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Research Article

Growth and Physiological Responses of *Miscanthus* × *Giganteus* to Combined Salinity and Drought Stress

Ghulam Murtaza[1], Muhammad Baqir Hussain[1*], Suleman Haider Shah[1,4], Muhammad Usman Jamshaid[1,2], Gulzar Akhtar[3], Muhammad Yasir[1]
[1] Department of Soil and Environmental Sciences, MNS University of Agriculture, Multan, Pakistan.
[2] Department of Outreach and Continuing Education, MNS University of Agriculture, Multan, Pakistan.
[3] Department of Horticulture, MNS University of Agriculture, Multan, Pakistan.
[4] Department of Agriculture (Extension and Adaptive Research), Government of Punjab, Murree, Pakistan.

ABSTRACT

Miscanthus × *giganteus* is a high-yielding, perennial C_4 grass recognized for its potential as a lignocellulosic biomass crop with a net-positive carbon footprint, ideal for sustainable bioenergy production. Its adaptability to grow on marginal lands, such as those in arid and semi-arid regions of Pakistan, highlights its potential to reduce pressure on fertile agricultural areas. However, salinity and water scarcity are major limiting factors in these environments. A three-month pot trial was conducted at MNS University of Agriculture, Multan, using a completely randomized factorial design with four replicates with the objective, to assess the effects of salinity and drought stress on *Miscanthus* growth, physiology and nutrient uptake. Treatments included two salinity levels i.e., 4 dS m^{-1} (S1, moderate salinity) and 6 dS m^{-1} (S2, high salinity), two irrigation regimes i.e., 100% field capacity (FC1) and 50% field capacity (FC2), and a non-saline control. Results showed that control plants under full irrigation (FC1) had significantly higher growth, biomass, photosynthetic traits, and nutrient uptake compared to stress treatments, with up to 65% higher biomass and substantially reduced electrolyte leakage. In contrast, plants under S2 with FC2 exhibited a 65% reduction in biomass. Nitrogen and phosphorus levels increased under moderate salinity (S1) with full irrigation (FC1), while potassium declined under all stress treatments. Overall, FC1 mitigated stress impacts significantly better than FC2. These findings suggest that *Miscanthus* can tolerate moderate salinity if sufficient water is available, making it a viable crop for saline-prone arid regions. Future studies should focus on long-term field evaluations and the development of effective management practices to enhance the resilience of *Miscanthus* × *giganteus* to salinity and drought stresses.

Keywords: Biomass accumulation, Combined abiotic stress, *Miscanthus* × *giganteus*, Nutrient uptake, Photosynthetic traits, Salinity and drought.

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Correspondence
Muhammad Baqir Hussain
baqir.hussain@mnsuam.edu.pk

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INTRODUCTION

Miscanthus × *giganteus*, a sterile hybrid C_4 perennial grass, has gained recognition for its high biomass yield and potential to contribute to net-negative carbon emissions, positioning it as a strong runner for sustainable bioenergy production (He et al., 2024). Its capacity to grow on degraded and low-input soils makes it particularly suitable for cultivation on marginal lands. However, environmental stresses such as salinity and drought significantly impact its productivity. Scientific research indicates that elevated salt concentrations cause a notable decline in biomass, with a 50% reduction observed at 10.65 dS m^{-1} NaCl. Despite this, physiological traits such as photosynthesis and water use efficiency show resilience

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under moderate salinity conditions (Stavridou et al., 2020). Abiotic stresses including drought and salinity disrupt yield potential through morphophysiological and biochemical alterations (Arias et al., 2023). To address these challenges, the EU OPTIMISC project identified stress-tolerant *Miscanthus* genotypes capable of maintaining biomass quality across diverse environments (Lewandowski et al., 2016), offering a pathway for sustainable energy systems under variable climatic conditions.

