

### **INFLUENCE OF SALINITY WITH DIFFERENT Cl:SO<sub>4</sub><sup>2-</sup> RATIOS ON WHEAT (*Triticum aestivum* L.) GROWTH**

#### **Abstract**

A significant threat to sustainable agriculture is the continuous increase in salt-affected areas. Salt stress causes land degradation, ecological imbalance, environmental pollution and reduces crop production. It changes different physical and chemical processes in the plant body, such as seed germination and different nutrients and water uptake. Wheat is the main cereal crop and primary diet for one-third of humans. It can tolerate the salinity effect to some extent, but its production reduces significantly at a higher level of salinity. Salt-affected soils have more salts that are easily solubilized in water and exchangeable sodium than the average soil. These salts are ionized and produce different cations (Mg<sup>2+</sup>, Ca<sup>2+</sup>, and Na<sup>+</sup>) and anions (Cl<sup>-</sup>, CO<sub>3</sub><sup>2-</sup>, SO<sub>4</sub><sup>2-</sup>, and HCO<sub>3</sub><sup>-</sup>). Sulfate salinity is also toxic for plant growth as chloride salinity. This research aims to assess the effect of salinity with various Cl:SO<sub>4</sub><sup>2-</sup> ratios on wheat growth. A pot trial was conducted on wheat by creating salinity with different levels of chloride and sulphate (4:1, 3:1, 2:1, 1:1, 1:2, 1:3, and 1:4). The experimental design was a complete randomized design (CRD). Different physicochemical parameters of the soil before and after crop harvesting was determined. Different growth parameters will also be determined. Data were analyzed using statistical software. Based on these results, it was determined that both types of salinity reduced the wheat growth significantly, but chloride type salinity has a more injurious effect than sulphate salinity. It was concluded that chloride ion has more toxic effects than sulphate ion for wheat growth and development.

**Keywords:** Wheat, salinity, chloride, sulfate, abiotic stress

#### **1. Introduction**

These days, water shortages and environmental degradation are significant issues. Salinization and urbanization pose a danger to the long-term viability of agriculture (Gould *et al.*, 2021). Salinity may lead to a decrease in agricultural productivity, an imbalance in the ecosystem, and changes in physiochemical soil characteristics (Machado and Serralheiro,

2017). The annual increase in salinized area is estimated at 10 % each year by factors like high surface evaporation, low precipitation, breakdown of the rocks, poor cultural activities, and irrigation with water having more salts (Kumar and Sharma, 2020). A saline-sodic soil has electrical conductivity ( $EC_e$ ) of saturated extract more than  $4 \text{ dS m}^{-1}$  and 15 exchangeable sodium percentage (ESP) at  $25^\circ\text{C}$ . The production of most of the crops is reduced at this  $EC_e$  and ESP (Munns, 2005). Consequently, the Plant undergoes morphological, biochemical, and physiological modifications (Wang *et al.*, 2021). Seed germination is inhibited by soluble salts, which restrict seeds from taking water from the soil due to osmotic action (Tavares *et al.*, 2021).

Salinity and sodicity result in a decrease in crop production, ecological imbalance, and change in soil physiochemical properties, which ultimately causes low income, soil erosion, and environmental pollution (Gould *et al.*, 2021).

More than half of the world's irrigated land and over a quarter of the world's cultivated land are suffering from salt stress. About 14 % of Pakistan's irrigated land has been salinized, and salinity is responsible for 64 % of the country's output losses. There are now only 23 million hectares (23 million acres) of land used for farming (Sanower and Sultan, 2019; Syed *et al.*, 2021). Almost 3 percent of irrigated land in Punjab, 18 percent in Sindh, and 2 percent in the NWFP suffer from severe surface salinity (Syed *et al.*, 2021). Pakistan's geographic size is estimated to be 79.61 million square miles, however only roughly 4.5 million square miles is salt affected (Tavares *et al.*, 2021).

Chlorine and sulphur both are essential nutrients for plants but due to their higher concentration and mobility in irrigated water caused toxicity as a micronutrient it is crucial to regulate the osmotic pressure, photosynthesis, and enzyme action (Reich *et al.*, 2015; Geilfus, 2018). Sulphur is essential to produce several biomolecules, including methionine, cysteine, chlorophyll, proteins, and the oil content of seeds, among others. Understanding how sulphur interacts with other macro components is critical to maximise crop yields (Aziz *et al.*, 2019; Geilfus, 2018). There is an adverse association between salinity and germination rate of seeds. When there is an increase in salinity and sodicity, the germination rate can be reduced and delayed (Yan *et al.*, 2019). Salinity causes to reduce the osmotic potential, protein and nucleic acid metabolism, uses of stored food by seeds (Helal and Mengel, 1979, