super 2, Soper 6, Soper 7, and Soper 9, are varieties that have not been extensively evaluated under saline stress conditions. The uniqueness of these sorghum varieties lies in their distinct morphological and physiological traits, which may contribute to varied adaptive responses under saline conditions. Given the absence of sorghum varieties specifically adapted to saline soils, research on plant responses to salinity is essential for developing effective selection strategies. This study aims to identify the most adaptive sorghum varieties with high yield and quality in saline soils based on morpho-physiological and biochemical traits.

MATERIALS AND METHODS

Research location

This research was conducted in the field of the Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, Medan, Indonesia (observation of morphological traits). Analysis of physiological traits was conducted in the Laboratory of Physiology and Plant Tissue Culture, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara. Analysis of biochemical traits was conducted in the Laboratory of Food Chemical Analysis, Faculty of Agriculture, Universitas Sumatera Utara. The research was conducted from March to September 2023.

Materials

There are six varieties of sweet sorghum tested, namely Suri 3, Super 1, Super 2, Soper 6, Soper 7, and Soper 9. Sorghum varieties were obtained from the Maros Cereal Research Center, South Sulawesi. The planting medium was saline soil from coastal rice fields at Tanjung Rejo Village, Percut Sei Tuan District, Deli Serdang District, North Sumatra, Indonesia, with soil characteristics presented in Table 1.

Methods

The experimental design used was a non-factorial Randomized Block Design (RBD), which tested 6 sorghum varieties (Suri 3, Super 1, Super 2, Soper 6, Soper 7, and Soper 9) under saline soil conditions with a natural salinity level of 6.59 dS/m based on soil Electrical Conductivity (EC) analysis results (Table 1). This salinity level is categorized as moderate to high (Soil Survey Staff 2014) and was selected because it represents the actual conditions of marginal coastal land in Tanjung Rejo Village, Deli Serdang District, North Sumatra, Indonesia.

Each variety was planted in a 40 × 40 cm polybag filled with saline soil without chemical modification. Two seeds were planted per polybag, and each variety was repeated three times (3 replicates). Plants were given basic fertilizer consisting of Urea (3.2 $g.ha^{-1}$), TSP (1.6 $g.ha^{-1}$), and KCl (0.8 $g.ha^{-1}$). Plant maintenance was carried out manually and chemically, depending on the condition of the plants in the field.

Observations were made on morphological traits (plant height, number of leaves, stem diameter, fresh weight, dry weight, flowering age, harvest age, panicle length, panicle weight, number of seeds per panicle, seed weight per panicle, weight of 100 seeds), physiological traits (chlorophyll content a, b, and total, number and density of stomata, and thickness of the leaf cuticle), and biochemical traits of seeds (water content, ash, lipid, amylose, and protein).

Table 1. Results of coastal rice field soil analysis

Soil characteristics	Value	Criteria
Coarse sand	1%	-
Fine sand	4%	-
Silt	60%	-
Clay	42%	-
N	0.32%	Moderate
C organic	3.07%	Moderate
P total	726.76 mg/kg	High
P Bray II	10.39 mg/kg	Low
Cation Exchange Capacity (CEC)	20.14 cmol/kg	Moderate
Ca	3.85 cmol/kg	Low
Mg	7.50 cmol/kg	High
K	0.90 cmol/kg	High
Al	0.61 cmol/kg	
Cl	664.26 ppm	Very high
Exchangeable Sodium Percentage (ESP)	17.28%	Very high
Electrical Conductivity (EC)	6.59 mmhos/cm	High
pH H_2O	6.93	
pH KCl	6.51	

Data analysis

Morphological and production data were analyzed using Analysis of Variance (ANOVA), with the means further compared and separated with a Tukey's test at $\alpha = 5\%$ (Hanafiah 2016).

RESULTS AND DISCUSSION

Screening of sorghum varieties to salinity stress based on morphological traits involves various evaluations of morphological traits of sorghum plants under saline conditions to identify varieties that show tolerance to salinity stress. This can be done by observing differences in growth, plant height, number of leaves, stem diameter, fresh weight, and dry weight of sorghum plants (Table 2).

The analysis results showed that the difference in varieties had a significant effect on plant height. The Suri 3 variety showed the best growth response with an average height reaching 231.33 cm, followed by Super 1 and Soper 6. Meanwhile, Soper 7 showed the lowest height, which was 176.67 cm, indicating a relatively lower adaptability to saline conditions. The varieties studied have a special capacity to react to their environment. According to Birhan et al. (2020), tall plant genotypes have great potential as genetic resources for forage development, while the characteristics of short plants contribute significantly to plant adaptation to drought conditions (Seyoum et al. 2019). Salt-tolerant plants are known to employ various defense strategies to survive and support growth under salinity stress. One key mechanism is maintaining cellular ion homeostasis, especially the Na^+/K^+ ratio, which is vital for plant function under saline conditions (Wakeel et al. 2011; Pantha et al. 2023). Furthermore, plants have evolved complex defenses to mitigate the harmful effects of ROS generated by salinity stress (Sachdev et al. 2021).