In Pakistan, *Miscanthus* × *giganteus* holds promise as a bioenergy crop suited to saline and arid soils where traditional crops struggle. It can be cultivated on lands degraded by poor water quality and excessive fertilization, thus relieving pressure on fertile agricultural areas. Research has shown that this species can tolerate moderate salinity and persist in water-deficient soils, enabling its use in marginal and wasteland zones (Steinhoff-Wrześniewska et al., 2022). In such restricted environments, *Miscanthus* × *giganteus* offers a sustainable solution for expanding bioenergy resources without compromising food security. However, its biomass productivity declines sharply in highly saline soils, with root development and leaf photosynthesis particularly affected (Pidlisnyuk et al., 2022). Combined drought and salinity stress exacerbates physiological limitations by inducing photoinhibition and altering metabolic activity (Stavridou et al., 2019). Given these limitations, immediate research attention is needed to understand and enhance stress tolerance mechanisms in *Miscanthus* × *giganteus*, especially under combined abiotic stress. Developing effective management strategies and identifying resilient genotypes are essential steps for optimizing its role in sustainable agriculture and energy production in regions facing climate-related resource constraints.

The physiological and biochemical responses of *Miscanthus* × *giganteus* to salt stress combined with drought have not been thoroughly studied. Multiple stress factors influence the growth, biomass composition, and stress tolerance of *Miscanthus* × *giganteus* (Stavridou et al., 2019). Several area specific cultivation methods remain immature because scientists lack knowledge about this species' natural reaction to drought and salt existence. *Miscanthus* × *giganteus* cultivation for maintaining bioenergy operations across harsh environmental zones require detailed studies of its growth patterns, biological responses to salinity and drought stresses. Studies focusing on genetic selection of tolerant varieties, along with the examination of morphological and photosynthetic changes, contribute to the development of optimal management practices for marginal lands (da Costa et al., 2019). Soil and climate information maintenance allows stakeholders to make precise land management decisions that enable accurate estimates of plant yields.

Water irrigation and soil salt concentrations determine how *Miscanthus* × *giganteus* grows and develops its physiological traits. Research was conducted to find out whether marginal land would support plant cultivation when faced with dual abiotic stress (Liang et al., 2022). The research studied *Miscanthus* × *giganteus* development and physiological processes along with macro elements content under conditions of salt stress and dry weather to determine its tolerance for marginally stressed environments (Jeżowski 2017). Research confirms that irrigated *Miscanthus* × *giganteus* tolerates minor salinity conditions (Stavridou et al., 2020; Steinhoff-Wrześniewska et al., 2022). Saline environments support plant growth and biomass accumulation, making them suitable areas for bioenergy production (Liang et al., 2022). Therefore, the current study was planned with the objectives of evaluating the growth performance, physiological responses and nutrient uptake of *Miscanthus* × *giganteus* under combined salinity and drought stress. The study specifically hypothesized that full irrigation (100% field capacity) would mitigate the adverse effects of salinity (4 and 6 dS m^{-1}) on plant development, while limited water availability would intensify stress responses, reducing biomass and physiological efficiency.

MATERIALS AND METHODS

Experimental Design and Treatments

A research trial was conducted at MNS University of Agriculture Multan in its B Block Lath House facilities in spring 2024. The experiment used twenty-four pots containing seven kg of ground, and sieved soil for its physical properties (sand 60%, silt 25%, clay 15%, with a pH of 7.8 and EC of 1.2 dS m^{-1}). There were four replications for each treatment in the experimental design that utilized a Completely Randomized Design (CRD). The applied treatments included salinity stress (CK (CK), S1 4, and S2 6 dS m^{-1}) and drought stress (100% and 50% field capacity). The 100% field capacity (FC1) treatment was used for optimal soil moisture without inducing waterlogging. Field capacity was first determined gravimetrically by saturating a representative soil sample and allowing it to drain for 48 hours. The amount of water retained after drainage was considered 100% FC. To maintain this level in pots, soil moisture was monitored daily using a moisture meter, and water was added each morning to replenish losses due to evapotranspiration. This practice ensured that the soil remained near field capacity but was not oversaturated. Although *Miscanthus* × *giganteus* shows some tolerance to waterlogging, irrigation was carefully controlled to avoid continuous saturation and to maintain

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adequate aeration in the root zone. Treatments were imposed 30 days after sowing, and plants were grown for 60 days before harvesting to assess growth, physiological and biochemical parameters.

Data Collection and Analysis

Plant height was measured from the base of the stem at soil level to the tip of the highest fully extended leaf blade, not including any flag leaves or senescent leaf tips. Only the main culm was measured in each plant. The number of leaves was recorded as the total number of fully expanded, green leaves on the main stem at the time of final harvest (60 days after treatment imposition). Leaves smaller than 5 cm or undergoing senescence were not counted. No side branches were present under the given pot conditions, and *Miscanthus* × *giganteus* maintained a single stem growth habit in all treatments during the trial. Stomatal conductance, sub-stomatal conductance, and photosynthetic rate were measured using a CIRAS system (CIRAS-3 Portable Photosynthetic System SW Version 2.00 Console Serial Number: C3F0255 via PP System, Amesbury, MA, USA) under full sunlight conditions. Chlorophyll and carotenoid content were analyzed spectrophotometrically after extraction with an acidified organic solvent (Grzeszczuk 2020). Relative water content (RWC) was measured based on fresh, turgid, and dry weights, effectively distinguishing between genotypes under water stress conditions (Islam et al., 2023). Electrolyte leakage analysis was performed by using the method, where leaf discs were incubated in distilled water and conductivity was measured before and after autoclaving to quantify total electrolyte content (Krzyżak et al., 2023).

A nutrient analysis of plant samples was performed using standard procedures. Total nitrogen (N) was determined by the Kjeldahl method, in which plant samples were digested with concentrated sulfuric acid and a catalyst mixture; the resulting ammonium was distilled and quantified through titration with standardized acid (AOAC Official Method 978.04). Phosphorus (P) content was determined using the molybdenum method, is measured spectrophotometrically at 410 nm. Potassium (K) concentration was measured using a BWB flame photometer. Calcium (Ca) was analyzed via atomic absorption spectrophotometry (AAS) at 422.7 nm after sample digestion with nitric-perchloric acid (AOAC Official Method 968.08). Soil samples were further analyzed for extractable phosphorus using Olsen's method and extractable potassium through ammonium acetate extraction (De Vega et al., 2021). Soil texture was assessed using the hydrometer method following dispersion in sodium hexametaphosphate (Dascalu et al., 2022). Data of parameters were statistically analyzed by using Statistics 8.1. Means were separated using the least significance difference LSD test (Steel et al*.,* 1997) at α = 0.05.

RESULTS

Salinity and drought stress significantly influenced the physiological, morphological, and biochemical attributes of *Miscanthus* × *giganteus*. A decline in shoot length, leaf length, and leaf number was observed under increasing salinity and drought conditions. Chlorophyll and carotenoid contents were 22% and 10% reduced in response to stress, indicating impaired photosynthetic efficiency. Relative water content decreased by up to 35% under drought and salinity stress, while electrolyte leakage increased by up to 30%, suggesting higher membrane damage. Macronutrient (NPK) uptake was also affected in stressed plants, with reduced nitrogen, phosphorus, and potassium concentrations. The photosynthetic rate declined progressively with increasing salinity and water deficit, demonstrating the adverse effects of abiotic stress on plant metabolism.

The results indicated that *Miscanthus* × *giganteus* possessed stress tolerance properties but its growth suffered from elevated salinity conditions and extended drought periods. All physiological, biochemical, and morphological parameters were statistically compared against the CK FC1 treatment, which served as the reference baseline for determining significance using the LSD test at $p \leq 0.05$. The plant shows distinct responses to different stress levels according to statistical verification.

Plant Growth Parameters

Plant height, leaf length, and number of leaves per plant were measured under salinity treatments (CK, S1, S2) and two irrigation levels (FC1, FC2). Plant height and leaf size along with number of leaves per plant serve as crucial indicators for assessing plant vigor in stressful conditions. This makes them vital for examining how soil additives and soil water conditions affect plant development.

Researchers use plant height as an indicator of vegetative and root development, as well as nutrient assimilation capacity. The plants grown in FC1 of the CK soil (untreated) reached their maximum height at about 125 cm, per Table 1. These specific conditions indicate optimal growing situations because they combine local soil and an adequate water supply. A significant decrease in plant height was observed in the S2 soil amendment treatment in contrast to S1 and S2. Under moisture-limited conditions (FC2), plant height decreased across all treatments, underscoring the

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detrimental effect of water stress. However, under FC1 (saturated) conditions, the CK treatment showed the greatest plant height, likely due to the absence of salinity stress.

Leaf length is a useful morphological trait reflecting photosynthetic capacity and physiological status under varying environmental inputs. As shown in Table 1, the longest leaves (42 cm) were observed in the CK soil at FC1, while S1 and S2 treatments led to shorter leaves (35 cm and 30 cm, respectively). Under FC2, a marked decline in leaf length occurred across all treatments, with the greatest reduction seen in the S2 treatment, where leaf length dropped below 30 cm. Despite the reduced moisture, plants in the CK soil maintained longer leaves than those in the amended soils, suggesting better water retention or nutrient availability in the original soil. Leaf count per plant is another key parameter, often linked to photosynthetic potential and overall biomass accumulation. Table 1 shows that the highest leaf number (12 leaves per plant) was recorded in the CK soil under full water availability. In contrast, the lowest count (6 leaves) was found in the S2 treatment under 50% field capacity. The trend remained consistent across both moisture conditions: the CK soil supported higher leaf production than S1 and S2, emphasizing the potential limitations of the modified soils in supporting vegetative expansion under both optimal and water-deficient conditions.

Table 1. Effects of salinity (S1: 4 dS m^{-1}, S2: 6 dS m^{-1}) and drought (FC1: 100%, FC2: 50%) on plant growth traits of *Miscanthus × giganteus*

Treatments		Plant Height (cm)	Leaf Length (cm)	Leaves per plant (No.)
FC1	CK	121.7 a	41.3 a	11.3 a
	S1: 4 dS m^{-1}	105.3 b	34.7 c	9.0 c
	S2: 6 dS m^{-1}	94.5 c	30.3 d	7.5 d
FC2	CK	92.8 c	38.0 b	10.0 b
	S1: 4 dS m^{-1}	80.6 d	32.3 d	7.8 d
	S2: 6 dS m^{-1}	67.8 e	26.5 e	6.0 e

The treatment means sharing similar letters are not significantly different from each other according to LSD at $P \leq 0.05$.

Physiological Measurements

Understanding plant physiological responses under varying environmental conditions provides insight into their adaptability and overall health. In this study, three fundamental gas exchange parameters, photosynthetic rate, stomatal conductance, and sub-stomatal conductance, were evaluated under two water levels: 100% field capacity (FC1), representing optimal moisture availability, and 50% field capacity (FC2), simulating drought conditions. The treatments applied, control, S1, and S2, likely represent differences in soil composition or amendment type, which can influence plant physiology through their impact on soil structure, nutrient dynamics, and microbial interactions. Photosynthesis, quantified as µmol CO_2 m^{-2} s^{-1}, is a primary measure of plant productivity and is sensitive to soil quality and water status. As shown in Table 2, the highest photosynthetic rate was recorded in plants grown in CK soil under FC1 conditions, indicating optimal carbon assimilation. Lower but comparable rates followed this in S1 and S2. Under limited water conditions (FC2), photosynthetic rates declined across all treatments, with the most pronounced reduction observed in S2. This suggests that S2 treated plants were more adversely affected by water stress, likely due to less favorable root-soil interactions or reduced physiological plasticity. The consistent superiority of the CK treatment under both moisture conditions highlights its better suitability in supporting photosynthetic efficiency.

