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Growth Characterization of Sugarcane (*Saccharum* spp.) under Salinity and Drought Stresses at the Seedling Stage

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Abstract

This study aimed to assess the combined effects of salinity and drought stresses on the growth and physiology of sugarcane. The pot experiment was carried out in the Autumn cropping season of 2021 under the polyhouse conditions at the Vietnam National University of Agriculture. The experiment consisted of four treatments: non-stress treatment (control), drought stress, salt stress, and salt and drought stress (combined stress). Five weeks after transplanting, salt stress was applied first for four weeks and followed by drought stress for another two weeks. The results showed that under the impact of stresses, sugarcane growth was inhibited with decreases in plant height, number of leaves, Fv/Fm, SPAD, and the fresh and dry weights of roots and stems. The growth and physiology indicators were the lowest under the combined effects of salinity and drought stress.

Keywords

Combined stress, drought, salinity, sugarcane growth

Introduction

Sugarcane (*Saccharum* spp.), a perennial grass of the family Poaceae, is a major sugar-producing crop in tropical and subtropical regions. In Vietnam, sugarcane is mostly grown in the provinces of Northern Central Vietnam, such as Thanh Hoa and Nghe An, the Central Highlands, and the Mekong Delta. According to the Vietnam Sugar Association, Vietnam harvested 141,906ha of sugarcane with an average cane production rate of 69.3 tons ha^{-1} in the 2022/23 season (Thuy Loan, 2023). Currently, sugarcane is considered an important industrial crop that is actively contributing to transforming the agricultural landscape, increasing economic efficiency, and improving the ecological environment.

Due to its long growth cycle from germination to ripening, sugarcane faces many adverse environmental conditions that affect its growth and yield. Drought and salinity stresses are caused by climate change and affect the morphological and physiological behaviors

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of sugarcane (Kaushal, 2019; Garcia *et al.*, 2020). Drought negatively changes a range of growth parameters such as increasing tillering, leaf discoloration, rolling of leaves, leaf folding and shredding, and reducing leaf area (Shrivastava & Srivastava, 2006; Karinki & Sahoo, 2019). Many physiological traits, such as the leaf chlorophyll content, photosynthetic rate, and stomatal conductance, among others, are markers for the selection of genotypes tolerant to drought stress (Silva *et al.*, 2012; Basnayake *et al.*, 2015). In terms of root traits, the depth and volume of roots are also considered as important criteria for selecting drought-tolerant genotypes (Smith *et al.*, 2005).

Salinity stress is also a major abiotic stress that influences sprout emergence, nutritional balance, and growth, leading to reductions in biomass production and sugar yield. The cane height, leaf area, and biomass are the traits most affected. Salinity coupled with subsequent drought are severe problems for the coastal area of Vietnam. The early growth stages of sugarcane, namely germination, tillering, and cane formation, are more sensitive than the later stages. Vasantha *et al.* (2017) reported that leaf area index, SPAD, and chlorophyll fluorescence efficiency are affected the most in the formative and grand growth stages under salinity stress (EC = 8 dS m^{-1}).

Previous studies have been conducted to assess the effects of drought or salinity stresses on sugarcane growth and the losses or improvements in cane growth under such conditions. Dinh *et al.* (2023) reported on the individual and combined effects of individual stresses (saline or drought) on the growth and physiological parameters of sugarcane. Jaiphong *et al.* (2016) showed that flooding had more negative effects on plant growth but was followed by drought stress. However, none of the studies have presented a comparative evaluation of cane grown under both consecutive drought and salinity conditions. Thus, this study aimed to assess the morphological changes that occur in sugarcane under both salinity and subsequent drought conditions.

Materials and Methods

Materials

Twenty-five-day-old sugarcane seedlings were used in this experiment. The sugarcane seedlings were propagated from ten-month-old healthy stalks of the commercial ROC10 cultivar.

Experimental design

The experiment was carried out in a greenhouse facility at the Vietnam National University of Agriculture, Hanoi, Vietnam. Single sets were germinated on soil trays for 25 days, and then each seedling was grown in individual pots containing 10kg of soil mixture (3 alluvium soil: 1 sand, v/v). In the salinity stress treatment, each pot was watered with 25mL of salinized nutrient solution containing sodium chloride (100mM) starting on the fifth week and continuing for four weeks. The combined stress treatment consisted of salinity stress starting on the fifth week and continuing for four weeks, followed by drought stress for two weeks (**Figure 1**). The drought stress treatment was initiated in the ninth week and lasted for two weeks. After the stress treatments, the pots were regularly watered during the recovery period. Pots were arranged in a factorial in a randomized complete block design with three replications. To assess the physiological traits, the chlorophyll fluorescence efficiency (Fv/Fm) and SPAD were recorded weekly at 10:00 a.m. on the first full leaf from the top by an Opti-Science Chlorophyll Fluorometer OS-30p (Hudson, USA) and a SPAD 502 Plus Meter (Minolta, Japan), respectively. The actively growing roots and leaf samples were sampled on the 8^{th}, 10^{th}, and 14^{th} weeks for biomass estimation. Sugarcane growth was scored based on the measurements of plant height and the number of leaves every week.

Data analysis

Data recorded for sugarcane growth were analyzed with analysis of variance (ANOVA) using IRRISTAT 5.0. Different means were

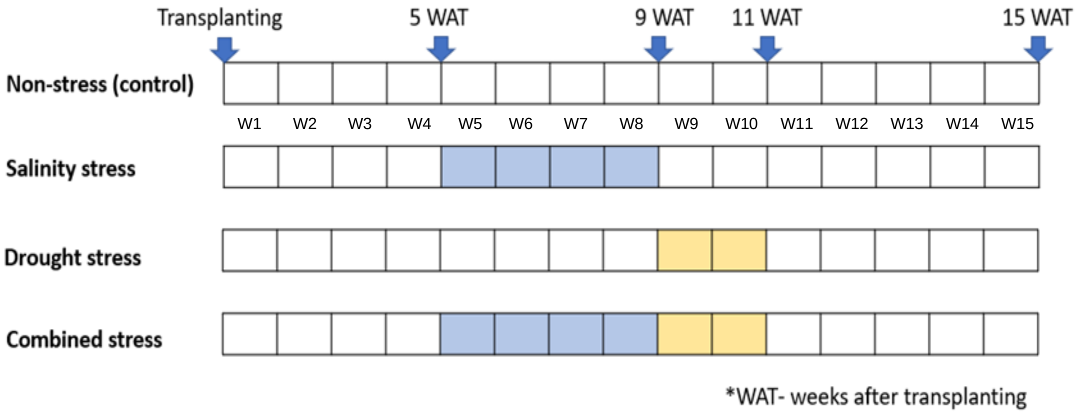


Figure 1. Timeline of stress treatment on sugarcane

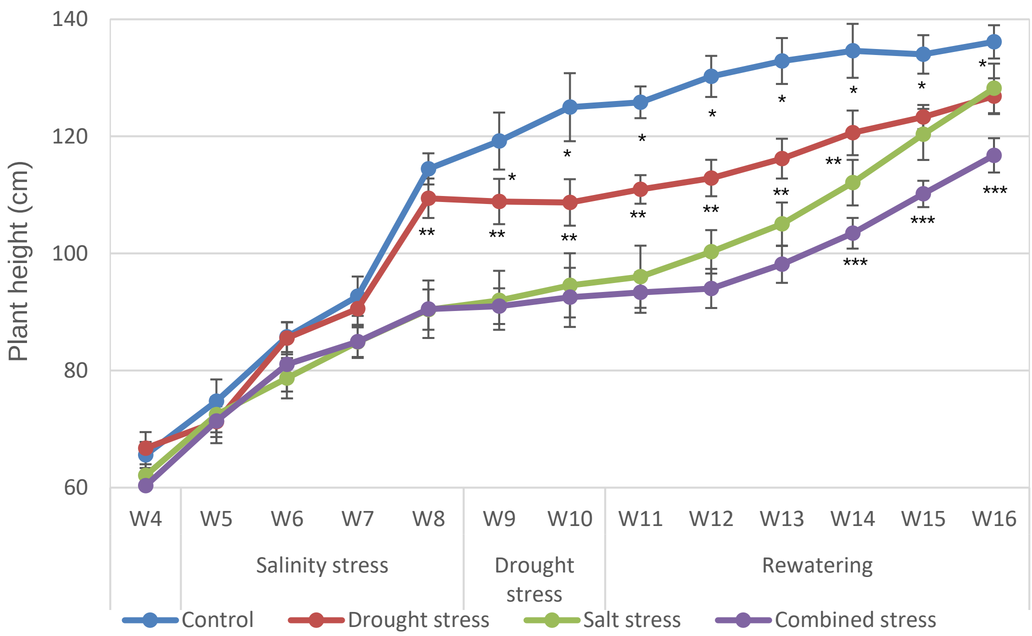
compared using Duncan's Multiple Range Test at $P \leq 0.05$.

Results

Effects of salinity stress and drought stress on the growth dynamics of sugarcane

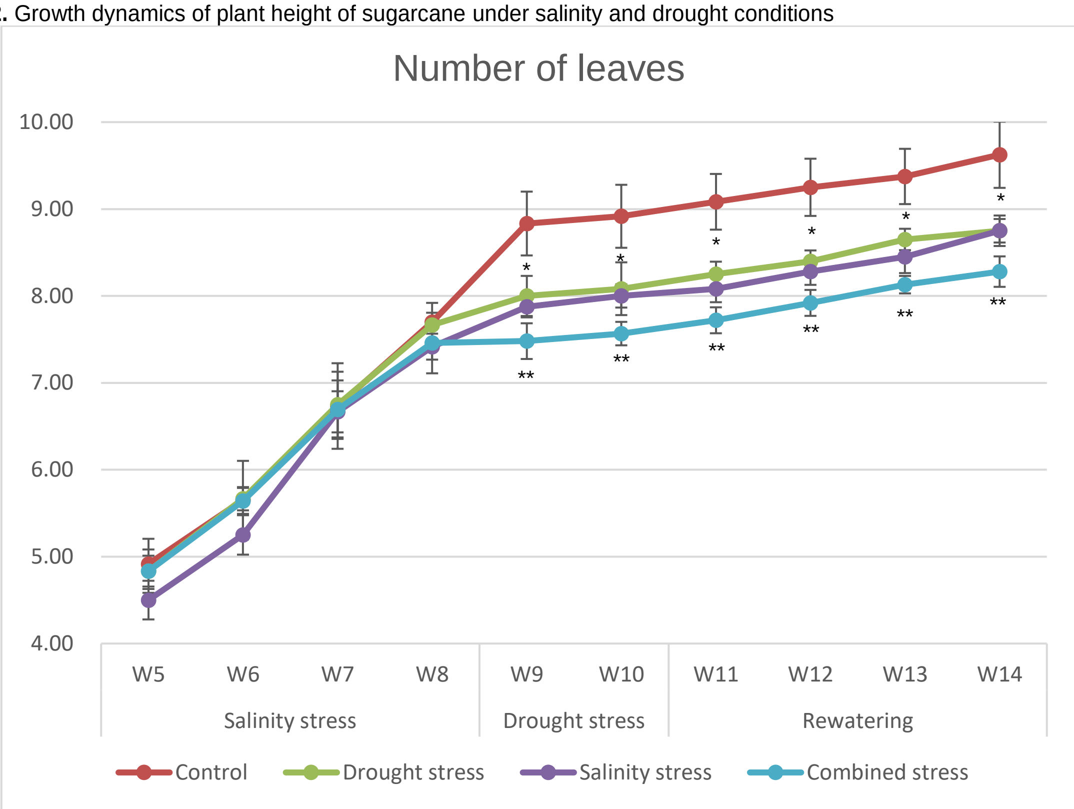
During the salt stress period, a great reduction was observed in the growth rates of the salinity stress and combined stress conditions compared to the control (**Figure 2**). In the treatments treated with drought stress, plant height ceased to increase. During the rewatering stage, the plant height of all the stress conditions increased at different rates. The plant height in the combined stress condition recovered the least (14% lower compared to the control condition).

After the salinity stress period, there were no significant differences in the number of leaves among all the treatments. The number of leaves in all the treatments increased steadily to 7.46



*Note: W- weeks after transplanting; *, **, ***: The means are significantly different between the control and other treatments; drought stress and salt stress; and salt stress and combined stress at P ≤0.05, respectively.*

Figure 2. Growth dynamics of plant height of sugarcane under salinity and drought conditions



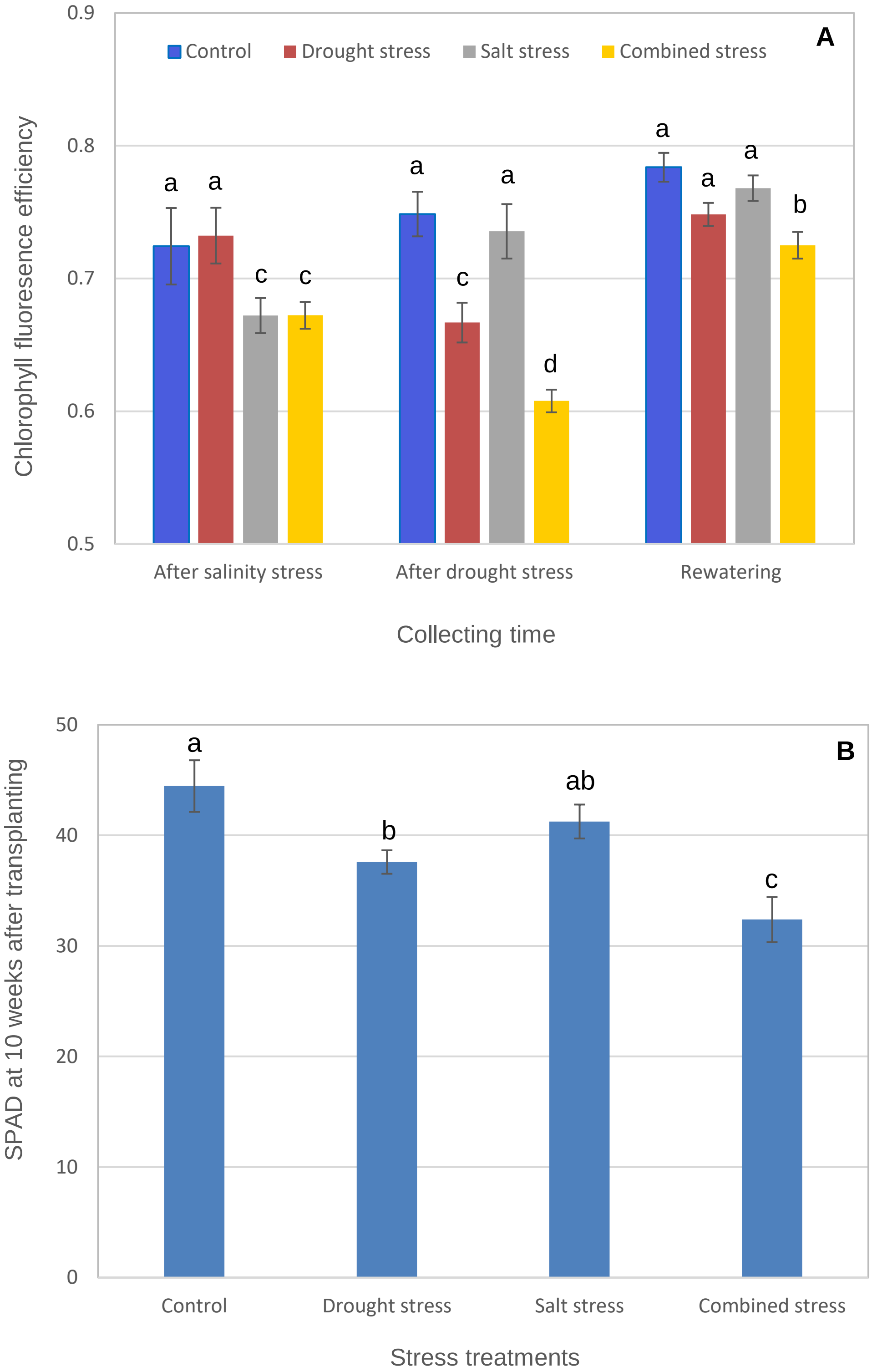
*Note: W- weeks after transplanting; *, **: The means are significantly different between the control and other treatments, and the salt stress and combined stress at P ≤ 0.05, respectively.*

