Journal of Experimental Biology and Agricultural Sciences, December - 2022; Volume – 10(6) page 1441 – 1452

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Elucidation of the morpho-physiological traits of maize (*Zea mays* L.) under salt stress

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Received – August 21, 2022; Revision – November 23, 2022; Accepted – December 08, 2022
Available Online – December 31, 2022

DOI: http://dx.doi.org/10.18006/2022.10(6).1441.1452

KEYWORDS

Maize

Salt stress

Morpho- physiological traits

Respiration

Transpiration

ABSTRACT

Agriculture is an essential sector for the increasing world population, hence the need for more food production. However, the aim of increasing food crop production is mostly suppressed by abiotic stresses such as drought and salinity. Salinity is a major limiting factor that inhibits the potential of plant growth and productivity worldwide. Hence, understanding the mechanisms behind plant stress response is important for developing new biomarker approaches that will increase salt tolerance in crops. To survive, plants exhibit various morphological, physiological, and biochemical processes when faced with saline conditions. This study was carried out to explore and evaluate the morphological and physiological effects of salinity on maize grown in the absence/presence of NaCl, followed by measurement of the various growth parameters at the end of a treatment cycle. Results of the study revealed that salt stress significantly decreased growth parameters such as plant height, leaf number, leaf width, leaf area, leaf length, and shoot (weight and length). On the other hand, salinity decreased physiological traits such as stomatal count, stomatal density, transpiration, and respiration rates. This study has shown the negative effects of salt stress on the morphology and physiology of maize. These findings can be used as a reference tool in stress response studies focusing on salt stress pathways in maize and other related crops.

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Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).

1 Introduction

Like any other living organism, plants also experience several abiotic and biotic stresses. For these reasons, plants are the most significant organisms to study as they are more vulnerable to morphological, physiological, and molecular changes when subjected to variable environmental conditions. Major environmental conditions that impact the growth, development, and survival of plants include extreme temperatures, drought, and salinity (Aslam et al. 2015; Alkahtani 2018). These stresses can cause damage either individually or in combination, however, extensive damage is mostly experienced when the stresses are in combination since different stress factors are considered additive or cumulatively interactive (Rafique et al. 2020).

Salinity is among the foremost problems in crop production since it strongly inhibits plants from exhibiting their genetic potential globally (Kaushal and Wani 2016; Singh et al. 2018; Singhal et al. 2021). Lack of adequate rainfall, poor irrigation, and secondary soil salinization mostly causes and intensify salinity stress (Alkahtani 2018). Consequently, this salt stress inhibits plant growth through osmotic and ionic effects; however, different plant species have established mechanisms to survive these effects (Khosravinejad et al. 2008). Under severe states, salinity decreases the average yields of major crops such as wheat, rice, cotton, and maize worldwide (Bartels and Sunkar 2005). Notably, various morphological, physiological, and biochemical processes allow certain crops to resist or adapt to these severe conditions. These resistance mechanisms may typically be in a form of escape, avoidance, or tolerance (Muhammad et al. 2015).

The interference of uptake and transport of essential nutrients caused by ion toxicity or low water potential results in high osmotic stress, which is a potential effect of salt stress on crop growth. Physiological processes such as photosynthesis, respiration, starch metabolism, and nitrogen fixation are also highly affected by saline conditions, leading to quantifiable loss of crop productivity (Farooq et al. 2015). Plant physiological and biochemical responses to salt stress and its tolerance mechanisms have been a major focus for plant scientists (Zhu et al. 2012). To this point, current knowledge about the key driver processes involved in plant adaptations to abiotic stress conditions, particularly salinity, is still very limited. Therefore, there is a need to understand the mechanisms of maize response and tolerance/adaptation strategies to salinity. On that point, information on the effects of salt stress in maize from germination to harvest stages has so far been partially presented, hence this study then focused on the morphological and physiological responses of this crop plant, particularly its photosynthetic, transpiration and respiratory processes, using some laboratory cultured maize (*Zea mays*) plants under salt conditions.

Maize is one of the third most important cereal crops after rice and wheat, which is grown in mild, sub-tropical, and tropical regions worldwide (Shekhar and Singh 2021). It belongs to the Poaceae family, which is moderately sensitive to salt stress (Maas and Hoffman 1977; Maas et al. 1983; Chinnusamy et al. 2005; Farooq et al. 2015). It is generally grown in numerous countries and acts as a key crop with multipurpose roles including human consumption, animal feed, and bioenergy production (Muhammad et al. 2015). In addition, maize is considered as a great crop model used for multiple investigations such as the determination of genetic components of certain crop plants and their stress adaptation mechanisms - this is due to its significant metabolism adapted for survival in extreme environmental conditions (Rafique et al. 2020). Apparently, maize responds negatively to salt stress as demonstrated by a decreased germination rate, stunted growth, reduced photosynthesis, and less productivity (Farooq et al. 2015; EL Sabagh et al. 2021).

Salt stress causes a serious financial strain on the agricultural farming sector due to reduced crop yield (Munns and Gilliham 2015). Moreover, it is a major obstacle to global food security considering that maize is a dominant crop plant used as a staple diet, animal feed, and energy source. Consequent to population growth and high limiting standards in several areas in arid and semi-arid regions, increasing crop yield has become a controversial topic concerning food security, specifically in Africa (Hussein et al. 2007; Pholo 2009).