Stomatal conductance (mmol H_2O m^{-2} s^{-1}) reflects the degree to which stomata are open, thereby regulating transpiration and the influx of CO_2 for photosynthesis. Table 2 illustrates that those plants under FC-100 maintained higher stomatal conductance than those under FC2, demonstrating more favorable conditions for gas exchange. Among the treatments, the CK consistently showed the highest values under both moisture levels, while S2 exhibited the lowest. This pattern underscores the potential of the CK soil to support better water balance and stress management, likely due to its structural and compositional properties that favor sustained physiological functioning. Sub-stomatal conductance (µmol mol^{-1}), which estimates the CO_2 concentration in the intercellular leaf spaces, representing the plant's ability to use internal CO_2 for carbon assimilation. The data in Table 2 shows that sub-stomatal conductance reached its peak levels under FC2 conditions in all treatments. According to the analysis results the CK treatment maintained better internal CO_2 diffusion and utilization performance than treatments S1 or S2. A clear decrease was observed across all treatments under FC2 conditions, with S2 showing the most substantial effect. The decline in photosynthetic capacity results from restricted stomatal opening and impaired mesophyll conductance.

The research indicates that CK soil treatments under wet conditions maintain better physiological performance, yet S2 treatments under drought stress most notably restrict gas exchange parameters. Physical resilience depends heavily on suitable soil amendments and irrigation approaches when operating in environments prone to stress situations.

Photosynthetic Pigment Content

The photosynthetic pigments chlorophylls and carotenoids act as light energy harvesters while preventing plant oxidative harm. Under FC1 conditions total chlorophyll content reached its highest point whereas the CK treatment showed the most considerable accumulation according to Table 3. Water stress at FC2 reduced chlorophyll content among all treated plants because it decreased their photosynthetic capacity. Under stress conditions the chlorophyll content was higher in S1 and S2 treatments when compared to the CK treatment although the increase was not significant enough to indicate substantial CK over stress.

The data in Table 3 demonstrate that water conditions had minimal impact on carotenoid content levels. The examined water medium of FC2 demonstrated no alteration or produced a slight increase in carotenoid content with emphasis on the S2 treatment. The observed changes in carotenoid levels seem to indicate that plants adapt this way because these pigments protect photosynthetic structures from ROS during stressful conditions. The different patterns of chlorophyll reduction and carotenoid stability indicate that plants utilize double mechanisms to respond to stress. Recovery of chlorophyll content lowers photosynthesis but the augmented carotenoids help protect plant tissue through ROS scavenging. The data signifies that S1 and S2 treatments demonstrate promise for drought mitigation through their effect on stress-related pigment responses.

Electrolyte Leakage and Relative Water Content

Survival of plants in drought stress conditions depends on preserving membrane health and enough water content. Analyzing electrolyte leakage and relative water content (RWC) is an essential indicator of plant stress responses towards water deficit. The extent of membrane damage can be measured through electrolyte leakage, although it arises mostly from oxidative stress during dehydration periods. Relative water content details the plant's ability to protect the water content necessary for cellular processes. Multiple indicators serve the dual purpose of measuring drought tolerance capacity in crops and locating traits among genotypes and treatment groups that show enhanced resilience to stress.

The data in Table 2 indicates that membrane damage from water stress significantly rose when water reached 50% field capacity (FC2) for all experimental groups. The membrane stability of plants in S1 and S2 treatments proved superior to the CK due to their declining electrolyte leakage levels. The experiments showed a large decrease in RWC when the plants were under FC2 stress because their RWC levels dropped into the range of 30-40%, while well-watered plants (FC1) maintained RWC values between 60-80%. Water retention levels in S1 and S2 proved superior against the CK set under stress conditions. The research shows that S1 and S2 possess superior physiological characteristics, making them viable markers to enhance drought tolerance objectives through breeding or management initiatives.

