

SALT INDUCED POSITIVE AND NEGATIVE EFFECTS ON GERMINATION AND OTHER PHYSIOLOGICAL TRAITS OF *SORGHUM VULGARE* SEEDLINGS

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Abstract

Salinity has long been noted as a great limiting factor to crop productivity. Sodium chloride (NaCl) proportion in saline soil of Pakistan causes ion toxicity to various plant species. Sorghum is known as ancient salt tolerance species in cereals. Irrigation with saline water is a limiting factor that causes reduction in production of several crops. Salt stress negatively influences the growth, development and metabolic pathways of plants. The current study was planned to investigate the tolerance of sorghum to salinity (NaCl) in a lab experiment carried out under Completely Randomized Design. Five different salt concentration levels (0, 2.5, 5, 10 and, 15g L⁻¹) were applied to quantify the impact of salt stress. The morphological attributes were recorded on germination count, germination %, coleoptile length, plant length, shoot and root length, fresh weight of plant, root and shoot as well as dry weight of shoot & root. Physiological parameters viz., chlorophyll content (µg cm²), leaf area (cm²), plant growth rate (g m⁻² day⁻¹), specific root, shoot and plant length and root shoot ratio were checked. Significant differences were observed after two weeks and four weeks + 2 days of seedling emergence. Higher salt concentrations over 2.5 gL⁻¹ adversely affected all the studied parameters. However, low salt concentration level (2.5 gL⁻¹) had a stimulatory impact on germination (80 %) and other traits of sorghum. i.e., coleoptile, plant, root & shoot length etc. Maximum coleoptile length (6.98 cm) was recorded after 15 days and root length (29.02 cm) after 30 days at 2.5 gL⁻¹ of NaCl saline solution.

Key words: Salinity stress, Sorghum, Physiology of seedling.

Introduction

Sorghum vulgare or Jawar is a C4 ancient cereal crop that has been grown in Egypt since ancient times. It is grown in tropical and subtropical countries including Pakistan, India, China, and Africa. Sorghum is cultivated as animal feed and as food crop, it is also important as a source for energy generation. It is whole grain alternative for gluten intolerance. Sorghum was taken as a crop of arid areas as it can withstand extreme weather conditions for agricultural purpose (Promkhambut *et al.*, 2010). Sorghum can withstand salinity upto 6.8- 4.5 dSm⁻¹. A further per unit increase of soil salinity may result in 16% reduction in grain yield. As it is a C4 crop it has the ability to sustain growth under harsh conditions (Calone *et al.*, 2020). The sorghum forage or byproduct contains protein, mineral nitrogen free extract, crude fat and carbohydrates. The sorghum varieties rich in lignocellulose contents makes it a potential crop for the energy production (Mathur *et al.*, 2017; Reddy *et al.*, 2005). Its extract is rich in fermentable sugars and can be directly converted to ethanol (Reddy *et al.*, 2005). We have to increase the grain yield of cereals for fast growing population in Pakistan with all biotic and abiotic stresses for food security. Salinity is the most crucial threat to crop production due to shortage of irrigated lands (Farheen and Mansoor, 2020).

The stress due to NaCl is among the most devastating aspects, significantly affecting plant growth and development (Ashraf *et al.*, 2008; 2009). Globally, higher salt concentration in soils is a major problem worldwide. The sodium ion is absorbed from the soil by root cells and moved to other organs of the plant by xylem and phloem. The sodium ion concentration greater than threshold limit of crop disturbs the growth of plants and development of

organs ultimately leading to death of plants (Tester & Davenport, 2003; Davenport *et al.*, 2005; Zafar *et al.*, 2015). Gradual increase in salt content in the soil due to irrigation water and fertilizers is known as salinization, salt stress produce not only changes in biochemical processes in plants but also have determinant influence on growth, germination and physiological traits (Ramzan *et al.*, 2019). In general water salinity act as brutal climatic trauma. Salinity reduces water up take ability that results in growth retardation in plants (Seeman & Critchley, 2012).

In genus sorghum some species exhibit reduced leaf area and pigment contents, they exhibit osmotic stress damage even at low concentration of salts and show wilting, yellowing, leaf tip burns and senescence (Lutts *et al.*, 1999; Iqbal *et al.*, 2009). Salt stress is the major factor in reducing the development of crops and their economic output (Zafar *et al.*, 2015). Saline stress is considered to be the cause of low germination percentage, reduced growth, decline in leaf expansion rates and biological mass of plants by affecting the enzymes and cell metabolism (Zafar *et al.*, 2018). The prime cause of low germination in various crops in the soil salinity. In soil salinity, NaCl is more injurious to plant growth and development (Lashari *et al.*, 2022). Seed germination is the first activity of growth of seed embryo which is susceptible to water and soil salinity. However, establishment of seedling in saline condition is the ability for determining crop growth and its economic yield (Hayat Ullah *et al.*, 2019).

To screen plant species against various salt concentrations is a promising approach towards salt tolerance potential. The current work is planned to find out the effect of different salt concentrations on germination, physiology and associated seedling growth of sorghum.

Material and Methods

The study was carried out to evaluate the effect of different concentrations of salts at Department of Agronomy, Faculty of Agriculture, Gomal University, Dera Ismail Khan, Pakistan. This experiment was performed to decipher the response of various NaCl salt concentrations on the seed germination, physiology and growth of sorghum. The sterilized soil sample of 2.5 kg was taken in pots and moistened with required concentration according to treatments. The experimental layout was a completely randomized design (CRD) having three replications. Sorghum seeds were purchased from the registered supplier of seed industry. About 10 seeds were used in each pot filled with clay and irrigated with four levels of saline solutions (2.5, 5, 10 and 15 g L⁻¹). The control plants contained deionized water only. Experiment was maintained for one month to collect the data for different parameters. One plant from each replicate was removed to examine various growth and physiological attributes. The following parameters were studied during the course of experimentation viz. Germination (%), Coleoptile length (cm), shoot, root and plant length (cm), Fresh and dry weight (g), chlorophyll content ($\mu\text{g cm}^{-2}$) was measured by SPAD photometer (Model 502, Japan), leaf area (cm²), plant growth rate (gm⁻² day⁻¹), fresh weight of root and shoot (g), shoot and root dry weight (g), specific root, shoot and plant length (cm), and root shoot ratio.

Soil features used for experimental purpose.

Soil characteristic	Unit	Value
pH	-	8.00
Electrical Conductivity (EC)	dS m ⁻¹	1.70
Sodium Adsorption Ratio (SAR)	-	10.15
Sand	%	40.10
Silt	%	20.80
Clay	%	39.1
Soil Textural	-	Sandy clay loam

Statistical analysis

Data for the individual traits was investigated statistically by subjecting it to the Analysis of Variance technique (ANOVA) by using the software (Statistix 8.1) according to the completely randomized design (CRD). Least significant difference (LSD) test helped to compare variations among the treatments (Steel *et al.*, 1997).

Results and Discussion

Germination (%) of sorghum as affected by different salt concentration: Germination of seed is the most sensitive time of plant emergence and growth especially in abiotic stress conditions like sodium enriched soils. During germination process, the internal metabolic changes taking place in seed provides the fundamental structure for successive plant growth and expansion as the seed imbibes water and in turn is a source of energy to the developing embryo. The data presented in (Table 1) revealed that salt concentration had significant effect on germination. High saline solution had inhibitory effect on sorghum germination (%) over control and T2 (2.5 g L⁻¹). Maximum

germination (%) was obtained in T2 (2.5 g L⁻¹). It means halo priming up to some limits promotes germination in sorghum. Our investigation is in line with Ullah *et al.*, (2019). They pointed out that wheat germination was declined due to increment in salt concentration. Low salt concentration (Halo priming) is very important in starting metabolic development in the seeds of any crop as it in our study. Rehman *et al.*, (2019) assumed that halo primed seeds attained outstanding performance due to repair mechanism, reformation of membrane, osmotic pressure and well-organized system. Bafeel *et al.*, (2012) reported that upto 0.46 MPa of saline environment sorghum seeds were able to germinate well. Higher saline concentration could be inhibitory to the seed emergence of sorghum. In our study, when salinity was more than 2.5 g L⁻¹. Germination percentage was decreased sharply as reported by Chen *et al.*, (2012). They enunciated the conditions of germination and salinity were the major factors for inhibiting seed germination of plants. Halopriming help in germination of wheat (Rizwan *et al.*, 2021).

Coleoptile length of sorghum as affected by different salt concentration: Coleoptile is that part of embryo which brings the plumule towards light. Moreover, coleoptile lengths favor the seed germination of the plant in deep sowing with moisture deficient soils. It is often assumed that deep seedling encourages deep rooting as well as promoting better anchorage, better nutrient and water uptake. Seed germination in sorghum begins with emergence of radicle or primary root that grows downward and coleoptile that grows upwards. Salinity causes the slower rate of metabolic activities and rate of elongation of roots and shoots development (Panday & Sinha, 2016). Various salt concentrations have non-significant impact on coleoptile length of sorghum (Table 2). It is totally inherited character which is not influenced by abiotic stress. Maximum coleoptile length was recorded in T2 (2.5 g NaCl) in 15 days after emergence while the minimum coleoptile length was recorded in T4 (10 g NaCl). Low salinity accelerated the process of growth, which mainly depended on ion homeostasis during 15 days of emergence. While after 30 days of emergence, salinity decreased the coleoptile length of sorghum. It might be due to emergence of proper root-shoot system of sorghum plants. Lashari *et al.*, (2022) described the reasons behind it. They explained the role of chloride ion in plants growth and its impact in physiological mechanism and uptake of essential ions i.e., nitrates, phosphate and sulphate. Salinity increases the osmotic potential, it hinders the seedlings ability to absorb water and decrease cell growth. It also affects the germination of seeds through toxic effect of Na and Cl ions.

Root length (cm) of sorghum as affected by different salt concentration: Root is that portion which is directly concerned with salinity. Hence, higher concentration of salt badly affects the root length. Various salt concentrations did not impact the root length of sorghum significantly after 15 days of emergence but after 30 days, treatment differences were significant (Table 3). All salt concentrations uniformly affected the root length. Optimum root length was recorded in T2 (2.5 g NaCl) after 30 days while, shorter root length was recorded in T5 (15 g NaCl) after 30 days. Plants behave

differently to salt stress depending upon their tolerance to salinity by altering the uptake of water and changing water balance of plants, ultimately affecting the transpiration, metabolic processes, respiration, photosynthesis, regulation in uptake of ions, stomatal exchange of gases, morphology and anatomy of plants and balance between plant hormones (Khatoun *et al.*, 2010; Ibrahim *et al.*, 2007). Maximum gain in root length was found in T2 (2.5 g L⁻¹) salt solution while gain was minimum in T4 (10 g L⁻¹) and T3 (5 g L⁻¹). The NaCl salinity stress disturbed the water balance including osmotic and turgor pressure, ionic, nutritional quality of plants and increased the concentration of Na⁺ ions thereby affecting the growth of plants especially reduction in root length. (Naseer *et al.*, 2017).

Table 1. Effect of different concentration of salts on germination of sorghum.

NaCl (g L ⁻¹)	Means	
	Seed germination count (No.)	Germination (%)
T ₁ (Control)	7 b	70 b
T ₂ (2.5)	8 a	80 a
T ₃ (5)	6 bc	60 bc
T ₄ (10)	5 c	50 c
T ₅ (15)	5 c	50 c

Means with same letter do not differ significantly at 5% significance level

Table 2. Effect of different concentration of salts on Coleoptile length (cm).

NaCl (g L ⁻¹)	Means (cm)		Loss in coleoptile length
	15 Days	30 Days	
T ₁ (Control)	6.40 ^{NS}	6.53 ^{NS}	-0.13
T ₂ (2.5 g L ⁻¹)	6.98	6.70	-0.28
T ₃ (5 g L ⁻¹)	6.35	6.03	-0.35
T ₄ (10 g L ⁻¹)	5.03	5.01	-0.023
T ₅ (15 g)	5.02	4.34	-0.68

Non-significant (ns); Means with similar letters do not differ significantly at 5% probability level

Table 3. Effect of different concentration of salts on root length (cm).