Varietal differences have a significant effect on the number of plant leaves. The six varieties tested produced varying average leaf numbers, ranging from 11.33 to 14.67, where the Suri 3 variety had the highest number of other varieties. These differences in the number of leaves reflect genetic variation between varieties in the ability to form a canopy and support photosynthetic activity. Photosynthesis is a vital metabolic process that regulates plant growth and has been identified as one of the primary targets affected by salinity stress (Pessarakli 2018; Pan et al. 2021). Salinity adversely affects photosynthesis by disrupting stomatal operation, pigment synthesis, chloroplast development, gas exchange, electron transport, membrane structure, and enzyme activity, ultimately reducing plant productivity (Ashraf and Harris 2013; Pan et al. 2021; Amombo et al. 2022).

Stem diameter was also observed as one of the indicators of plant structural strength in facing environmental stress. The results of stem observations showed that there was variation in diameter between varieties. Three varieties, namely Suri 3, Super 1, and Soper 6, showed the highest stem diameter of 1.70 cm, which indicated a stronger stem structure and potentially more resistant to salinity stress and mechanical stress factors. In addition to its role in biofuel production, stem diameter also reflects the structural strength of the plant, contributing to its uprightness and resistance to lodging. This aligns with the findings of Kong et al. (2020), who stated that plant height and stem robustness are key factors determining bioenergy production potential. Stem diameter of sorghum can be affected by salinity levels, with higher salinity generally leading to a decrease in stem diameter. However, the extent of this effect can vary depending on the specific sorghum variety and other factors like irrigation frequency and environmental conditions. Some studies indicate that sorghum can tolerate moderate salinity levels without significant changes in stem diameter.

The assessment of biomass accumulation and growth efficiency of each variety can be obtained through observations of fresh weight and dry weight of the plant. The results of the observation showed that the Suri 3 variety showed the highest results in both parameters, with a fresh weight of 272.53 g and a dry weight of 49.43 g, reflecting excellent growth capacity even under the influence of salinity. High fresh weight indicates that the variety possesses the capacity to sustain turgor pressure and metabolic activity under saline conditions. However, dry weight provides more stable picture of the actual structural growth efficiency. Salinity stress generally inhibits plant growth through two main mechanisms: osmotic stress, which interferes with water uptake, and ionic toxicity, which inhibits physiological activities (Zhao et al. 2020). Plants that are able to maintain vegetative growth and still accumulate dry biomass under these conditions are considered to be salinity-tolerant. Thus, the differences in fresh weight and dry weight in the tested varieties indicate variations in morphophysiological adaptation strategies to salinity stress.

Salinity, particularly high concentrations of NaCl, significantly reduces both fresh and dry weight in sorghum plants. This reduction is observed across different sorghum genotypes, with higher salinity levels leading to more substantial decreases in biomass. Salinity also affects other growth parameters like plant height and leaf size. Many studies have reported that Dehnavi et al. (2020) genotypes that are able to maintain higher germination rates under saline conditions tend to have better salt tolerance, resulting in greater biomass and yields. Kaymakanova et al. (2008) also reported that salinity stress can decrease intercellular CO_2 concentrations, ultimately inhibiting photosynthesis due to stomatal closure. Furthermore, high salt levels can cause Na^+ accumulation, which subsequently inhibits the transport of important ions such as nitrate (NO^{3-}). This disruption results in a decrease in nitrogen-containing compounds, thus inhibiting plant growth and biomass accumulation (Chien et al. 2009).

Table 2. Morphological traits of six sorghum varieties under salinity stress

Variety	Plant height (cm)	Number of leaves (leaves)	Stem diameter (cm)	Fresh weight (g)	Dry weight (g)
Suri 3	231.33 a	14.67 a	1.70	272.53	49.43
Super 1	224.67 a	14.00 ab	1.70	223.97	34.53
Super 2	205.33 ab	11.33 c	1.57	205.51	37.26
Soper 6	215.33 ab	14.00 ab	1.70	206.85	37.57
Soper 7	176.67 c	13.67 ab	1.60	157.38	14.67
Soper 9	190.67 bc	12.67 bc	1.57	162.44	35.63

Note: Numbers followed by different letters in the same column and row indicate significant differences according to Tukey's test at α = 5%

Table 3. Production traits of six sorghum varieties under salinity stress

Variety	Flowering age (day)	Harvest age (day)	Panicle length (cm)	Panicle weight (g)	Number of seeds per panicle (seed)	Seed weight per panicle (g)	Weight 100 seeds (g)
Suri 3	60.00 c	107	20.83 ab	52.60	1520.67	44.74	3.01 c
Super 1	63.33 bc	115	22.17 a	38.88	1140.00	36.27	3.17 c
Super 2	72.67 a	128	18.83 abc	27.97	763.00	25.21	3.36 c
Soper 6	63.33 bc	115	21.50 a	57.62	1358.00	52.79	3.98 b
Soper 7	64.67 b	115	16.00 c	36.78	712.00	32.75	4.60 a
Soper 9	64.00 b	115	17.50 bc	50.10	969.33	45.32	4.67 a