Table 2. Effects of salinity (S1: 4 dS m^{-1}, S2: 6 dS m^{-1}) and drought (FC1: 100%, FC2: 50%) on growth traits of *Miscanthus × giganteus*

Treatments		A (μmol CO_2 m^{-2} s^{-1})	RWC (%)	EL (%)	g_s (mol m^{-2} s^{-1})	C_i (mol m^{-2} s^{-1})
FC1	CK	13.45 a	72 a	25.8 f	25.9 a	237.2 a
	S1: 4 dS m^{-1}	9.30 c	62 b	28.1 d	22.9 b	237.3 a
	S2: 6 dS m^{-1}	8.50 d	59.5 c	30.8 b	19.2 d	211.8 b
FC2	CK	10.08 b	55.75 d	26.8 e	23.3 b	210.3 b
	S1: 4 dS m^{-1}	7.68 e	51.25 e	29.1 c	21.3 c	218.3 b
	S2: 6 dS m^{-1}	6.83 f	48.5 f	31.6 a	18.3 d	171.7 c

Where A; Photosynthetic Rate, RWC; Relative water content, EL; Electrolyte leakage, g_s; stomatal conductance and Ci; Sub-stomatal conductance. The treatment means sharing similar letters are not significantly different from each other according to LSD at $P \leq 0.05$

Nutrient Analysis NPK

Nutrient homeostasis is fundamental to plant growth and stress adaptation, especially under drought conditions where nutrient uptake and transport are often impaired. Among essential macronutrients, nitrogen (N) supports protein synthesis and chlorophyll formation, phosphorus (P) is vital for energy transfer and root development, and potassium (K) regulates osmotic balance and stomatal function. Understanding how these nutrients respond to water deficits can reveal key physiological traits contributing to drought resilience and nutrient-use efficiency. Genotypes that maintain stable nutrient levels under stress are more likely to support sustained metabolic activity, delayed senescence, and better recovery after stress events.

As shown in Table 3, nitrogen concentration decreased significantly under water stress (FC2), with the sharpest decline observed in the CK treatment. However, S1 and S2 maintained higher Nitrogen levels under FC2, indicating better nitrogen uptake or conservation under limited water availability. Table 3 presents a similar pattern for phosphorus, where S1 and S2 again retained higher P levels than the CK under FC2, suggesting more effective phosphorus acquisition despite reduced soil mobility. In Table 3, potassium concentration generally declined under increasing stress. CK FC1 maintained the highest K levels, while S1 and S2 treatments showed reduced concentrations, especially under FC2. This reduction is consistent with known effects of salinity and drought, where ionic stress interferes with potassium uptake and transport, often due to sodium competition and impaired membrane function. The declining K pattern further supports the notion that plant growth and nutrient homeostasis were strongly inhibited under combined abiotic stress. These traits sustain growth and improve yield stability under adverse climatic conditions, making them valuable targets for breeding climate smart, resource efficient cultivars.

Table 3. Effects of salinity (S1: 4 dS m^{-1}, S2: 6 dS m^{-1}) and drought (FC1: 100%, FC2: 50%) on NPK, ionic balance and leaf pigments of *Miscanthus* × *giganteus*

Treatments		N (%)	P (mg g^{-1})	K (mg g^{-1})	Na (mg g^{-1})	Ca (mg g^{-1})	Carotenoid (mg g^{-1})	Chlorophyll (SPAD)
FC1	CK	1.19 a	0.788 b	67.9 a	215 d	80 a	3.60 a	65.25 a
	S1: 4 dS m^{-1}	1.14 b	0.818 a	55.43 c	285 b	72.3 cb	3.53 ab	62.50 a
	S2: 6 dS m^{-1}	1.09 b	0.77 b	49.38 e	324 a	65 de	3.23 abc	53.75 c
FC2	CK	0.60 c	0.425 d	60.45 b	169 e	77.5 ab	3.15 bc	65.00 a
	S1: 4 dS m^{-1}	0.55 d	0.453 c	54.65 cd	215 d	69.8 cd	3.10 c	58.00 b
	S2: 6 dS m^{-1}	0.48 e	0.395 e	51.88 de	271 c	62.8 e	2.91 c	48.00 d

The treatment means sharing similar letters are not significantly different from each other according to LSD at $P \leq 0.05$.

Analysis of Sodium and Calcium

Plant survival depends on maintaining proper ion balance for adaptation to both drought and salinity conditions under abiotic stress. Excess sodium is toxic and disrupts cellular processes and calcium is Important for membrane stability, signal transduction and stress signaling. The balance between these ions directly affects plant growth, stress perception, and tolerance mechanisms. Disruptions in Na^+/Ca^{2+} equilibrium under limited water availability are commonly associated with impaired root function and altered membrane permeability. Therefore, genotypes that efficiently regulate these ions under water stress exhibit enhanced physiological stability and survival potential.