NaCl (g L ⁻¹)	Means		Gain in root length (cm)
	15 Days	30 Days	
T ₁ (Control)	8.74 ^{NS}	27.23 a	18.05
T ₂ (2.5)	6.97	29.02 a	22.05
T ₃ (5)	10.34	23.01 b	12.67
T ₄ (10)	6.81	18.05 c	11.24
T ₅ (15)	6.75	13.2 d	19.95

Non-significant (ns); Means with similar letter do not differ significantly at 5% probability

Table 4. Effect of different concentrations of salts on shoot length (cm).

NaCl (g L ⁻¹)	Means		Gain in shoot length (cm)
	15 Days	30 Days	
T ₁ (Control)	12.28 ^{NS}	27.29 ^{NS}	15.01
T ₂ (2.5)	16.03	25.32	9.29
T ₃ (5)	16.73	24.23	7.49
T ₄ (10)	11.62	22.34	10.72
T ₅ (15)	10.23	21.35	11.12

ns = Non-significant

Shoot length (cm) of sorghum as affected by different salt concentrations: Different salt concentrations non-significantly influenced the shoot length of sorghum (Table 4). It may be due to the fact that single variety of sorghum was used in the experiment. However, visual observations were recorded. Maximum shoot length was observed in T3 (5g NaCl), while minimum shoot length of plant was recorded in T5 (15g NaCl) (Table 4). Low salt concentration positively affected the absorption of nutrients. This is due to the fact that plants adapt themselves to saline environment by exhibiting anatomical adaptations in their structure. However, they also exhibit physiological changes and compartmentalization of toxic ions in vacuole (Flowers & Colmer, 2008) and maintenance of turgor by osmotic adjustments (Abouleila *et al.*, 2012). However, maximum gain in shoot length (cm) was recorded in untreated check (Table 4). While, minimum gain in shoot length was recorded in T3 (15 g L⁻¹) followed by T4 and T5, respectively. It may be due to the fact that water uptake maintains turgor pressure, mitosis and transport of nutrients as shown by Abouleila *et al.*, (2012). Salih *et al.*, (2022) described that salinity stress significantly influenced plants physiological process and inhibited seedling growth attributes such as shoot and root length and biomass.

Plant length (cm) of sorghum as affected by different salt concentrations:

In the current study in sorghum, plant length after 15 days and 30 days was tested under different salt concentrations. Significant differences within the respective columns were found in 15 days and 30 days after germination in plant length (Table 5). Maximum values were recorded in control where pure water was applied in both readings. While, maximum gain in plant length was registered in T2 (2.5 g L⁻¹) it means slightly saline water had stimulatory effect on plant length. Salt in low concentration had positive impact on absorption of aqueous nutrients content whereas, higher concentrations of salt cause ex-osmosis in plants which reduced the growth and plant length (Yang *et al.*, 2009). Bafeel *et al.*, (2012) evaluated the sorghum emergence and seedling growth under saline environment. Their experimental outcomes exhibited significant reduction in plant length and above ground volume biomass and stock as salinity level was enhanced gradually (Table 5). However, the plant length of sorghum was greatly affected by various concentrations of NaCl showing their harmful effects on growth of sorghum plants. The results of our experiment are in accordance with the findings of Islam *et al.*, (2007) and Farheen *et al.*, (2020). They observed a reduction in plant length by an increase in the induced NaCl salt stress.

Fresh weight (g) of sorghum as affected by different salt concentrations:

Different salt concentrations significantly influenced the plant fresh weight of sorghum on both dates (Table 6). The highest fresh biomass was observed in T1 (control), while the lowest value was recorded in T5 (15 g NaCl). The data on the fresh biomass of seedlings exhibited significant variations among all sorghum treatments. Fresh weight (g) was decreased with the increase in salt application. A reduction in fresh biomass of sorghum seedlings under different NaCl concentrations was a

deviation to the findings of Amjad *et al.*, (2007) and Khan *et al.*, (2009), they reported that NaCl seed priming or halopriming did not show variations in fresh biomass of seedlings. The fresh biomass of sorghum seedlings behaved differently under various concentrations of NaCl stress. A reduction in the seedling fresh biomass was observed in high salt treatments, might be due to ex-osmosis in plants during plant growth. However, low salt concentration (T₁ & T₂) after 30 days of emergence remained statistically unaffected. Over all, remaining all concentration had inhibitory effect in this regard. This gradual decrease in fresh weight by increasing salt concentration is due to the fact that the maximum energy is utilized to synthesize compounds by photosynthetic process. Early plant growth and seedling was adversely affected by the increase in concentration of NaCl stress. The decrease in growth and plant fresh biomass attributed with sodium chloride has been documented by Aytac *et al.*, 2014.

Table 5. Effect of different concentrations of salts on plant length (cm).

NaCl (g L ⁻¹)	Means		Gain in plant length (cm)
	15 Days	30 Days	
T ₁ (Control)	27.08 a	55.72 a	35.2
T ₂ (2.5)	23.03 ab	49.67 b	26.63
T ₃ (5)	20.02 b	40.72 c	13.64
T ₄ (10)	18.44 bc	34.58 cd	16.14
T ₅ (15)	10.56 c	27.86 d	17.3

Means with same letter do not differ significantly at 5% probability

Table 6. Effect of different concentrations of salts on fresh weight (g).

NaCl (g L ⁻¹)	Means		Increase in fresh weight (g)
	15 Days	30 Days	
T ₁ (Control)	1.53 a	105.62 a	104.08
T ₂ (2.5)	0.68 b	104.73 a	104.05
T ₃ (5)	0.45 b	83.92 b	83.48
T ₄ (10)	0.40 b	55.76 c	55.35
T ₅ (15)	0.32 c	54.20 c	53.88

Means with same letter do not differ significantly at 5% probability

Table 7. Effect of different concentrations of salts on dry biomass (g).

NaCl (g L ⁻¹)	Means		Gain in dry weight (g)
	15 Days	30 Days	
T ₁ (Control)	0.15	19.2 a	19.05
T ₂ (2.5)	0.36	21.2 a	20.83
T ₃ (5)	0.27	15.76 b	15.48
T ₄ (10)	0.06	10.21 c	10.14
T ₅ (15)	0.05	6.11 d	6.05

Means with same letter do not differ significantly at 5% probability

Table 8. Effect of different concentrations of salts on chlorophyll contents (SPAD unit) .