Note: Numbers followed by different letters in the same column and row indicate significant differences according to Tukey's test at α = 5%

Figure 1. Growth performance of six sorghum varieties under salinity stress at: A. Vegetative, and B. Generative stages

After evaluating the morphological response that reflects the initial ability of the plant to adapt to salinity stress, followed by examining production traits that provide a clear picture of the efficiency of resource allocation to the generative phase, which is strongly influenced by environmental stress, production traits showed variation among sorghum varieties in responding to salinity stress. The Suri 3 variety had the earliest flowering and harvesting age, along with the highest number of seeds per panicle, but with the lowest 100-seed weight. In contrast, the Soper 9 variety showed the highest 100-seed weight, despite having fewer seeds. The variety Soper 6 showed a winning combination of the highest panicle weight and high 100-seed weight, indicating good production efficiency under salinity conditions (Table 3).

The flowering age of sorghum can affect its response to salinity stress. While sorghum is generally considered more tolerant to salinity than many other crops, certain stages, like flowering, are more sensitive to water stress, which can be exacerbated by salinity. In this study, the fastest flowering and harvest age was in the Suri 3 variety (60 days), followed by Super 1 and Soper 6, while the longest flowering and harvest age was in the Super 2 variety (72.67 days) (Table 3). Salinity stress can affect the reproductive phase of plants in ways that vary across species. In some plants, such as sorghum, salinity causes delayed flowering (Bhosale et al. 2025), while in salt-tolerant species, accelerated flowering can occur (Oh et al. 2014). However, even in tolerant plants, salinity can still potentially reduce pollen fertility, seed formation, and seed viability (Boscaiu et al. 2025). Overall, the combination of osmotic stress and ion toxicity due to salinity creates secondary stresses that negatively impact germination, growth, and overall plant development (Liang et al. 2018; Laamari et al. 2023).

Panicle length is one of the important reproductive morphological parameters in assessing the yield potential of sorghum plants. Longer panicles generally have the potential to produce more seeds so that they can be used as an indirect indicator of productivity under stress conditions. The results showed variations in panicle length between the varieties tested. The Super 1 variety had the highest panicle length of 22.17 cm, followed by Soper 6 (21.50 cm) and Suri 3 (20.83 cm), while Soper 7 variety shows the shortest panicle length. Generally, salinity stress can negatively impact sorghum panicle length, reducing it significantly. However, certain sorghum varieties exhibit greater tolerance to salinity, suggesting potential for breeding for salinity resistance. Landi et al. (2017) revealed that various sorghum cultivars exhibited different responses to high soil salt levels, both under water stress and non-water stress conditions, resulting in varying yield reductions. Salinity is known to disrupt the physiological development of sorghum plants, primarily through inhibition of photosynthesis, transpiration, and stomatal conductance (Arif et al. 2020; Webber et al. 2022).

Under salinity stress, the ability of plants to continue producing panicles with high weight indicates physiological efficiency and stress resistance, especially in the generative phase. The highest panicle weight was obtained in Soper 6 (57.62 g) and Suri 3 (52.60 g), followed by Soper 9 (50.10 g). On the other hand, the lowest panicle weight was recorded in Super 2 (27.97 g), indicating a sharp decline in productivity due to possible physiological disturbances due by salinity stress. The high panicle weight of the Soper 6 and Suri 3 varieties indicates that both are able to maintain productivity even in limited environmental conditions, such as water and nutrient availability disrupted by salinity. This strengthens the indication that both varieties have good physiological and morphological adaptation mechanisms on marginal land.

Salinity negatively impacts sorghum panicle weight by reducing grain weight, the number of filled grains per panicle, and overall panicle yield. According to Hailu et al. (2020), high salinity inhibits seed germination and early root growth due to osmotic pressure, which has a detrimental effect because it interferes with the plant's ability to absorb nutrients to support healthy growth optimally. Soil salinity decreases the yield potential of various crops. Kausar and Gull (2019) reported that the results of previous research showed a similar pattern, where the administration of 100 mM NaCl caused a decrease in grain weight per panicle, number of panicles per plant, grain yield per plant, and 1,000-grain weight.

There was significant variation among varieties in terms of 100-seed weight, where Soper 9 variety showed the highest value (4.67 g), followed by Soper 7 (4.60 g) and Soper 6 (3.98 g). This indicates that these varieties have good physiological ability in maintaining seed filling even under stress conditions. This adaptation can be associated with the capacity of the variety in regulating osmoregulation (Dourado et al. 2022), maintaining photosynthetic activity (Yang et al. 2020), and the efficiency of assimilate transport to seed organs during the reproductive stage (Atta et al. 2023). This finding is in line with previous reports that higher seed weight under salinity conditions is an important indicator of tolerance, as it reflects the ability of plant to maintain carbon and nitrogen supplies for seed development (Amombo et al. 2022).