As shown in Table 3, sodium concentration significantly increased under FC2 across all treatments, with values rising to nearly 350 units particularly in the CK genotype. This trend reinforces the role of water deficit in exacerbating Na accumulation. However, S1 and S2 treatments displayed lower sodium levels under stress, indicating a more controlled uptake or active exclusion strategy. In Table 3, calcium content was consistently higher under FC1, with notable declines under FC2, especially in the control. The inverse relationship between Na and Ca levels suggests an antagonistic uptake mechanism likely mediated by ion transporters or signaling proteins. The ability of S1 and S2 to maintain lower Na and higher Ca under stress conditions points to their enhanced ionic regulation capacity, which is crucial for maintaining membrane integrity and signal transduction under drought. A proper focus on traits related to ion balance must be integral when creating new crop varieties able to survive drought and salinity conditions.

DISCUSSION

This research trial examined how *Miscanthus* × *giganteus* reacts morphologically, biochemically, and physiologically to combine salinity and drought stress conditions when grown on marginal lands. Generally, research results indicate that biomass production can continue if salinity levels are balanced and adequate water supply. Water scarcity and severe salinity effects harm plant development and physiological function. Numerous previous studies confirm that *Miscanthus* × *giganteus* shows average resistance to environmental stresses while being susceptible when exposed to severe drought and salinity conditions at once. There was a significant reduction in plant characteristics such as plant height, leaf length, and leaf number as field capacity decreased in conjunction with increasing salinity (Table 1). The best plant heights and maximum leaf numbers emerged under conditions where field capacity reached 100%, thus proving that water access is fundamental in promoting plant growth. The combined stress from severe salinity stress (6 dS m^{-1}) and FC2 levels caused a 65% decrease in biomass production.

The research conducted showed that *Miscanthus* × *giganteus* experienced substantial biomass reduction and physiological limitations under combined salinity and drought in the present study. Similar drought-induced impacts on photosynthesis, growth, and biomass were reported by (De Vega et al., 2021), though their work did not include salt stress. *Miscanthus* genotypes showed significant decreases in biomass and plant height between high and low saline-alkaline soil areas because poor soil quality limits physiological development (Zheng et al., 2022). Photosynthetic inhibition increased consistently with the intensification of stress levels, as indicated by physiological measurements. Maximum photosynthetic rates and stomatal conductance levels occurred among CK plants that received full irrigation; however, the most serious combined stress caused minimal photosynthetic activity. Stomatal and sub-stomatal channels regulated their conductance activities when drought and salinity stresses occurred thereby affecting CO_2 intake and internal transport capabilities. Although (Lutts et al., 2024) reported that *Miscanthus* × *giganteus* exhibited seasonal variation in physiological traits when grown on metal-contaminated soils, the elevated sodium content in soil was not sufficient to induce classic salt stress. Their findings suggest that photosynthetic reduction and stomatal conductance changes were more likely linked to heavy metal toxicity and soil physicochemical interactions rather than salinity alone. The plants became more efficient in water use through stomatal regulation (Liang et al. 2022). The research on *Miscanthus* × *giganteus* plants under merged stress conditions verified that physiological regulation failure influenced both carbon assimilation process and saccharification traits. The decline in photosynthetic system integrity under stress conditions activated an increase in stress, reducing the number of pigments in the photosynthetic system (Ain et al., 2022). When compared to other irrigation levels the S2 treatment decreased watering level caused carotenoid levels to remain steady or possibly increase (Table 3).