Several studies have investigated salt stress effects on the developmental growth stages of maize, however, there is no comprehensive study that has evaluated the physiological responses of this crop to salinity, specifically concerning its respiratory and transpiration rates (Farooq et al. 2015). To the best of our knowledge, there is limited information on the evaluation aspects of these physiological parameters to salinity, more specifically in the QN701 cultivar. It is, therefore, imperative to understand the salinity concentration that triggers/modulates respiration and transpiration rates in maize. This study may perhaps assist to clarify the mechanisms of salt stress on transpiration and respiration in maize. This study focused on the assessment of salt stress responses and/or adaptation systems in a drought-tolerant maize cultivar (QN701) at the morphological (e.g., root morphology and plant growth) and physiological (stomatal quantity, leaf respiration, and transpiration rates) levels when irrigated with saline or non-saline solutions. The acquisition of morphological and physiological responses of crops to salinity is valuable data that can be utilized for breeding programs and advisory purposes by plant breeders.

2 Materials and methods

2.1 Plant material and seed sterilization

This study was conducted at the Plant Biotechnology Research Laboratory, Department of Botany, North-West University, South

Africa. The *Zea mays* (QN701) seed cultivar utilized in this study was acquired from Quality Seed (Dalton, KwaZulu-Natal, RSA). This cultivar is regarded as a white single cross hybrid that is non-GMO, suitable for irrigation and dry land conditions and is normally used for grain and silage production. The selected cultivar has been reported to be of excellent yield potential and disease-resistant (Quality Seed). A total of 30 seeds were selected for uniformity in terms of size and physical appearance, and 5 seeds per plant pot during experimentation. The seeds were surface sterilized following the procedure described by Dikobe et al. (2021), where they were collected in a 50 ml falcon tube with 70% (v/v) ethanol and vortexed for a minute, followed by further decontamination with 1.25% (v/v) sodium hypochlorite solution (bleach) for 10 minutes. Immediately after surface sterilization and decontamination, the seeds were washed three times with 3 ml of sterile distilled water. The washed seeds were allowed to imbibe in sterile distilled water at room temperature for 20 minutes, to promote rapid germination.

2.2 Germination of seedlings and treatment growth conditions

Five seeds were sown in each of the 6 plastic plant pots (16 cm diameter), filled with a 3:2 (v/v) mixture of sterile organic soil (Culterra, Muldershift, South Africa) and vermiculite (serial# SMC-9001, Rajasthan, India). The intended maize plants were randomly grown under greenhouse conditions of long days (16-hour days) and short nights (8-hour nights) at a temperature of 25/22°C day/night. The sown seeds were irrigated at every 2 days intervals with 200 ml of sterile tap water until germination was initiated on day 7. Plant treatment commenced on the 8th day after germination, whereby the plants were separated into two groups i.e. T_C= Control, and T_E= 100 mM NaCl.

The non-salt stressed plants (control) were irrigated at every 2 days intervals with 200 ml sterile tap water while the salt-stressed plants (experiment) were irrigated with 200 ml of 100 mM NaCl solution, for 28 days. After 28 days (treatment period), plants were harvested to measure the morphological growth traits and physiological parameters. Each treatment group was conducted in three independent biological replicates (n = 3).

2.3 Measurement of shoot and leaf functional traits

The protocols as described by Huang et al. (2019) were used for measurements, wherein leaf length was defined as the distance between the leaf base and leaf apex, which is at the junction of the petiole and leaf blade. Leaf width was defined as the maximum distance between the edges of the blade that is perpendicular to the straight line through the leaf apex and leaf base. The number of leaves per plant was manually counted. Similarly, a ruler measured the plant heights, leaf widths, and shoot lengths in cm. Leaf area was calculated using the formula: leaf area= leaf length × leaf width × K, whereby K = 0.75 as a coefficient constant (Musa and Usman 2016). The shoot fresh weights were measured with an electrical weighing balance (Radwag, model # PS 750/C/2, Lasec, Midrand, South Africa) in grams. All the morphological measurements were taken from three biological replicates of each treatment (n = 3).

2.4 Measurement of the physiological parameters

2.4.1 Leaf stomatal density

The leaf stomatal density was determined following the Xu and Zhou (2008) method, which expresses the number of stomata per unit leaf area from smooth leaves with little to no leaf hairs. The leaf epidermal structures of both the abaxial and adaxial surfaces of fleshy expanded maize leaves from the control and treatment plants were identified following the method described by Volenikova and Ticha (2001). A thin layer of clear nail polish was spread on each surface (abaxial and adaxial) and allowed to dry. A strip of clear sticky tape (approximately 12 mm x 20 mm) was placed over the dried leaf for both the abaxial and adaxial sides and pressed down to form a leaf impression. The sticky tape was peeled off and placed on a microscope slide and immediately viewed under 400x magnification using a Primo Star light microscope (Carl Zeiss Microscopy, Germany), and images were captured with a digital camera coupled to the microscope (Axiocam 208 color, Zeiss, Germany). The stomatal density of each leaf was recorded per unit area, as the number per square mm. The microscope diameter of the field of view (FOV) was 0.05 mm and its area was calculated using the formulae as described by Volenikova and Ticha (2001):

$$\text{FOV} = \frac{\text{field number}}{\text{magnification number}}$$

$$\text{Area of FOV} = \pi r^2$$

where π = 3.14 and r^2= radius of the field of view

Therefore, to determine the stomatal density, the below formula was used:

$$\text{stomatal density} = \frac{\text{number of stomata in entire FOV}}{\text{Area (mm}^2\text{)}}$$