NaCl (g L ⁻¹)	Means		Gain in chlorophyll content
	15 Days	30 Days	
T ₁ (Control)	14.17 a	49.53 a	35.36
T ₂ (2.5)	15.91 a	48.23 ab	32.31
T ₃ (5)	13.80 a	47.56 ab	33.75
T ₄ (10)	9.41 b	35.65 b	26.24
T ₅ (15)	9.43 b	29.90 c	20.47

Means with same letter do not differ significantly at 5% probability

Dry weight (g) of sorghum as affected by different concentrations of salt: Results showed that dry weight showed non-significant differences among sorghum treatments after 15 days of emergence but significant differences were found after 30 days of emergence (Table 7). Salt concentration exhibited that highest dry weight in T₂ (2.5 g NaCl) in both observations, while minimum plant dry weight was recorded in T₅ (15 g NaCl). In our experiment the reduction in dry biomass of sorghum seedlings under NaCl stress was supported by the findings of Amjad *et al.*, (2007) and Khan *et al.*, (2009) who reported non-significant effect on dry biomass of sorghum seedling which responded differently under NaCl stress. However, in our study deceleration in dry weight occurred with alleviated levels of salt stress in sorghum seedling in both readings i.e. 15 days and 30 days after emergence. Control expressed better results except treatment 2 (2.5 g saline solution). Results of this research indicated that salt stress upto certain limit exhibited a sharp decline in sorghum growth, showing a considerable decrease in biomass of sorghum plants as reported by Ullah *et al.*, (2019). High salt stress causes imbalance of ions and disturbs plant metabolic processes and ultimately reduce plant growth and biomass (Zafar *et al.*, 2018). Lashari *et al.*, (2022) noted the impact of sodium chloride on little millet and chloride salt accumulated in the plants and reduced its dry weight.

Chlorophyll content of sorghum as affected by different salt concentration: Chlorophyll content were measured by SPAD photometer. Chlorophyll absorbs energy in the visible range of the spectrum (i.e. light) and changes the radiant energy into chemical form of energy (Sinha, 2014). Different salt concentrations significantly affected the chlorophyll content of sorghum after 15 and 30 days of emergence (Table 8). The maximum chlorophyll content was noticed at T₂ (2.5 g NaCl) while the lowest was recorded at T₄ (10 g NaCl) after 15 days of emergence. However, readings from T₁ to T₄, recorded non-significant differences in this regard. Chlorophyll contents were declined as salinity proceeded. Interpretation of data conclude that chlorophyll contents are not affected at low level of NaCl salinity while at higher salinity, chlorophyll contents are badly influenced. Similar results were recorded in the study of Ehsen *et al.*, (2017). They observed that plant suffering from salinity stress, usually minimized their growth. Photosynthesis is also sensitive to salt stress above a limit for a particular species. A decreasing trend in the amount of chlorophyll pigments was observed with difference in NaCl environment by Naseer *et al.*, (2017). Yamane *et al.*, (1992) reported that synthesis of chlorophyll and photosynthetic processes were badly affected by different salt concentration. High salt concentration badly affected the chlorophyll formation, disturbed the photosynthetic attributes and decreased chlorophyll contents as described by Zafar *et al.*, (2015). Perez *et al.*, (2021) provided reasons that salinity stress causes for reduction in chlorophyll contents. Total chlorophyll content decreased in *Ocimum basilicum* L. as NaCl content was increased. Decline of this pigment may be due to several factors, which may cause reduction in leaf water content which causes many biochemical changes affecting the physiological development and altered the plant metabolism.

Table 9. Effect of different concentrations of salts on leaf area (cm²).

NaCl (g L ⁻¹)	Means		Gain in leaf area
	15 Days	30 Days	
T ₁ (Control)	75.30 a	189.40 a	114.1
T ₂ (2.5)	62.32 b	187.20 a	124.88
T ₃ (5)	54.56 c	118.63 b	64.07
T ₄ (10)	47.91 c	97.65 c	49.74
T ₅ (15)	36.31 d	72.34 d	36.03

Means with similar letter do not differ significantly at 5% probability

Table 10. Effect of different concentrations of salts on plant growth rate (g day⁻¹ m⁻²).

NaCl (g L ⁻¹)	Means		Gain in PGR
	15 Days	30 Days	
T ₁ (Control)	2.1 a	3.7 a	1.6
T ₂ (2.5)	2.3 a	2.9 ab	0.6
T ₃ (5)	1.7 ab	2.1 b	0.4
T ₄ (10)	1.5 ab	2.0 b	0.5
T ₅ (15)	1.0 b	1.3 c	0.3

Means with similar letter do not differ significantly at 5% probability

Table 11. Effect of different concentrations of salts on root fresh biomass (g).

NaCl (g L ⁻¹)	Means		Gain in root fresh weight
	15 Days	30 Days	
T ₁ (Control)	0.90 a	50.1 a	49.2
T ₂ (2.5)	0.45 b	47.2 a	46.75
T ₃ (5)	0.27 c	43.3 b	43.3
T ₄ (10)	0.23 c	29.6 c	29.37
T ₅ (15)	0.21 c	28.5 c	28.29

Means with similar letter do not differ significantly at 5% probability

Table 12. Effect of different concentrations of salts on shoot fresh biomass (g).

NaCl (g L ⁻¹)	Means		Gain in fresh weight of shoot
	15 Days	30 Days	
T ₁ (Control)	0.72 a	58.2 a	57.48
T ₂ (2.5)	0.67 a	59.3 a	58.63
T ₃ (5)	0.32 b	45.6 b	45.28
T ₄ (10)	0.33 b	28.2 c	27.87
T ₅ (15)	0.29 b	23.7 c	23.41

Means with similar letter do not differ significantly at 5% probability

Table 13. Effect of different concentrations of salts on root dry biomass (g).