High salinity levels significantly inhibit seed germination, while lower levels may initially promote it, as seen in some studies. The impact on seedling growth, including root and shoot dry weight, is also negative, with the most severe effects observed at higher salt concentrations. Based on the salinity tolerance classification proposed by Shakeri and Emam (2018) and Dehnavi et al. (2020), grain sorghum is classified as moderately tolerant to salinity stress. However, sorghum genotypes exhibit variable responses to salinity. Although the salinity tolerance threshold of sorghum is close to that of wheat, the rate of yield decline in sorghum tends to be sharper.

In addition, there is a clear trade-off between the number of seeds and seed weight in the sorghum varieties tested, where the Suri 3 variety produced the highest number of seeds per panicle (1,520.67) but had the lowest 100-seed weight (3.01 g). Conversely, Soper 9 and Soper 7 produced relatively fewer seeds per panicle (969.33 and 712.00) but had the highest 100-seed weight (4.67 g and 4.60 g). This phenomenon indicates a trade-off between seed quantity and quality, which is common in plants that adjust resource allocation under abiotic stress (Huot et al. 2014). This pattern shows that sorghum varieties allocate assimilates to maximize the number of seeds or increase individual seed weight, depending on their genetic background and stress response strategy.

This trade-off has important implications from a breeding perspective. Varieties with a higher number of seeds but smaller seed size (Suri 3) can be targeted for total yield improvement, especially in environments where maximizing harvest index is critical. Meanwhile, varieties with fewer but heavier seeds (Soper 9 and Soper 7) may be more suitable for markets or processing industries that prioritize seed size and quality. Therefore, balancing these traits is crucial in breeding programs to align with yield potential and end-use requirements. This phenomenon has also been reported in cereals under abiotic stress conditions, where the trade-off between seed number and seed weight is related to assimilation partitioning during the reproductive stage (Šerá and Šerý 2004; Sadras 2007; Gambín and Borrás 2010). Seed number tends to have a greater influence on total yield (Boyles et al. 2016), while seed size can make a significant contribution and may offer prospects for further yield improvement (Yang et al. 2009).

In the context of plant physiology, adaptation to salinity stress is strongly influenced by the ability of varieties to regulate physiological processes such as transpiration, photosynthesis, and cellular protection. The physiological traits observed include the number and density of stomata, cuticle thickness, and chlorophyll content. Based on Table 4, Suri 3 and Soper 6 varieties that previously showed high production performance also had the highest cuticle thickness, higher total chlorophyll, lower stomatal number, and relatively low stomatal density. These characteristics indicate physiological efficiency in minimizing water loss and maintaining photosynthetic activity in saline environments, which directly contributes to more optimal yields. In contrast, Super 2 and Soper 7 varieties showed the lowest values in cuticle thickness and chlorophyll content, indicating weaker adaptation potential to saline conditions.

The number of stomata in sorghum plants affects tolerance to salinity stress. In this research, sorghum plants with a higher number of stomata are Super 2, and the lower number of stomata are Suri 3, Soper 6, and Soper 7 (Table 4). Sorghum plants with a lower number of stomata tend to be more tolerant to salinity because stomata help the transpiration and gas exchange processes, which are important for suppressing the negative impacts of salinity. Ma et al. (2020) explained that plant responses to salinity typically follow a biphasic pattern, with an initial phase resembling drought stress caused by osmotic imbalance, followed by a later phase characterized by toxic ion accumulation. In response to drought stress, plants are generally categorized as isohydric, meaning they reduce water loss through stomatal closure, or anisohydric, meaning they adapt through osmotic adjustment mechanisms. Based on a study by Dourado et al. (2022), sorghum is included in the anisohydric group because it can adapt to drought through osmotic adjustment, which may also contribute to its tolerance to salinity conditions.

The study revealed no significant reduction in stomatal density with increasing salinity levels. As shown in Suri 3 (Figure 2.A), Super 1 (Figure 2.B), and Soper 6 (Figure 2.D), smaller stomata were observed, and their density appeared to rise in association with reduced leaf size. Drake et al. (2013) noted that smaller stomata exhibit faster dynamic behavior, enhancing long-term water use efficiency and minimizing the risk of hydraulic disruption by enabling rapid stomatal opening and closure in response to environmental fluctuations. Similarly, Peel et al. (2017) reported an inverse relationship between stomatal density and leaf area, particularly leaf width, and suggested that salinity-induced leaf size reduction may increase stomatal density due to higher cell packing per unit area. Nevertheless, the stomatal index remains relatively constant at the cellular level. Since stomata are formed early during leaf development, their density peaks in young leaves and gradually stabilizes as the leaf expands, irrespective of cell number or surface area.