2.4.2 Transpiration and respiration rates

Various physiological traits such as photosynthesis, transpiration, and respiration were measured by following the method described by Dikobe et al. (2021), using an LCpro-SD infra-red gas analyzer (IRGA) (ADC BioScientific, Hertfordshire, UK). The physiological readings were taken under randomized design by clamping a leaf into the leaf chamber of the IRGA. Measurements were then taken from the leaf adaxial

surface on three independent biological replicates for each treatment group (T_C and T_E). The resultant readings, displayed on the device's screen, were taken for 3 minutes at 10-second intervals. The following parameters were measured: photosynthetic rate (A) ($\mu mol.m^{-2}.s^{-1}$) and transpiration rate (E) ($mol.m^{-2}.s^{-1}$) under ambient temperature (25-27°C). Photosynthesis and transpiration graphs were then constructed by plotting the obtained response values against time. To measure the respiration rate, negative values from the photosynthetic responses were recorded and used to plot a graph against time.

2.5 Statistical data analysis

Analysis for all the morphological and physiological parameters was based on the means of three independent replicates, where corresponding responses for each process were subjected to one-way analysis of variance (ANOVA) (Super-Anova, Statsgraphics Version 7, 1993, Statsgraphics Corporation, USA). To verify the significance of variations between treatments, the means (n = 3) were separated using *post hoc* Student Newman Kuehls (SNK), multiple range test ($p \leq 0.05$).

3 Results

3.1 Morphological impact of salinity stress on maize

The effects of salinity on maize plants were initially analyzed on the appearance of T_C (non salt stressed plants) and T_E (salt-stressed plants) (Figure 1). Various morphological growth changes between the T_C and T_E were observed and recorded after 28 days of treatment, wherein T_E phenotypically showed some noticeable changes in plant growth attributes compared to T_C. Numerous morphological traits were induced by salt stress as indicated in Table 1.

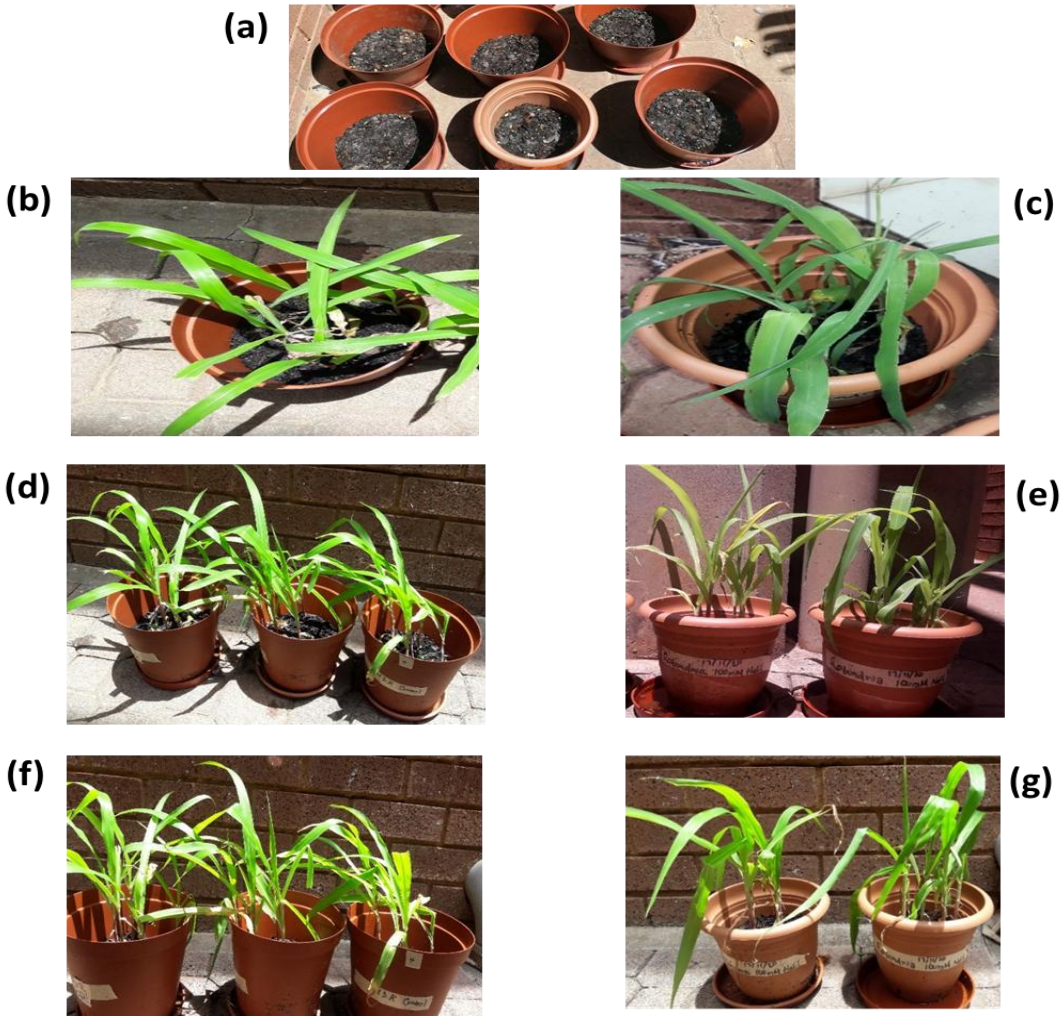

Figure 1 Morphological responses of Zea mays to salt stress; the phenotypic appearance of maize plants in response to salt stress (a) planting pots and soil used to sow the seeds, (b) non-salt stressed plants on day 8, (c) salt stressed plants on day 8, (d) non-salt stressed plants on day 18, (e) stressed plants on day 18, (f) non-salt stressed plants on day 28, and (g) salt stressed plants on day 28

Table 1 The effects of salt stress on the morphological parameters of maize plants exposed to 0 mM and 100 mM NaCl treatment for 28 days.