NaCl (g L ⁻¹)	Means		Gain in dry weight of root
	15 Days	30 Days	
T ₁ (Control)	0.085	4.3	4.21
T ₂ (2.5)	0.053	4.2	4.14
T ₃ (5)	0.028	3.6	3.57
T ₄ (10)	0.026	3.2	3.17
T ₅ (15)	0.024	2.6	2.57

Means with similar letter do not differ significantly at 5% probability

Leaf area (cm²) of sorghum as affected by different salt concentration: Leaf area of sorghum decreased significantly from T₁ (control) to maximum concentration T₅ (15 gm) after 15 days and 30 days of emergence. In (Table 9) maximum leaf area per plant was (cm²) registered in control, where no exogenous salt was applied. Leaf area values were dropped significantly in concentration of low NaCl to high NaCl levels. Severe saline stress reduces growth of plants, development and photosynthesis by reducing the leaf area (Hu *et al.*, 2013). Low salts present in irrigation water promote the growth by increasing leaf area after 30 days of emergence (Table 9). The reduction in leaf area per plant was due to both ionic stress and osmotic stress due to maximum salt uptake that disturbed the metabolism pathways of plants (Shereen *et al.*, 2015) and growth of sorghum. Perez *et al.*, (2021) also confirmed our results.

Plant growth rate (g day⁻¹ m⁻²) of sorghum as affected by different salt concentration: Plant growth rate (g day⁻¹ m⁻²) of all treatment was influenced significantly in both reading of 15 days interval (Table 10). Maximum plant growth rate after 15 days of emergence was found in T₂ (2.5g) followed by T₁ (Control) after 30 days' data showed that control (T₁) is superior and followed by T₂ (2.5 gm). Maximum gain in plant growth rate was also found in T₁ plants. The plants grown under salinity stress faces ionic stress too and exhibit many changes in their physiology. This is usually due to the presence of inorganic salt as well as buildup of osmolytes which affects plant growth rate and development (Shereen *et al.*, 2015). The Na⁺ accumulation in plant tissues directly influence the membrane system, water uptake and cell's organelles, thus declining the growth rate and increasing the chance of anomalous development (Siringam *et al.*, 2011). It is observed by other experiments too that salinity disturbs the plant growth rate and photosynthesis due to the excessive uptake of Na⁺ by plant parts (Ashraf & Haris, 2013). Peraz *et al.*, (2021) described the role of salinity in the growth of plants. They argued that plant growth and development was influenced by abnormal metabolic, biochemical and physiological process of the plant, and water mobility declined in the body.

Root fresh biomass (g) of sorghum as influenced by different concentrations of salt: Two weeks and 4 days old seedlings were uprooted, their roots were separated and data was recorded, analyzed according to the CRD. The results are presented in (Table 11). In the current investigation, growth of sorghum root was not influenced by medium levels of salinity. T₁ (Control) and T₂ (2.5 g) remained insignificant to each other at both readings. However, maximum gain in fresh weight (g) of roots was recorded in control. All saline treatments from T₂ to T₅ dropped fresh weight gradually after 30 days of emergence. Decreased growth of sorghum roots under high NaCl treatments was mainly due to the less intake of water by roots, reduced plant growth rate and lower photosynthesis as described by Abideen *et al.*, (2014). Sorghum growth in slightly or moderate salinity stress produced long and thick roots. In treatments T₂ (2.5 g) and T₃ (5g) gain in root fresh weight showed similar results with comparatively less reduction in their root fresh weight.

Table 14. Effect of different concentrations of salts on Shoot dry biomass (g).

NaCl (g L ⁻¹)	Means		Gain in shoot dry biomass
	15 Days	30 Days	
T ₁ (Control)	0.09 ^{NS}	0.67 a	0.58
T ₂ (2.5)	0.08	0.72 a	0.64
T ₃ (5)	0.05	0.53 b	0.48
T ₄ (10)	0.04	0.34 c	0.3
T ₅ (15)	0.02	0.21 d	0.19

Non-significant (ns); Means with similar letter do not differ significantly at 5% probability

Table 15. Effect of different concentrations of salts on specific root length (cm/gm).

NaCl (g L ⁻¹)	Means		Loss in specific root length
	15 Days	30 Days	
T ₁ (Control)	102.78 ^{NS}	6.33 ^{NS}	-95.67
T ₂ (2.5)	131.50	6.90	-124.6
T ₃ (5)	369.39	6.39	-362.61
T ₄ (10)	261.92	5.64	-255.36
T ₅ (15)	281.25	5.07	-275.69

Ns =Non-ignificant

Table 16. Effect of different concentrations of salts on specific shoot length (cm/gm).

NaCl (g L ⁻¹)	Means		Loss in specific shoot length
	15 Days	30 Days	
T ₁ (Control)	136.47 ^{NS}	40.73 ^{NS}	-95.27
T ₂ (2.5)	200.7	35.16	-164.84
T ₃ (5)	334.66	45.71	-288.29
T ₄ (10)	290.67	65.70	-224.3
T ₅ (15)	511.5	101.66	-409.84

NS= Non-significant

Table 17. Effect of different concentrations of salts on specific plant length (cm/gm).

NaCl (g L ⁻¹)	Means		Loss in specific plant length
	15 Days	30 Days	
T ₁ (Control)	127.76 ^{NS}	2.90 ^{NS}	-124.86
T ₂ (2.5)	62.81	2.34	-60.47
T ₃ (5)	99.07	2.58	-96.49
T ₄ (10)	276.41	3.38	-272.62
T ₅ (15)	185.26	4.55	-180.45

Ns =Non-significant

Table 18. Effect of different concentrations of salts on root shoot ratio.

NaCl (g L ⁻¹)	Means		Gain in root shoot ratio
	15 Days	30 Days	
T ₁ (Control)	0.711 ^{NS}	0.997 ^{NS}	0.28
T ₂ (2.5)	0.434	1.146	0.17
T ₃ (5)	0.618	0.949	0.27
T ₄ (10)	0.585	0.807	0.22
T ₅ (15)	0.659	0.618	-0.04

Ns =Non-significant

Shoot fresh biomass (g) of sorghum as affected by different salt concentration: Similar trend of shoot fresh weight (g) of 15 days old seedlings and 30 days seedlings was observed in root fresh weight (g) of sorghum. In (Table 12) the highest increase in shoot fresh biomass (g) was found in control (T₁) followed by T₂ (2.5 g NaCl) in the seedlings. However, maximum gain in fresh weight of sorghum at 15 days interval was registered in T₂ (2.5 g NaCl). Aqueous NaCl solution of various concentrations negatively affected the shoot fresh weight except T₂ (2.5 g NaCl), similar observations were reported by Abideen *et al.*, (2014). Nagao *et al.*, (1999) suggested that by enhancing the photosynthesis in any plant, one could increase its shoot fresh weight, dry weight and ultimately increase its economic yield. While Ashraf & Haris (2013) argued that plant fresh weight, shoot fresh weight and plant growth rate were badly affected by salt stress due to reduction in photosynthesis and growth.