Table 4. Physiological traits of six sorghum varieties under salinity stress

Variety	Number of stomata (stomata)	Stomata density (mm^2)	Cuticle thickness (µm)	Chlorophyll content		
				Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)
Suri 3	7	35.669	30.20	13.5359	6.7195	20.3555
Super 1	10	50.955	15.34	12.9719	6.5627	19.6305
Super 2	11	56.051	9.38	8.7124	4.4442	13.221
Soper 6	7	35.669	23.72	12.6828	6.838	19.6146
Soper 7	7	35.669	15.25	7.4616	3.9288	11.4456
Soper 9	8	40.764	21.27	8.4708	4.3995	12.9329

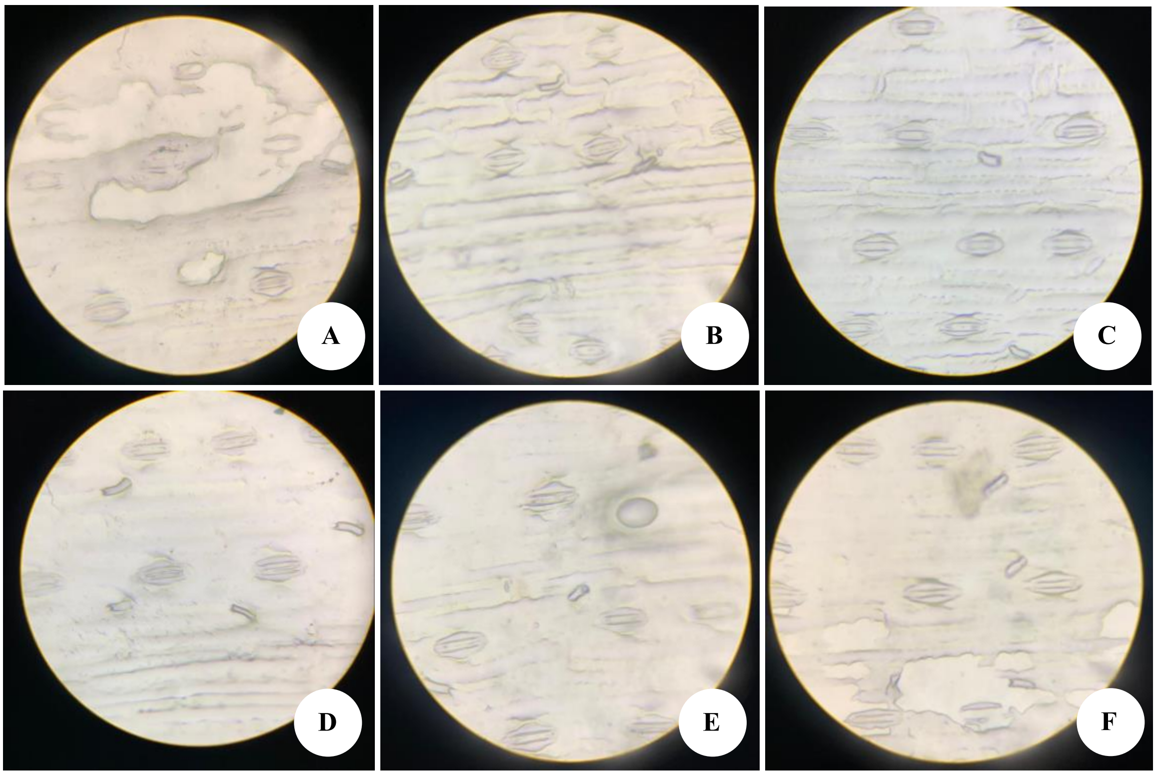

Figure 2. Leaf stomata of six sorghum varieties under salinity stress observed under a light microscope at 400× magnification: A. Suri 3, B. Super 1, C. Super 2, D. Soper 6, E. Soper 7, F. Soper 9