Figure 3. Growth dynamics of the number of leaves of sugarcane under salinity and drought conditions

leaves/plant in the salinity stress treatments, and around 7.70 leaves/plant for the non-salinity stress treatments (**Figure 3**). During the drought stress period, the changes in the number of leaves in the four treatments had significant differences. At the end of drought stress, the highest number of leaves was found in the control condition (9.08 leaves/plant) and the lowest in the combined treatment (7.72 leaves/plant). After rewatering, the largest number of leaves was still in the control with 9.63 leaves/plant, whereas the combined stress treatment had the fewest number of leaves with only 8.28 leaves/plant.

Effects of salinity stress and drought stress on the physiological traits of sugarcane

Chlorophyll fluorescence efficiency (Fv/Fm) is a parameter that reflects the physiological state of the photosynthetic apparatus under adverse conditions. After the salinity period, the control condition had the highest Fv/Fm value, while the salinity and combined stress treatments had the lowest values (**Figure 4A**). After the drought stress period, the low Fv/Fm of the combined stress continued, whereas it recovered in the saline stress treatment. In the drought stress treatment, the Fv/Fm reduced to 0.66, significantly lower than in the control and saline stress treatments, but noticeably higher than the Fv/Fm in the combined treatment. At the recovery stage, the Fv/Fm values of all the stress treatments recovered. Although the Fv/Fm of the stress treatments were still lower than the control, a significant difference was found only in the combined stress treatment. For SPAD chlorophyll indexes, after the stress period (10 weeks after transplanting), significant reductions were found in the stress treatments in comparison to the control, especially in the combined stress treatment (**Figure 4B**).



Note: The means followed by different letters are significantly different at P ≤0.05

Figure 4. Effects of salinity and drought stresses on chlorophyll fluorescence efficiency (A) and SPAD (B) of sugarcane plants

Effects of salinity and drought stresses on the root and stalk biomasses of sugarcane

After the salinity treatment, the fresh and dry weights of the roots under the salinity and combined treatments decreased in comparison to the control with reduction rates of 14.07% and 14.98% in the fresh weights of the salinity and combined stresses, respectively. Root dry weights of the salinity and combined stresses showed decreases of 24.54% and 29.45%, respectively, compared to the roots of plants grown under normal conditions (Table 1). Similarly, there were reductions in both the stalk fresh and dry weights in the salinity stress and combined stresses by ranges of 23.97-31.46% and 24.28-31.23%, respectively (**Table 2**). The data showed that the difference between the salinity stress and control was statistically significant ($P < 0.05$).

After the drought stress period, the fresh weights of the roots showed significant reductions in the water stress treatments. The highest reduction was found in the combined stress treatment by 32.03%, followed by the drought stress treatment by 18.78%. There was a recovery in the root fresh weight of the saline stress treatment but it was still significantly lower than the control (**Table 1**). There were similar reductions in the dry weights of roots under the drought, salinity, and combined stresses. After a short period of rewatering, the root accumulation in the salinity stress still decreased, but at a lower rate than the other stress treatments.

The fresh weights of the stalks varied from 17.05g in the combined stress treatment to 27.55g in the control treatment, and the dry weight of the stalks varied from 5.76g to 8.23g, respectively. Both drastic reductions were obtained in the combined stress treatments (**Table 2**). There was differential sensitivity to stress individually and in combination. The reductions

Table 1. Effects of salinity and drought stress on the fresh and dry weights of sugarcane roots

Collecting time	Stress treatment	Fresh weight (g)	Dry weight (g)	The relative reduction rate (%)	
				Fresh weight	Dry weight
After salinity stress	Control	8.81^{b}	1.63^{b}	-	-
	Drought	8.31^{b}	1.51^{b}	-	-
	Salinity	7.57^{a}	1.23^{a}	14.07	24.54
	Combined stress	7.49^{a}	1.15^{a}	14.98	29.45
	$LSD_{0.05}$	0.81	0.35		
	CV%	6.4	6.5		
After drought stress	Control	11.02^{b}	2.43^{b}	-	-
	Drought	8.95^{a}	1.83^{a}	18.78	24.69
	Salinity	10.08^{a}	2.18^{a}	8.53	10.29
	Combined stress	7.49^{a}	1.66^{a}	32.03	31.69
	$LSD_{0.05}$	3.36	0.51		
	CV%	14.9	7.7		
Re-watering	Control	17.7^{b}	2.87^{b}	-	-
	Drought	15.68^{b}	2.51^{b}	11.41	12.54
	Salinity	15.11^{b}	2.48^{b}	14.63	13.58
	Combined stress	11.37^{a}	1.95^{a}	35.76	32.06
	$LSD_{0.05}$	1.23	0.50		
	$CV_{\%}$	3.7	10.1		

Note: The means followed by different letters in the same column are significantly different at P ≤0.05

Table 2. Effects of salinity and drought stress on the fresh and dry weights of sugarcane stalks

Collecting Time	Stress treatment	Fresh weight (g)	Dry weight (g)	The relative reduction rate (%)	
				Fresh weight	Dry weight
After salinity stress	Control	23.15^{b}	4.1^{b}	-	-
	Drought	22.43^{b}	3.77^{b}	-	-
	Salinity	17.62^{a}	2.81^{a}	23.97	31.46
	Combined stress	17.53^{a}	2.82^{a}	24.28	31.23
	$LSD_{0.05}$	2.45	0.95		
	CV%	3.5	5.9		
After drought stress	Control	27.55^{c}	8.23^{b}	-	-
	Drought	23.13^{b}	7.26^{b}	16.04	11.79
	Salinity	21.44^{b}	6.57^{b}	22.17	20.17
	Combined stress	17.05^{a}	5.76^{a}	38.11	30.01
	$LSD_{0.05}$	2.00	0.77		
	CV%	3.7	7.3		
Re-watering	Control	45.90^{c}	10.70^{c}	-	-
	Drought	33.08^{b}	8.56^{b}	27.93	20.00
	Salinity	32.38^{b}	8.01^{b}	29.46	25.14
	Combined stress	28.78^{a}	7.16^{a}	37.30	33.08
	$LSD_{0.05}$	2.72	0.7		
	CV%	6.6	10.2		

Note: The means followed by different letters in the same column are significantly different at P ≤0.05

were 11.79% and 20.17% in dry weight, and 16.04% and 22.17% in fresh weight under the drought and salinity stresses, respectively, while the reductions in the combined treatment were highest with the rates of 30.01% in dry weight and 38.11% in fresh weight.

At the rewatering stage, there were recoveries in the fresh and dry weights of roots in the stress treatments. However, the three stress-affected roots showed decreasing patterns against the normal-grown roots. In the combined stress conditions, the decrease in the fresh weight of the roots was 35.76% whereas in the drought and salinity stresses, decreases of 11.41% and 14.63% were observed in comparison to the control, respectively (**Table 1**). The decreases were obtained in the dry weights of roots exposed to stress. The data revealed that the fresh and dry weights of the roots were statistically significant between the stressed and unstressed conditions. Similarly, the fresh and dry weights of stalks slowly recovered during post-stress growth with rewatering. The recovery in both traits was the slowest in the combined stress (**Table 2**).

Discussion

Drought and salinity result from climate changes that greatly affect sugarcane productivity (Kumar *et al.*, 2023). They affect not only the morphology but also the physiology, biochemistry, and expression of genes related to a plant's response to these stresses (Vasantha *et al.*, 2017; Dinh *et al.*, 2018, 2023; Brindha *et al.*, 2019; Misra *et al.*, 2019; Meena *et al.*, 2020; Zelm *et al.*, 2020; Silva *et al.*, 2022). Both salinity and drought stress affect the growth and yield of sugarcane, especially when stresses occur in the early growth stages of the crop (Dinh *et al.*, 2018).

In our experiment, the salt treatment at an early stage for four weeks showed a significant decrease in plant height and number of leaves between the stressed and unstressed conditions.