Root dry biomass (g) of sorghum as affected by different salt concentration: The effect of low to high salt concentration on dry matter accumulation in sorghum remained non-significant in 15 days old seedlings (Table 13). However, observations after 30 days of emergence become significantly different from control to high salt concentrated solution (T₃ to T₅). Maximum gain in dry weight of sorghum was achieved in control (T₁). where only distilled water was applied. Data presented in table 11 unveiled the trend in dry weight of root from low to high NaCl concentration showing a gradual reduction in weight gain as an increase in NaCl amount in the irrigated water. Salinity causes inhibition in photosynthesis, transport of ions which may cause reduction in dry bio-mass accumulation in roots. Photosynthetic CO₂ fixation is reduced under high saline medium; however, the tolerance varies from species to species as reported by Bendaly *et al.*, (2016). Salinity affect the ionic regulation in plant tissues when Na⁺ get deposited and compete with K⁺ uptake. This difference in ionic state of any plant causes reduction in root and shoot biomass as described by EL-Fouly *et al.*, (2010).

Shoot dry biomass (g) of sorghum as affected by different salt concentration: Plant water requirements and its physiological growth are based on the weight of above ground dry harvested matter, so it is very important about shoot dry weight, like plant height and canopy to note the photosynthetic activities, growth and development. The data in (Table 14) revealed that different concentrations of NaCl had non-significant impact on 15 days old seedlings but it was significantly reduced the dry weight of sorghum seedlings after 30 days of emergence from T₃ (5g) to T₅ (15g). Maximum gain in shoot took place in T₂ (2.5g) after 15 days of interval. The harmful effect of NaCl stress increased steadily resulting in decreased dry biomass of seedlings. Another possible cause could be the inhibitory effect on Na⁺ and Cl⁻ ions on movement of water inside plant tissues resulting in declining in physiological processes and growth of sorghum plants. The reports are in accordance with the findings of Bendaly *et al.*, (2016).

Specific root length (cm/gm) of sorghum as affected by different salt concentration: There is less research directed to root than to other plant parts. Crop producers (Agronomist) seeking effective input to increase crop production are becoming vigilant about root growth and

development. Seedling after 15 and 30 days were uprooted, roots were separated and data were recorded and analyzed according to CRD design. The results are presented in table 15 showing that specific root length (cm/gm) was non significantly affected after 15 days and 30 days of emergence. A decrease in specific root length was noticed in 15 days' interval. However, maximum specific root length was found in T3 (5g) and maximum negative value (loss in specific root length) was also obtained in comparison of results of values obtained from 15 days and 30 days. It means NaCl solution of various concentrations inhibited the seedling growth of sorghum. If the soil solution is highly concentrated with Na⁺ and Cl⁻ ions, it increases the osmotic pressure and when it reaches in cell sap, water is not absorbed much and cause inhibitory effect on root and shoot development. It is one of the reasons, why the plants fail to grow in highly saline soil as described by Shereen *et al.*, (2015).

Specific shoot length (cm/gm) of sorghum as affected by different salt concentration: Data presented in (Table 16) showed specific shoot length of sorghum seedling after 15 days and 30 days of emergence. Data presented in Table (16) showed similar trend as shown in specific root length. A wide range of injurious effects on plant growth rate and specific root and shoot length by NaCl concentrations is described by Shereen *et al.*, (2015) and Ehsan *et al.*, (2017). They also reported that root and shoot dry biomass and their specific length was decreased with increase in NaCl concentrations. Photosynthesis and water status of leaf was gradually reduced with the increase in saline stress. However, data revealed that specific shoot length (cm/gm) was maximum during 15 days and 30 days after emergence. A loss in specific shoot length was observed in 15 days of interval in growth and development in sorghum.

Specific plant length (cm/gm) of sorghum as affected by different concentrations of salt: When the growth occurs in plants, its organs increase in size and dry weight. Specific plant length is a ratio between plant length and plant dry weight. It is an essential attribute to see the growth of a plant. The process of growth is influenced by various aspects regarding absorption of water and salts. The supply of saline water has direct relationship with the rate of growth and metabolic activities (Table 17) showed the specific plant length (cm/gm). Maximum values are recorded after 15 days' interval followed by 4 weeks plus two days. It means as growth proceeded, dry matter accumulation that increased the dry weight but root length enlargement was decreased with time. In the present investigation, various salt concentrations on sorghum decreased the specific plant length with increasing salinity after T2 (2.5 g NaCl). A high salt concentration in growth solution disturbed the uptake of necessary nutrients, physiology, biochemistry and metabolic pathways thus causing ionic disturbances and growth reduction (Del Amor *et al.*, 2001).

Root shoot ratio of sorghum as affected by different concentrations of salt: Table (18) showed the data of root shoot ratio of sorghum recorded at 15 days and 30 days after emergence. Data was found non-significant in both observations. Maximum values were recorded in T2 (2.5 gm) in 30 days' observation followed by T1 (control). In two weeks' observations, T1 (control) registered high ratio than other treatments. It was observed that saline water

decreased photosynthesis and accumulation of Na⁺ ion. Our findings are in accordance with the work of other researchers in which reduction and imbalanced growth and death of plants are mentioned under saline stress (Delgado *et al.*, 1994; Chartzoulakis *et al.*, 2002).

Conclusion

Various NaCl salt concentrations significantly affected the studied parameters. Saline water having less than 2.5 gm L⁻¹ NaCl (low concentration) may be applied for irrigation. Low concentration improved the germination, its associated traits and sustained growth by increasing chlorophyll contents and biomass of sorghum. While high salt concentration adversely affected the growth, physiology and metabolism of sorghum.

References

- Abideen, Z., H.W. Koyro, B. Huchzermeyer, M.Z. Ahmed, B. Gul and M.A. Khan. 2014. Moderate salinity stimulates growth and photosynthesis of *Pphragmites karka* by water relations and tissue specific ion regulation. *Environ. Exp. Bot.*, 105: 70-76.
- Abou-Leila, B., S.A. Metwally, M.M. Hussien and S.Z. Leithy. 2012. The combined effect of salinity and ascorbic acid on anatomical and physiological aspects of *Jatropha* plants. *Aus. J. Basic Appl. Sci.*, 6(3): 533-541.
- Amjad, M., K. Ziaf, Q. Iqbal, I. Ahmad, M.A. Riaz and Z.A. Saqib. 2007. Effect of seed priming on seed vigour and salt tolerance in hot pepper. *Pak. J. Agri. Sci.*, 44(3): 408-416.
- Ashraf, M. 2009. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotech. Adv.*, 27(1): 84-93.
- Ashraf, M. and P.J.C. Harris. 2013. Photosynthesis under stressful environment: an overview. *Photosynthesis*, 51: 163-190.
- Ashraf, M., X. Luan and T. Mc Nelly. 2008. Salinity tolerance in Brassica oil seeds. *Crit. Rev. Plant Sci.*, 23(2): 157-174.
- Aytac, Z., N. Gul mezoglu, Z. Sirel, I. Tolay and A.A. Torun. 2014. The effect of zinc on yield, yield components and micronutrients concentration in the seed of safflower genotypes (*Carthamus tinctorious* L.) in saline soils. *Biotechnol. Adv.*, 27: 84-93.
- Bafeel, S.O., I.A. Arif, M.A. Bakir, A.A. Al-Homaidan, A.H. Al Farhan and H.A. Khan. 2012. DNA barcoding of arid wild plants using rbcL gene sequences. *Genet. Mol. Res.*, 11(3): 1934-1941.
- Bendaly, A., D. Messedi, A. Smaoui, R. Ksouri, A. Bouchereau and C. Abdelly. 2016. Physiological and leaf metabolome changes in the Xerohalophyte species *Atriplex halimus* induced by salinity. *Plant Physiol. Biochem.*, 103: 208-218.
- Calone, R., R. Sanoubar, C. Lambertini, M. Speranza, L. Vittori Antisari, G. Vianello and L. Barbanti. 2020. Salt tolerance and Na allocation in *Sorghum bicolor* under variable soil and water salinity. *Plants*, 9(5): 561.
- Chartzoulakis, K., M. Loupassaki, M. Bertaki and L. Androulakis. 2002. Effect of NaCl salinity on growth, ion content and CO₂ assimilation rate of six olive cultivars. *Sci. Hort.*, 96(1): 235-247.
- Chen, S, S. J.J. Xing and H.Y. Lan. 2012. Comparative effects of neutral salt and alkaline salt stress on seed germination, early seedling growth and physiological response of halophyte species *Chenopodium glaucum*. *Afr. J. Biotech.*, 11: 9572-9581.
- Davenport, R., R.A. James, A. Zakrisson-Plogander, M. Tester and R. Munns. 2005. Control of sodium transport in durum wheat. *Plant Physiol.*, 137(3): 807-818.
- Del Amor, F., V. Martinez and A. Cerda. 2001. Salt tolerance of tomato plants are affected by stage of plant development. *Hort. Sci.*, 36: 1260-1263.

- Delgado, M., F. Ligeró and C. Lluch. 1994. Effects of salt stress on growth and nitrogen fixation by Pea, Faba bean, common bean and soybean plants. *Soil Biol. Biochem.*, 26: 371-376.
- Ehsen, S., R.F. Rizvi, Z. Abideen, I. Aziz, S. Gulzar, B. Gul, M.A. Khan and R. Ansari. 2017. Physicochemical response of *Zaleya pentandra* L. Jeffrey to NaCl treatment. *Pak. J. Bot.*, 49(3): 801-808.
- EL. Fouly, M.M., Z.M. Mobarak and Z.A. Salama. 2010. Improving tolerance of faba bean during early growth stages to salinity through micronutrients foliar spray. *Not. Sci. Biol.*, 2: 98-102.
- Farheen, J. and S. Mansoor. 2020. Morpho-biochemical response of *Vigna radiata* to salinity generated hydrogen peroxide stress. *Pak. J. Bot.*, 52(4): 1131-1135.
- Flowers, T.J. and T.D. Colmer. 2008. Salinity tolerance in halophytes. *New Phytol.*, 945-963.
- Hayat Ullah, B. Hussain, N. Muhammad, N. Uddin and N. Ali. 2019. Screening of wheat genotypes under osmotic stress at germination and seedling stage. *Int. J. Bot. Stud.*, 4(6): 23-30.
- Hu, X.W., Z.Q. Zhou, T.S. Li, Y.P. Wu and Y.R. Wang. 2013. Environmental factors controlling seed germination and seedling recruitment of *Stipa bungeana* in saline conditions. *Ecol. Res.*, 24: 559-564.
- Ibrahim, M., J. Akhtar, M. Younis, M.A. Riaz, M.A.U. Haq and M. Tahir. 2007. Selection of cotton (*G. hirsutum* L.) genotypes against NaCl stress. *Soil Environ.*, 26(1): 59-63.
- Ibrahim, M.E.H., A.Y.A. Ali, A.M.I. Elsiddig, G. Zhou, N.E.A. Nimir, G.H. Agbna and G. Zhu. 2021. Mitigation effect of biochar on sorghum seedling growth under salinity stress. *Pak. J. Bot.*, 53(2): 387-392.
- Iqbal, N., M. Ashraf and M.Y. Ashraf. 2009. Influence of exogenous glycine betaine on gas exchange and biomass production in sunflower (*Helianthus annuus* L.) under water limited conditions. *J. Agron. Crop Sci.*, 195(6): 420-426.
- Islam, M.Z., M.A.B. Mia, M.R. Islam and A. Akhter. 2007. Effect of different saline levels on growth and yield attributes of mutant rice. *J. Soil. Nat.*, 1: 18-22.
- Khan, H.A., C.M. Ayub, M.A. Pervez, R.M. Bilal, M.A. Shahid and K. Ziaf. 2009. Effect of seed priming with NaCl on salinity tolerance of hot pepper (*Capsicum annuum* L.) at seedling stage. *Soil Environ.*, 28(1): 81-87.
- Khatoon, T, K. Hussain, A. Majeed, K. Nawaz and M.F. Nisar. 2010. Morphological variations in maize (*Zea mays* L.), under different levels of NaCl at germinating stage. *J. Appl. Sci.*, 8(10): 1294-1297.
- Lashari, M.S., M.A. Bakht-un-Nisa, I. Rajpar, M. Ali, M.S. Jogi and T.A. Sial. 2022. Effect of salt stress on growth and biochemical properties of little millet (*Panicum miliaceum* L.). *Pak. J. Bot.*, 54(5): 1589-1594.
- Lutts, S., V. Majerus and J.M. Kinet. 1999. NaCl effects on proline metabolism in rice (*Oryza sativa*) seedlings. *Physiol. Plant.*, 105(3): 450-458.
- Mathur, S., A.V. Umakanth, V. A. Tonapi, R. Sharma and M.K. Sharma. 2017. Sweet sorghum as biofuel feedstock: Recent advances and available resources. *Biotech. Biofuels*, 10(1): 1-19.
- Nagao, K., T. Takahashi and K. Nakaseko. 1999. Differences between two spring wheat cultivars in effects of shading on dry matter production. *Jap. J. Crop Sci.*, 68(1): 29-33.
- Naseer, M., M. Hameed, A. Zahoor, F. Ahmed, S. Fatima, M.S. Aqeel, A.K. Shafique and M. Iftikhar. 2017. Photosynthetic response in Button wood (*Conocarpus erectus* L.) to Salt Stress. *Pak. J. Bot.*, 49(3): 847-856.
- Panday, S N. and B.K. Sinha. 2016. Biological nitrogen fixation in plant physiology. (Eds.): Panday, S.N. and B K Sinha. Vikas publishing House, Pvt. Ltd. E-28, Sector _8, Noida_20/30/ (up). India. pp. 363.
- Perez, R, J.J., M.B. Amador, N.A. Garibay, H.L. Montle, R.H.F. Espinoza and O.E. Puente. 2021. Influence of humates to mitigate NaCl induced adverse effect on *Ocimum basilicum* L., relative water content and photosynthetic pigments. *Pak. J. Bot.*, 53(4): 1159-1165.
- Promkhambut, A., A. Younger, A. Polthanee and C. Akkasaeng. 2010. Morphological and physiological responses of sorghum (*Sorghum bicolor* L. Moench.) to water logging. *Asian J. Plant Sci.*, 9(4): 183-193.
- Ramzan, M., A. Perveen, A.A. Shah and I. Hussain. 2019. Study of morphological and physiological aspects of *Alternanthera bettzickiana* under salt stress. *Sylwan J.*, 162(2): 201-207.
- Reddy, B.V., S. Ramesh, P.S. Reddy, B. Ramaiah, M. Salimath and R. Kachapur. 2005. Sweet sorghum-a potential alternate raw material for bio-ethanol and bio-energy. *Int. Sorghum Millet Newsletter*, 46: 79-86.
- Rehman, T.U., H.M. Ali, M.M. Janjua, U. Sajjad and W.M. Yan. 2019. A critical review on heat transfer augmentation of phase change materials embedded with porous materials/foams. *Int. J. Heat Mass Transf.*, 135: 649-673.
- Rizwan M., S. Ullah, S. Baloch, N. Latif, H. Shazed, R. Tahreem, A.A. Khakwani, I. Hussain and I. Ahmad. 2021. Impact of salicylic acid on wheat under cadmium stress in Pakistan. *Pure Appl. Biol.*, 11(1): 1-8.
- Salih, E.G.I., G. Zhou, A.M. Muddathir, M.E.H. Ibrahim, N.E. Ahmed, A.Y.A. Ali and I. Ahmad. 2022. Effect of seed priming with plant growth regulators on germination and seedling growth of argel (*Solenostemma argel*) under salinity stress. *Pak. J. Bot.*, 54(5): 1579-1587.
- Seemann, J.R. and C. Critchley. 2012. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species, *Phaseolus vulgaris* L. *Planta*, 164(2): 151-162.
- Shereen, A., M.U. Shirazi, M.A. Khan and S. Mumtaz. 2015. Salt tolerance potential of upland and low land rice in physiological perspectives. *Pak. J. Bot.*, 47(6): 2055-2061.
- Sinha, R.K. 2014. Photosynthesis in Modern Plant physiology. Narosa publishing House, India. 178.
- Siringam, K.N. Juntawong, S. Chaum and C. Kirdmanee. 2011. Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt sensitive rice (*Oryza sativa* L.), roots under isoosmotic conditions. *Afr. J. Biotech.*, 10: 1340-1346.
- Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and procedures of statistics. A biochemical approach, 3rd ed. McGraw Hill Book Co. Inc., New York, USA.
- Tester, M. and R. Davenport. 2003. Na⁺ tolerance and Na⁺ transport in higher plants. *Ann. Bot.*, 91(5): 503-527.
- Ullah, H., B. Hussain, N. Muhammad, N. Uddin and N. Ali. 2019. Screening of wheat (*Triticum eastivum* L.) genotypes under osmotic stresses at germination and seedling stage. *Int. J. Bot. Stud.*, 4(6): 23-30.
- Yamane, A., H. Nishmusa and J. Mizutani. 1992. Allelopathy of yellow field crass (*Rorippa sylvestris*). Identification and characterization of phytotoxic constituents. *J. Chem. Ecol.*, 18: 683-691.
- Yang, C.W., M.L. Zhang, J. Liu, D.L. Shi and D.C. Wang. 2009. Effects of buffer capacity on growth, photosynthesis, and solute accumulation of a glycophyte (wheat) and a halophyte (*Chloris virgata*). *Photosynth.*, 47: 55-60.
- Zafar, S., M. Y. Ashraf and M. Saleem. 2018. Shift in physiological and biochemical process in wheat with zinc and potassium under saline condition. *J. Plant Nutr.*, 41(1): 19-28.
- Zafar, S., M.Y. Ashraf, M. Niaz, A. Kausar and J. Hussain. 2015. Evaluation of wheat genotype for salinity tolerance using physiological indices as screening tool. *Pak. J. Bot.*, 47: 397-405.