Morphological Parameters	Control (0 mM NaCl)					Experiment (100 mM NaCl)				
	T_C	T_C	T_C	Mean ± SD	SEM	T_E	T_E	T_E	Mean ± SD	SEM
Plant height (cm)	53	45	36	40.50 ± 6.36	3.67	38	30	35	34.33 ± 4.04	2.33
Leaf length (cm)	36.5	30	28	31.50 ± 4.44	2.57	32	29	30	30.33 ± 1.53	0.88
Leaf area (cm^2)	76.5	81	75	77.55 ± 3.10	1.79	34.56	31.61	25.2	30.46 ± 4.79	2.76
Leaf width (cm)	2.1	2.7	2.2	2.33 ± 0.32	0.19	1.08	1.09	1.05	1.07 ± 0.02	0.01
Leaf number	15	17	16	16.00 ± 1.00	0.58	15	13	11	13.00 ± 2.00	1.15
Shoot length (cm)	14	11.5	13.6	13.03 ± 1.34	0.78	10	12.5	8.5	10.33 ± 2.02	1.17
Shoot fresh weight (g)	1.72	1.02	1.27	1.34 ± 0.35	0.2	1	1.4	0.9	1.20 ± 0.28	0.16
Leaf color	Bright green	Bright green	Bright green			Dark green with brown apex	Dark green with brown apex	Dark green with brown apex		

T_C: Treatment control; T_E: Treatment experiment; means ± SE of three independent experiments (n=3); SD: standard deviation; SEM: standard error of the mean ($p \leq 0.005$).

3.2 Effects of salinity on maize growth

The plant height, total number of leaves, leaf length, width, and area; shoot fresh weight and shoot length were measured to assess plant growth after 28 days of salt stress exposure (Figure 2). Plant height, leaf number, and leaf length did not show much variation (Figure 2a-c), whilst leaf width and leaf area significantly decreased for the treated seedlings (Figure 2d-e). Furthermore, shoot length and shoot fresh weight were measured between the control and experiment. A non-significant reduction in shoot length and shoot biomass was observed in salt-stressed plants as compared to the non salt stressed group (Figures 3a and b).

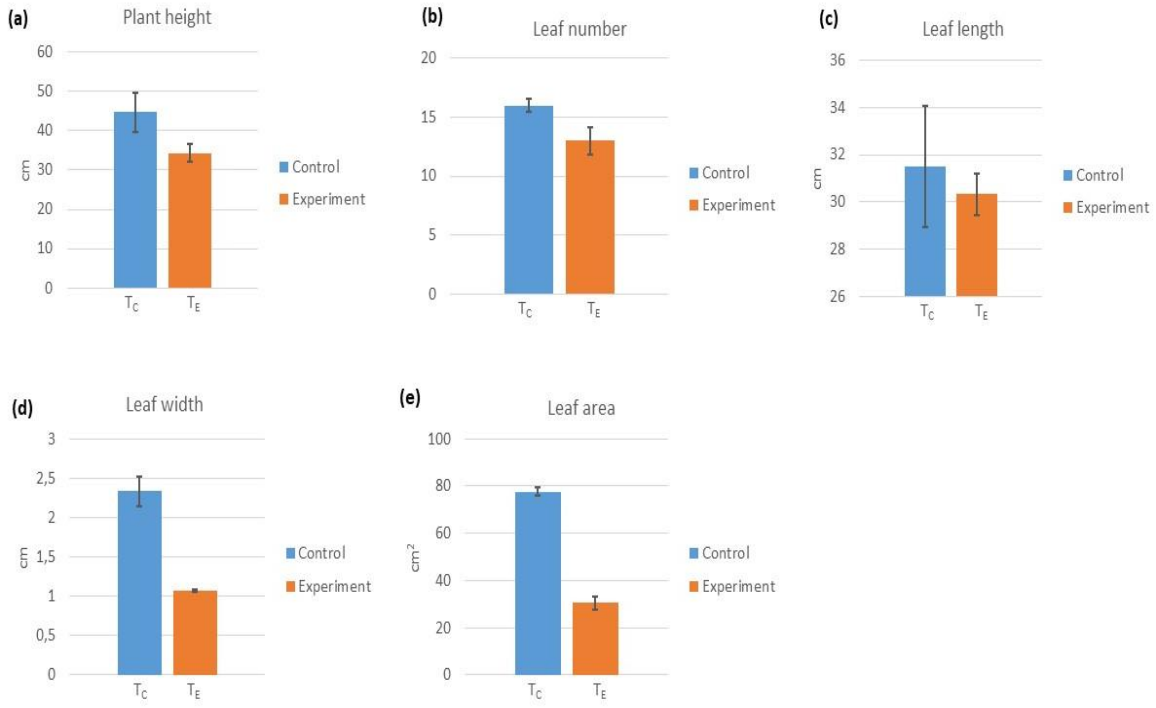

Figure 2 Effects of salt stress on growth parameters of maize; Growth traits of maize plants exposed to salt stress for 28 days at T_C (0 mM NaCl) and T_E (100 mM NaCl); Salinity decreased plant growth parameters including (a) plant height, (b) leaf number, (c) leaf length, (d) leaf width and (e) leaf area. Error bars represent the mean values of the standard error of three independent treatments (n = 3; $p \leq 0.005$)