The research showed that *Miscanthus* × *giganteus* preserved its pigment properties along with photochemical efficiency in moderate salt stress conditions (Stavridou et al., 2020). The plant suffered pigment loss because of photoinhibition together with osmotic stress during conditions of extreme salt stress. The assessment of plant pigments is an effective method to understand stress responses in *Miscanthus* plants grown on marginal soils (Malinská et al., 2020). Research evaluated membrane health and water management of the plant through electrolyte leakage along with relative water content (RWC) analysis (Table 2). The application of FC2 treatment led to cell deterioration and membrane deterioration across all experimental salinity concentrations yet displayed its maximum harm impact on plants stored within CK soil (Table 2). Membranes of plants under stress managed greater stability through S1 and S2 applications which also boosted their ability to maintain water content under adverse conditions. *Miscanthus* plants are maintained under reduced irrigation, likely due to the effect of reactive fertilizer applications (Lewin et al., 2025). Determining salt-tolerant plant varieties requires assessing their physiological characteristics that include membrane stability and water retention in addition to pigment content.

Drought and salinity stress caused essential nutrients including nitrogen (N), phosphorus (P) and potassium (K) to decrease because they limited root absorption along with physiological damage and disruptive effects produced by competing ions. The application of FC2 nutrient solution led to enhanced nitrogen and phosphorus absorption when plants were exposed to S1 and S2 treatments (Table 3). Therefore, stress adaptations within the root functions must have contributed to observed improvement. Stress tolerant *Miscanthus* genotypes displayed superior regulation of N and P during unfavorable circumstances (Xiao et al., 2024). A balanced compound mix in the FC2 solution directly enhanced both photosynthetic activity and biomass productivity. The capability of plants to use nutrients efficiently in stressing environments remains an essential breeding criterion for sustaining their growth in marginal soils.

The application of FC2 resulted in significant changes to sodium (Na) content in plants while they produced in untreated CK soil conditions (Table 3). The application of calcium led to a decrease in Ca levels because ion competition affected the regular functioning of ion transport processes (Table 3). Sodium concentration increased under both S1 and S2 treatments (Table 3), while calcium levels declined in parallel with increasing salinity (Table 3). This pattern suggests that the plants did not exhibit active sodium exclusion or calcium retention under stress. Instead, *Miscanthus* × *giganteus* may have undergone a stepwise physiological inhibition due to salt-induced osmotic imbalance and ion toxicity. The observed decline in chlorophyll content, photosynthetic rate, and potassium levels further supports this interpretation, as ion imbalances can disrupt chloroplast stability and enzyme function. Plants under moderate salinity (S1) showed slightly better performance than under S2, indicating a threshold effect rather than efficient ion regulation, as some physiological processes remain partially active before severe inhibition. These findings indicate the need for deeper physiological or molecular studies to confirm specific tolerance mechanisms.

The experimental data verifies that *Miscanthus* × *giganteus* shows tolerance against moderate salt concentrations as long as it receives sufficient water supply. This strategy offers a practical means to evaluate bioenergy development in saline and dry areas of southern Punjab, assuming that irrigation and soil management practices support plant resilience. Future research should pursue extended field tests with studies about genotype environment dynamics and utilizing microorganisms for stress tolerance management to optimize biomass production. The research demonstrates that *Miscanthus* × *giganteus* shows stress tolerance under certain conditions of abiotic stress exposure. Productivity experiences a severe reduction under severe salinity drought conditions but irrigated crops can withstand moderate salinity pressure.

CONCLUSIONS

The study highlights the ability of *Miscanthus* × *giganteus* to tolerate moderate salinity (4 -6 dS m^{-1}) under full irrigation (FC1), sustaining growth, photosynthesis, and nutrient uptake. However, combined salinity and drought (FC2) severely reduced biomass (65%), photosynthetic efficiency, and nutrient absorption, with sodium toxicity and calcium deficiency exacerbating stress. Optimal performance occurred in non-saline, well-saturated conditions, while S2 and FC2 caused the greatest decline in plant height, leaf development, and physiological function. Stable carotenoid levels under stress suggested a protective role, but chlorophyll and stomatal conductance dropped significantly. These findings confirm potential of *Miscanthus* for marginal, saline regions if irrigation is maintained, though stress limits productivity. Future research should explore long-term field trials and stress-tolerant genotypes to enhance resilience in arid bioenergy systems.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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