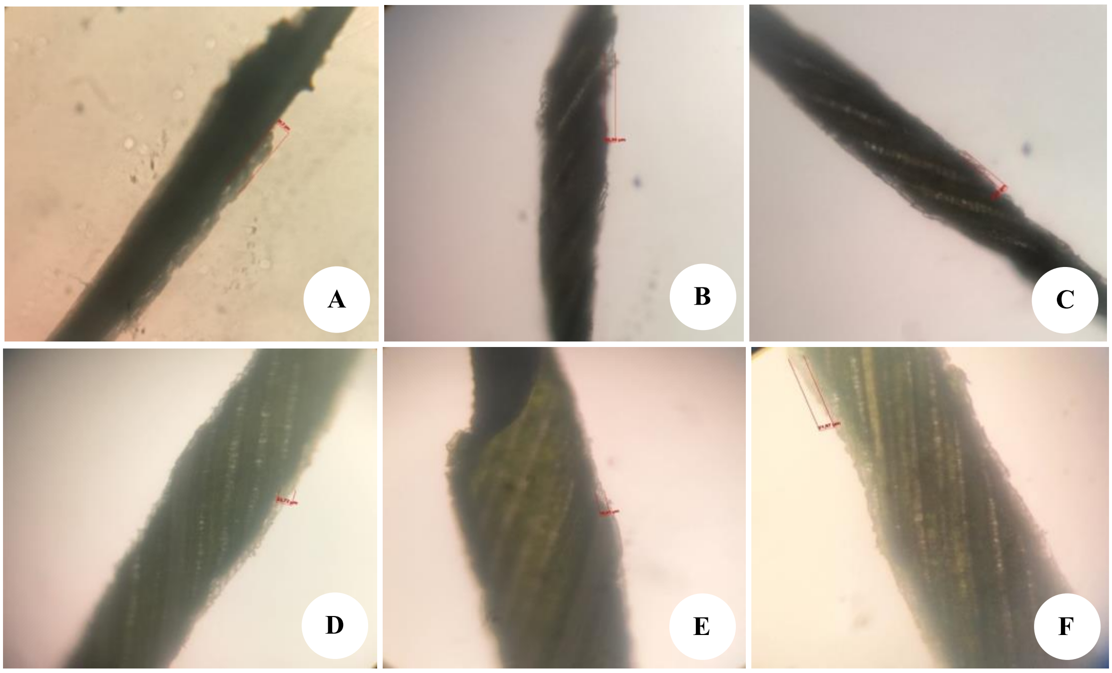

Figure 3. Leaf cuticle of six sorghum varieties under salinity stress observed under a light microscope at 400× magnification: A. Suri 3, B. Super 1, C. Super 2, D. Soper 6, E. Soper 7, F. Soper 9

Microscopic observations of leaf cross sections of six sorghum varieties showed differences in cuticle thickness under salinity stress conditions (Figure 3). The cuticle functions as an outer protective layer of the leaf that plays an important role in regulating transpiration and limiting the diffusion of salt ions into the inner tissues (Fernández et al. 2017). Microscopic observations showed that Suri 3 (Figure 3.A) and Soper 6 (Figure 3.D) varieties had relatively high cuticle thickness compared to other varieties, indicating potential morpho-anatomical adaptation to abiotic stress. High cuticle thickness is also closely related to increased plant tolerance to salinity stress because it can reduce the rate of water loss and slow the entry of toxic ions such as Na^+ and Cl^- into photosynthetic tissues (Srivastava 2022). In contrast, the Super 2 variety (Figure 3.C) showed the thinnest cuticle thickness, indicating a possible lower protective efficiency against salinity stress. Salinity causes changes in plant cells, one of which occurs in the cuticle, resulting in varying cuticle thickness. This is because salinity results in abnormal cell structures, including loss of membrane integrity, damage to the lamellae in the cytoplasm, vacuoles, cell walls, and intercellular spaces. This condition can increase the transpiration rate and penetration of salt ions into the tissue (Goodwin and Jenks 2007), thus accelerating plant physiological disorders. This variation in cuticle thickness reinforces the role of morphological mechanisms in sorghum's adaptation strategy to marginal environments, where varieties with thicker cuticles potentially have better resistance to salinity stress. From a breeding perspective, cuticle thickness has been reported to exhibit moderate to high heritability (Petit et al. 2017), indicating its potential as a reliable selection criteria in the development of sorghum genotypes with enhanced salinity tolerance.

Chlorophyll content measurements provide further insight into the photosynthetic capacity of the tested sorghum varieties. Suri 3 variety had the highest total chlorophyll content of 20.3555 mg/g, with chlorophyll a (13.5359 mg/g) dominating over chlorophyll b (6.7195 mg/g). Soper 6 and Super 1 also showed relatively high total chlorophyll contents of 19.6146 mg/g and 19.6305 mg/g, respectively. In contrast, Soper 7 recorded the lowest chlorophyll content (11.4456 mg/g), which may indicate lower photosynthetic capacity and limited adaptation to salinity stress (Table 4). High chlorophyll content is usually associated with the ability to maintain photosynthetic activity under stress conditions. Baiseitova et al. (2018) examined the effect of saline nutrient media on photosynthetic pigment content in several sweet sorghum varieties to identify the most tolerant varieties to chloride salinity. The results showed that increasing NaCl concentrations caused a decrease in chlorophyll a, chlorophyll b, and carotenoid levels in leaves. However, salinity-tolerant varieties maintained higher photosynthetic pigment content than susceptible varieties. This higher pigment content contributes to photosynthetic efficiency, which directly affects plant productivity. Thus, differences in chlorophyll content among varieties reflect genetic variation in physiological responses to salinity (Rauf et al. 2024) and provide a strong basis for selection of sorghum varieties tolerant to suboptimal environments.

In an effort to comprehensively assess the resistance of sorghum varieties to salinity stress, the biochemical traits of the seed are an important component that reflects the quality of yield and plant metabolic responses. The results of biochemical analysis showed that there were variations in water, ash, lipid, amylose, and protein contents among varieties. Soper 9 recorded the highest amylose and moisture content, while Super 2 and Soper 6 showed the highest protein content. Soper 7 had the highest lipid content and lowest water content, indicating efficient accumulation of secondary metabolites under saline conditions (Table 5).

Table 5. Biochemical traits of six sorghum varieties under salinity stress

Variety	Water content (%)	Ash (%)	Lipid (%)	Amylose (%)	Protein (%)
Suri 3	18.14	1.38	0.75	34.82	11.50
Super 1	15.42	1.27	0.68	31.49	11.03
Super 2	18.13	1.44	1.43	31.05	12.25
Soper 6	18.16	1.30	1.44	38.27	12.25
Soper 7	9.32	1.63	2.25	37.27	11.90
Soper 9	21.93	1.43	1.15	44.96	10.33

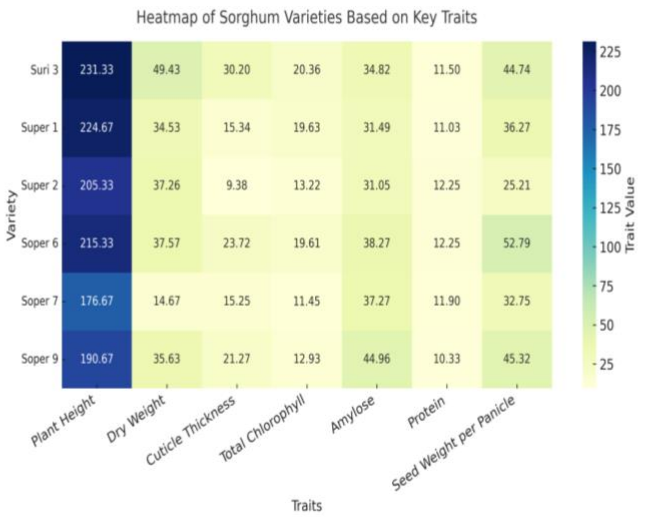

Figure 4. Heat map of six sorghum varieties with variations in morphological, physiological, and biochemical properties under salinity stress. The color gradient represents trait values, where darker blue indicates higher values and lighter yellow indicates lower values. The color scale bar (right) provides the numerical range of trait intensity

Sorghum has quite good tolerance to salinity stress. The results of the study showed that each sorghum variety has different biochemical traits (Table 5). The highest amylose content was found in the Soper 9 variety (44.96%), and the lowest amylose content was found in the Super 2 variety (31.05%). According to Ramadhan et al. (2020), amylose levels can be influenced by varieties, natural conditions, and where the plants originate. Salinity stress can affect amylose levels in sorghum, although the effects vary between genotypes. In line with Dehnavi et al. (2023), salinity stress can cause changes in amylose levels in sorghum starch. Some sorghum genotypes have a better ability to maintain stable amylose levels under salinity stress. Moreover, Soper 9 with high amylose content, suggesting a biochemical advantage that supports grain filling and quality under stress. These differences highlight that multiple tolerance mechanisms coexist among sorghum genotypes, and that breeding strategies should integrate both physiological performance and grain quality traits (Boyles 2016).

Besides, salinity can affect various aspects of sorghum plants, including their fat content. According to Kaboodkhani et al. (2024), salinity can disrupt various physiological processes, potentially influence lipid metabolism, and lead to changes in the overall fat content of the plant. In other studies, Ahmed (2022) specifically, salinity can interfere with the biosynthesis of lipids, leading to changes in fatty acid composition and potentially affecting the accumulation of storage lipids like oils and fats.

Salinity stress can significantly impact protein content in sorghum plants, with some studies showing both increases and decreases in specific protein types depending on the genotype and the level of salinity. While some research, such as that of Elsiddig et al. (2023) and Huang (2018), indicates that salinity can increase soluble protein content, other studies focus on specific proteins and their changes in expression under salinity stress. According to Chiconato et al. (2021) and Jha (2022), bioinformatics approaches enable the further identification of proteins involved in salinity stress responses. Protein expression variability under salinity stress is typically linked to specific biological functions and post-transcriptional or post-translational regulation, directly contributing to adaptive phenotypes through essential metabolic pathways. Therefore, specific protein functions play a more crucial role in salinity tolerance mechanisms than the total number of expressed proteins.

Genotypic response variations to salinity were identified in depth through a heatmap and Principal Component Analysis (PCA), which enabled the evaluation of correlations between traits and the identification of genotypic separation patterns based on salinity tolerance levels.

The heatmap visualization shows the distribution of values for several morpho-physiological and biochemical traits in six sorghum varieties tested under high salinity conditions (Figure 4). The color distribution pattern indicates that the Suri 3 and Soper 6 varieties exhibit high performance consistency across several parameters, particularly plant height, dry weight, cuticle thickness, total chlorophyll content, and grain yield per ear. This suggests that these two varieties demonstrate stronger tolerance mechanisms to salinity stress compared to other varieties. Conversely, Super 2 and Soper 7 exhibited lower values for several important parameters, such as total chlorophyll content, dry weight, and cuticle thickness, reflecting physiological disturbances caused by salt accumulation that hinder growth and photosynthetic efficiency (Novita et al. 2023; Wang et al. 2023). High amylose content was found in Soper 9. Still, this variety did not consistently show superior morphological performance or yield, suggesting that amylose content is not the primary indicator of salinity tolerance.

Cuticle thickness, total chlorophyll content, and dry weight appear to be the most sensitive and informative physiological indicators for distinguishing responses between varieties to salinity stress. A thicker cuticle is thought to play a role in reducing water loss and the penetration of toxic Na^+ ions (Taratima et al. 2023). Meanwhile, chlorophyll content is closely correlated with photosynthetic efficiency and leaf health status under salinity stress (Tsai et al. 2019).