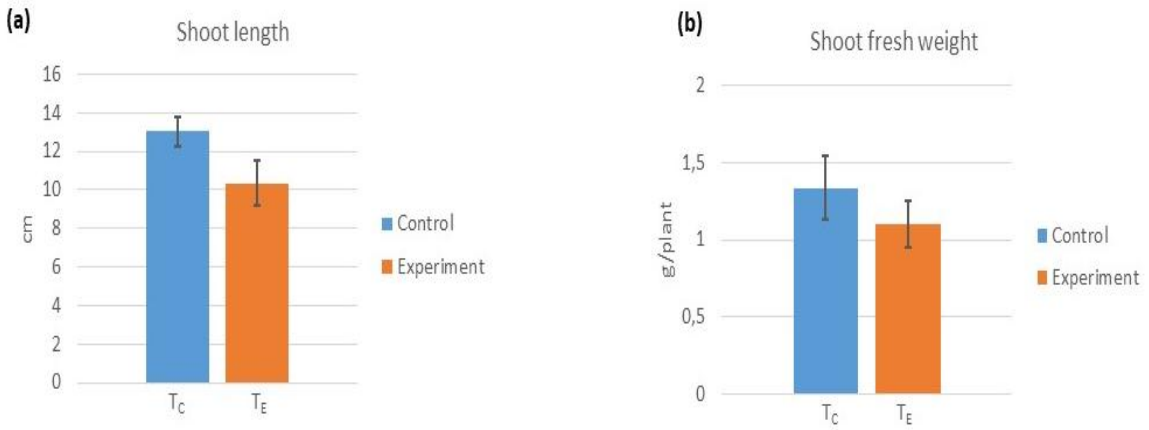

Figure 3 Effect of salt stress on growth attributes (a) Shoot length and (b) shoot fresh weight of maize plants grown under non-salt treatment T_C (0 mM NaCl) and salt treatment T_E (100 mM NaCl). Error bars indicate the mean values of the standard error of three independent seedling treatments ($n = 3$; $p \leq 0.005$)

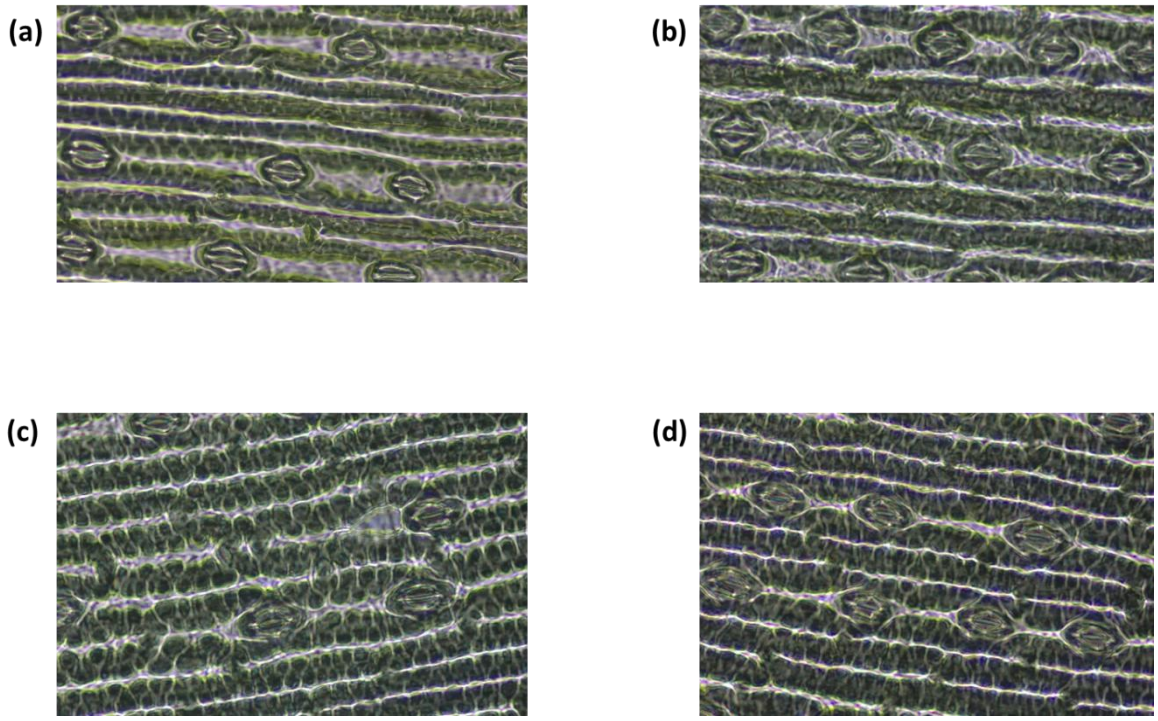

Figure 4 Microscopic images of the abaxial and adaxial surfaces of non-salt stressed (T_C) and salt stressed (T_E) maize leaves; the images were taken from leaf impressions of the (a) abaxial (lower surface) for T_C, (b) adaxial (upper surface) for T_C, (c) abaxial (lower surface) for T_E and (d) adaxial (upper surface) for T_E