This is because when plants are exposed to salt stress, their cells are dehydrated, affecting cell elongation and division, and resulting in decreased rates of root and leaf growth. Consequently, the oldest leaves gradually age and become dried, and new leaf generation is inhibited (Munns, 2002). In this study, after several weeks, it was clear that physiological traits such as SPAD, Fv/Fm, and the fresh and dry weights of stems and roots were reduced in this study. This may indicate that the osmotic and ionic stresses (Na^+ and Cl^-) caused by the salinity-influenced components of the photosynthetic machinery such as chlorophyll content and maximum quantum yield of PSII efficiency (Fv/Fm) sequentially impact plant growth and yield. The reductions in the SPAD reading index, chlorophyll fluorescence, root and stem dry matter, stalk height, and other yield parameters here are constitutive with some previous reports about sugarcane growth under salt stress such as Brindha *et al.* (2019), Silva *et al.* (2022), and Sharma *et al.* (2021). Vasantha *et al.* (2017) showed that chlorophyll content, fluorescence, and biomass production were reduced in drought and salinity stresses, individually and in combination.

In terms of drought stress, due to the induction of osmotic stress, the initial response of plants to drought and salinity stresses is nearly identical, like reductions in growth, stomatal apertures, and nutrient deficiencies (like K^+ and Ca^{2+}). In addition, drought stress here was treated at the tillering phase, which needs water for sugarcane growth. As a result, the growth of the sugarcane roots and leaves here was limited under drought stress. Among the various parameters, plant height, number of leaves, root length, and dry and fresh matter were most affected under drought stress as seen in previous research like Wang *et al.* (2003), Jangpromma *et al.* (2012), Hemaprabha *et al.* (2013), and Medeiros *et al.* (2013). Therefore, the combined stress between drought and salt would reduce sugarcane growth the most. Both salt stress and drought stress alter the plant's root system architecture, impair the growth of aboveground parts (Julkowska *et al*., 2014; Ranja *et al*., 2022), induce stomatal closure (Chaves *et al.*, 2009; Martin Stpaul *et al.*, 2017; Zahedi *et al.*, 2022) and leaf senescence (Pic *et al.*, 2002; Zhou *et al.*, 2023), and reduce plant water uptake, leading to limited plant growth (Fisarakis *et al.*, 2001) and the loss of yield at harvest. The growth reaction depends on the time and duration of the stress treatment, and the genotype reduces the effects of osmotic stress and absorbs ions (Na^+, Cl^-). Vasantha *et al.* (2017) indicated that the biomass reduction varied in some sugarcane genotypes. The reduction in total biomass was in the range of 26 to 65%, 43 to 75%, and 40 to 86% under drought, salinity, and combined stress conditions, respectively. Total dry matter in this study recorded reductions in drought, salinity, and combined stresses of 36.48%, 18.31%, and 49.55%, respectively. Thus, in this study, the total dry biomass values of sugarcane were within the above ranges, except for the sugarcane grown in saline conditions, which was lower than in Vasantha's experiment. This can be explained in that the sampling time in this experiment was two weeks after the salinity treatment. The sugarcane was rewatered to recover, which seemed to reduce the harmful effects of salinity. This is also the reason why after rewatering, compared to the control, the decline of total dry matter of sugarcane under saline conditions was the lowest (29.4%), followed by drought stress (32.0%,) and drought and salinity stress (56.7%). Dinh *et al.* (2018) also showed that the decline in the total dry matter during the recovery period was lowest in saline (35%) and drought (40%), and highest in drought and salinity (59%) conditions. This can be explained because the recovery time of rewatering for salt stress is two weeks before drought and drought-salinity stress. The visible recovery in both the roots and stalks in this study can be explained by the recovery of roots and leaves as shown in the previous research results of Dinh *et al.* (2018) on sugarcane under drought stress.

Conclusions

Drought and salinity stress significantly affected all the growth parameters of sugarcane, namely plant height, number of leaves, and the fresh and dry weights of roots and stalks. In general, the highest reductions were observed in plants grown under combined stress conditions and followed by the drought stress and salinity stress treatments. In addition, drought and salt stress also reduced the physiological parameters, namely SPAD and Fv/Fm. Partial sugarcane growth was recovered after rewatering, however, the recovery growth depended on the type of stress with the lowest growth parameters found in plants affected by salinity-drought stress. These findings of growth responses to these abiotic stresses may be a useful resource in the future breeding of salinity and drought tolerance in sugarcane.

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