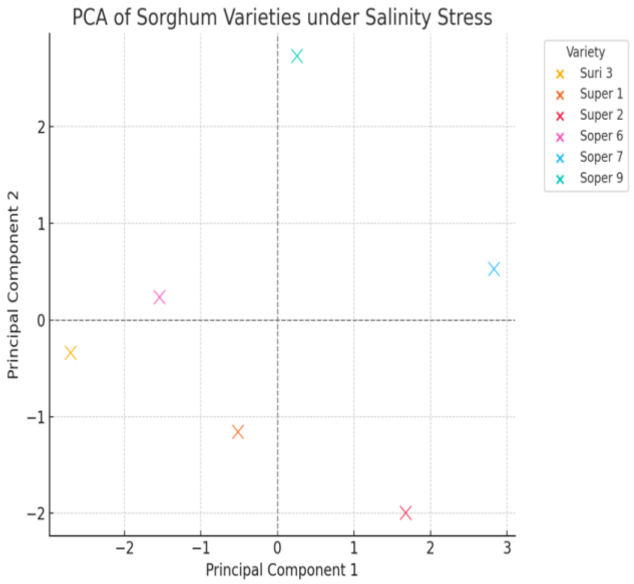

Figure 5. Principal component analysis (PCA) of six sorghum varieties based on morphological, physiological, and biochemical traits under salinity stress

Principal Component Analysis (PCA) was used to identify multivariate patterns and distributions of diversity among six sorghum varieties based on a number of morpho-physiological and biochemical traits under salinity stress. Visualization of the two principal components (PC1 and PC2) explains a significant proportion of the variance, with PC1 and PC2 cumulatively reflecting the main phenotypic differences between varieties (Figure 5). The PCA analysis explained a total variance of 84.82%, with PC1 accounting for 63.45% and PC2 for 21.37% of the total variation. PC1 was primarily associated with morphological and physiological traits such as plant height, dry weight, and chlorophyll content, whereas PC2 was influenced by biochemical parameters including total protein and amylose content. This indicates that the first two principal components effectively summarize most of the variability among the sorghum varieties under salinity stress.

The Suri 3 and Soper 6 varieties clustered in the negative PC1 area, indicating a stable combination of tolerance traits, including high cuticle thickness, relatively high total chlorophyll content, and high grain yield per panicle. This supports the claim that both are promising candidates for tolerant varieties. Similar studies have shown that the success of plant adaptation to salinity stress is strongly correlated with physiological features such as membrane integrity (Harsha et al. 2025) and photosynthetic efficiency (Hassan et al. 2024). Meanwhile, the PCA plot shows that Soper 9 and Soper 7 occupy extreme positions in the upper right and lower right quadrants of the graph, indicating unique characteristics that distinguish them from other varieties. Soper 9, located far in the upper right quadrant, is associated with high amylose content, medium cuticle thickness, and satisfactory grain yield. Conversely, Super 2 and Super 1 are located in opposite and distant areas, reflecting highly contrasting responses to stress, particularly in terms of photosynthesis and production.

The distribution pattern in the heatmap supports the differentiation of genotypic tolerance, where a combination of morpho-physiological and biochemical traits can be used as integrative markers for the selection of salt-tolerant sorghum varieties (Soltabayeva et al. 2021). A similar approach has also been successfully applied to other crops such as rice (Alkahtani and Dwiningsih 2023), wheat (Ashraf et al. 2023), and maize (Zamani et al. 2024). Furthermore, the clear separation between varieties in PCA indicates that these parameters can be used as marker traits in breeding programs to improve sorghum tolerance to salinity stress.

This study successfully identified the morpho-physiological and biochemical traits that distinguish the tolerance levels of six sorghum varieties to salinity stress. However, several important physiological indicators, such as proline accumulation, Relative Water Content (RWC), and Na^+/K^+ ratio, which are widely recognized as key markers of salinity tolerance, were not included in this study, and this should be recognized as a limitation of the present study. Therefore, further research is needed to integrate these physiological parameters with morpho-biochemical traits to gain more comprehensive understanding of variety responses to saline conditions.

In conclusion, differences in morpho-physiological and biochemical responses among sorghum varieties to salinity stress indicate the presence of genetic potential that can be exploited for the development of tolerant varieties. The use of multivariate approaches and data visualization successfully identified the Suri 3 and Soper 6 varieties as the most tolerant candidates to salinity, characterized by the highest dry weight (49.43 and 37.57 g, respectively), cuticle thickness (30.20 and 23.72 µm), total chlorophyll (20.3555 and 19.6146 mg g^{-1}), and protein content (11.50 and 12.25%) under saline conditions. In contrast, Super 2 exhibited the lowest performance across most traits, confirming its sensitivity to salinity. Key parameters such as dry weight, cuticle thickness, total chlorophyll, and protein content emerged as important indicators for variety selection. These findings provide a strong foundation for the development of adaptive sorghum varieties through functional trait-based breeding programs. Future research should expand across multiple environments and incorporate molecular and physiological markers (e.g., proline, RWC, and Na^+/K^+ ratio) to validate these tolerance traits.

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