3.3 Evaluation of the physiological parameters

3.3.1 Effects of salinity on the stomatal count

Following the successful assessment of the effect of salt stress on maize plant morphology, further physiological investigations were carried out to determine the stomatal count and density. Stomata located on both the abaxial and adaxial leaf surfaces displayed a substantial difference in T_C and T_E values, whereby T_C showed more stomata on both surfaces than T_E (Figure 4). Stomatal density was recorded from 2632 to 10000 mm^2 in the non-salt stressed leaves while ranging from 4 736 to 10 000 mm^2 in the salt stressed leaves (Table 2). In leaves of both plant groups (T_C and T_E), the adaxial (lower) surface displayed a higher number of stomata as compared to the abaxial (upper) surface (Figure 5a). Furthermore, salt stressed maize leaves (T_E) showed nonsignificant difference in the stomatal

Table 2 Morphological modifications of stomatal number and density for the non-stressed and salt stressed maize plants

Leaf sample	Magnification (ocular x objective)	Surface (upper/ lower)	FOV #	Number of stomata in entire FOV (mm^2)	Stomatal density stomata/mm^2
T_C	400x	Upper	1	16	8 421
	400x	Lower	1	19	10 000
T_C	400x	Upper	1	09	4 736.8
	400x	Lower	1	17	8 947.3
T_C	400x	Upper	1	05	2 632.0
	400x	Lower	1	07	3 684.2
T_E	400x	Upper	1	12	6 315.7
	400x	Lower	1	19	10 000
T_E	400x	Upper	1	13	6 842.1
	400x	Lower	1	14	7 368.4
T_E	400x	Upper	1	09	4 736.84
	400x	Lower	1	10	5 263.15

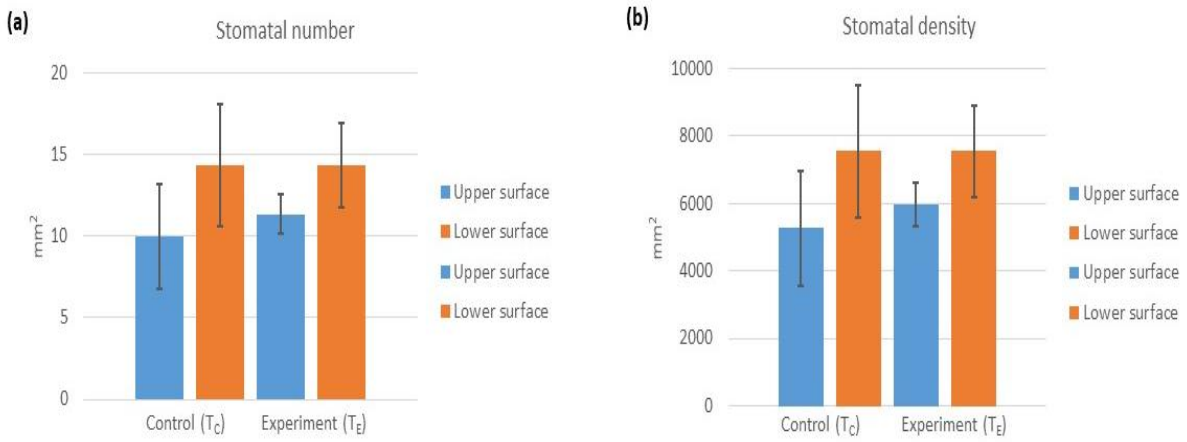

Figure 5 Assessed impact of salt stress on the (a) stomatal number and (b) stomatal density for the adaxial (upper) and abaxial (lower) surfaces of non-salt stressed (T_C) and salt stressed (T_E) maize leaves; Error bars indicate the mean values of the standard error of three independent seedling treatments ($n = 3$; $p \leq 0.005$).

number and density for both surfaces when compared to the leaves of non-salt stressed (T_C) plants (Figures 5a and b).

3.3.2 Effects of salt stress on transpiration rate

The effects of salt stress on transpiration rate were also analyzed in maize leaves. Salt stress significantly decreased the transpiration rate (Figure 6). Salt stressed leaves resulted in a moderate increase in the first 90 seconds, followed by a constant transpiratory response that was lower than those of the control (non-salt stressed) until the 150th second (Figure 6). Furthermore, salt stressed leaves displayed a moderate incline until the end of the reaction rate. Responses for the non-salt stressed maize leaves maintained a higher transpiration rate as compared to the salt stressed leaves (Figure 6).

3.3.3 Effects of salt stress on the respiration rate of maize

Salt stress significantly inhibited the respiration rate. Results presented in Figure 7 show a constant respiratory response in salt stressed plants that were lower than that of the control (non-salt treatment) in the first 60 seconds, followed by an unstable trend, where there was a steady increase and fell off for the next 120 seconds. Furthermore, responses for the non-salt stressed maize leaves maintained a higher respiration rate as compared to the salt stressed leaves (Figure 7).

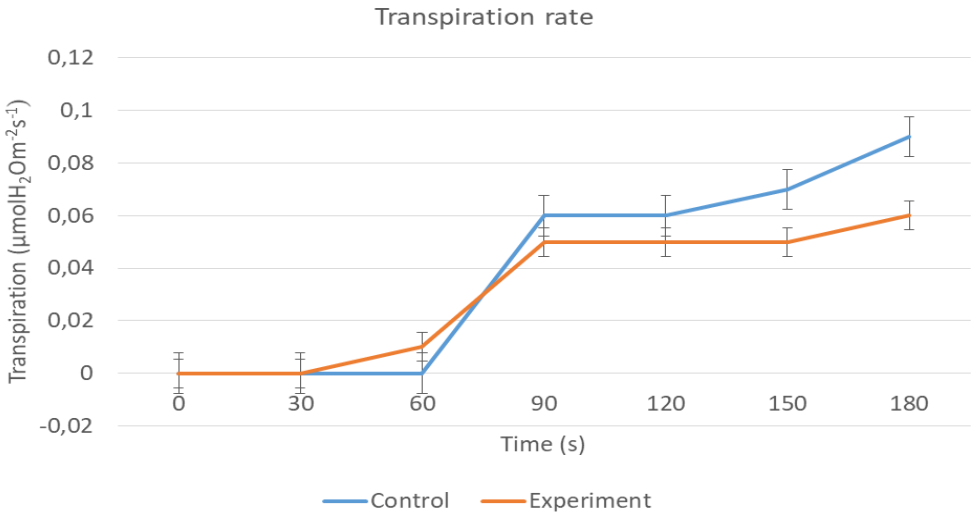

Figure 6 Comparative representations of the effects of salt stress on transpiration in maize; transpiration rates of *Z. mays* leaves treated with 0 mM NaCl (control) and100 mM NaCl (experiment) observed during the assaying process; Error bars indicate the standard error means (SEM) of three biological replicates (n = 3) for various response values ($p \leq 0.005$)

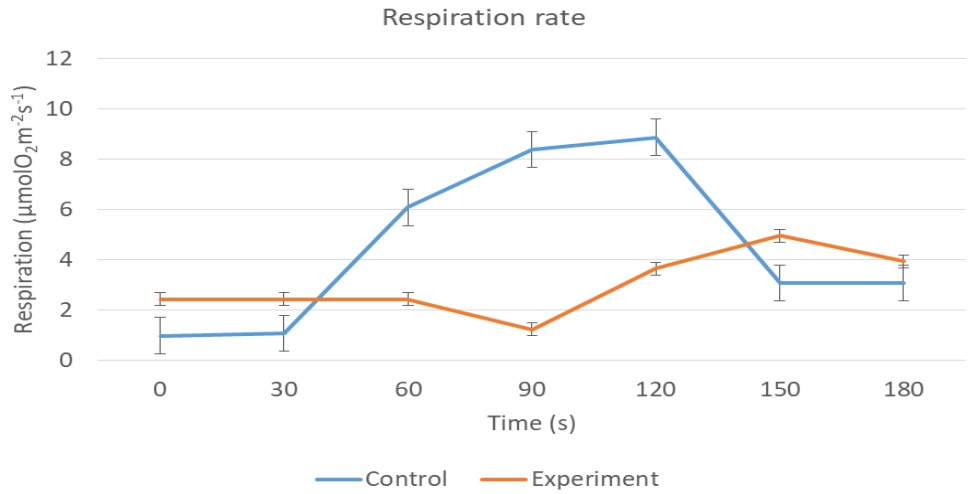

Figure 7 Comparative representation of the effects of salt stress on respiration in maize; respiration levels of *Z. mays* leaves treated with 0 mM NaCl (control) and 100 mM NaCl (experiment) observed during the assaying process. Error bars indicate the standard error means (SEM) of three biological replicates (n = 3) for various response values ($p \leq 0.005$)

4 Discussion

Salinity is an indispensable environmental challenge that restricts plants from reaching their complete genetic potential; therefore, salt-stress induces enormous growth restrictions on the morphological, physiological, and biochemical processes of plant species (Nazar et al. 2011; Hala et al. 2020). Since plants respond differently to environmental stresses, researchers have focused on various effects of salt-stress, in different plant species to understand their pathways that lead to tolerance mechanisms. Furthermore, salt stress causes water deficiency and imposes drought in plants. On that note, it was, therefore, necessary for this study to focus on the morphological and physiological processes of *Z. mays*, particularly the transpiration and respiration rates of plants exposed to salt stress over 28 days under greenhouse conditions. Elevated soil salinity affects agricultural production, thus having an impact on the economy (Zadeh and Naeini 2007).

This study has shown how salt stress significantly decreased the growth of the QN701 maize plants. Under salt stress, numerous morphological alterations were observed, whereby a negative relationship between salt stress and vegetative growth parameters such as leaf number, leaf area, and leaf weight were exhibited. Similar changes have been reported by various researchers in previous studies related to salt stress (El Sayed and El Sayed 2011; Ramezani et al. 2011; Alam et al. 2016; Dikobe et al. 2021).

Among the morphological traits, leaf color varied between the control and experiment (Table 1), wherein leaves of the salt-stressed plants (T_E) appeared dark green with brown apex whilst those of the non-salt stressed plants (T_C) were bright green (Figure 1b-e). Furthermore, various studies have supported the fact that salt stress induces a reduction in the number of leaves as indicated in *Brassica napus* L. and maize (Zadeh and Naeini 2007; El Sayed and El Sayed 2011). Whereas in this current study, the number of leaves in T_C were higher than those in T_E (Table 1), the reduction of leaf numbers in salt-stressed seedlings may be as a result of the deficiency in the uptake of water from the roots (Alam et al. 2016). Besides the reduction in the number of leaves that were observed (Figure 2b), the widths and lengths of the leaves showed a difference, whereby T_C was having larger wide leaves than T_E (Figure 2) while at the same time, shoot lengths (cm) were longer than those of T_C (Figure 3a). Hala et al. (2020) previously reported similar findings in pea plants under salinity.

According to Khodarahmpour et al. (2012), the reduction of seedling height is a common phenomenon in many crops grown under saline conditions, mostly as a result of osmotic effects even in salt-resistant plants (Wakeel et al. 2011). Sodium chloride reduced the growth rate of various morphological parameters including plant height, leaf length, width, and shoot weight whilst increasing shoot length. The reduction in plant height was highly evident for the salt stressed maize plants as compared to the control (Figure 2a). However, Mansour et al. (2005) suggested that the reduction in plant growth could be a salt stress coping mechanism that may assist plants with tolerance and energy-saving processes. Takemura et al. (2000) also confirmed that higher NaCl (mM) concentration lowers plant height (cm).

Negrao et al. (2017) suggested that the reduction in shoot growth is a familiar signal of salinity. The present study supports the above assertion, with a decrease in shoot length in experimental plants as a response signal to salt stress (Figure 3a). An increase in shoot growth mostly occurred due to turgor potential, which is reduced by water deficit caused by the accumulation of salts (high concentrations) in the soil. Salt-stress stimulates an increase in growth inhibitors and a decrease in growth promoters, hence water disturbance in salt-stressed plants limits the uptake of water or absorption of the required nutrients (Khatoon et al. 2010; Hala et al. 2020). The reduction in fresh weight due to salt stress has been studied by numerous researchers and appeared as a common phenomenon in most trees and crop plants (Gurbanov and Molazem 2009; Alam et al. 2016). In this study, a drastic reduction in shoot fresh weight (Figure 3b) was observed, which may be due to the disturbances in physiological activities under salt stress.

It was previously noted that salinity affected the expansion of leaves, which in turn limits the leaf area (Negrao et al. 2017). The decline in leaf area under salinity might be due to salt stress-induced reduction in plant fresh weights as leaves are the units of an assimilatory system (Hussain et al. 2013). The reduction in leaf area (Figure 2e) was observed and this may be associated with elongation and impaired cell division caused by the salt induced osmotic stress. Salt stress brings many changes in the physiological and biochemical processes in almost all the growth stages, one of the changes being reduced production of biomass (Alkahtani 2018). For instance, overall maize growth and plant development, including physiological parameters such as photosynthesis (P_n), transpiration rate (E), stomatal conductance (gs), and were heavily affected by salinity stress, which lead to crop yield losses (AbdElgawad et al. 2016). The transpiration rate is determined by the stomatal conductance (gs), which depends on the stomatal density (Camargo and Marenco 2011). In this study, leaf stomatal density declined with salt stress (Table 2). In a broader sense, the decrease in stomata density resulted in a decrease in transpiration rate, consequently reducing CO_2, which causes a reduction in the photosynthesis rate. However, Xu and Zhou (2008) suggested that although the stomatal density is closely related to the development of a leaf, the reaction ranges of cell number and size to stress depends on the time of leaf development. Although the stomatal density is mostly related to leaf area, it could be that increasing leaf thickness provides additional protective cells and plasticity for a given leaf area under drought conditions (Galme´s et al. 2007). The pattern of number of stomata was interesting, similar in the adaxial (upper) surfaces of both the control and experimental plants, whereas there was a decrease on the abaxial (lower) surfaces of the experimental plants (Table 2). Gill and Dutt (1982) associated low stomatal frequency with a high photosynthetic rate in beans (*Phaseolus vulgaris* L.), they further emphasized that surfaces with low stomatal frequency transpired less and had higher stomatal resistance than those with a higher stomatal frequency. The anatomy of the stomata for both the adaxial and abaxial surfaces was closed in response to salinity (Figure 4). Furthermore, the adaxial (upper) surfaces of the control and experimental plants displayed a low frequency of stomata as compared to the abaxial (lower) surfaces of the control and experimental plants (Figure 5).

The transpiration results suggest that the balance of water was improved with salt stress for the first 90 seconds (Figure 6) before the stress reduced the hydraulic conductivity, which then resulted in a decrease in water flow. The decline in water flow resulted in stomatal closure as a way of preserving water status in the leaves. The first 90 seconds of this study results corresponded to those of Negrao et al. (2017), which revealed that under salt stress, plants can tolerate salt by maintaining normal transpiration rates. The data presented in Figure 7 indicate that salt stress harmed respiration because respiration rates for the experimental (salt-stressed) plants were lower than those of the control (non-salt

stressed). Under salt stress levels, the seedlings unrestrainedly respired at low rates for the first 60 seconds, and then transpiration rates increased moderately for the last 90 seconds. The results may suggest that for seconds, respiration rates were increasing while the oxygen demand was high, and plant growth may have increased. According to Moud and Maghsoud (2008), respiration rates for plants under stress conditions are expected to be high. However, in our study, values were considerably low for respiration. This indicates that maize seedlings used great quantities of stored carbohydrates to maintain the development of organs under salinity stress. The decline in respiration rates in response to salt stress appears to be a systematic metabolic response that prevails under conditions, where salt severely restricts the availability of CO_2 inside leaf cells, therefore, creating the risk of secondary oxidative stress (Khosravinejad et al. 2008; Iqbal et al. 2020).

Conclusion

The salinity tolerance mechanism involves several complex responses at morphological, cellular, physiological, biochemical, and molecular levels. In conclusion, the present study indicated that salinity significantly affected maize's morphological and physiological traits. It induced a decline in the morphological parameters of the *Z. Mays* cultivar QN701, including plant height, leaf width, leaf height, leaf area, leaf numbers, shoot length, and shoot weight. Moreover, salt stress has shown a great effect on various physiological attributes including stomatal appearance, stomatal count, stomatal density, transpiration, and respiration rates, therefore, causing a significant decline in seedling growth and thus all growth attributes. Data presented in this study adds extra knowledge on the morphological and physiological responses of maize to salinity and could be utilized in the development of relevant biomarker strategies that can improve salt stress tolerance in maize as a major food crop.

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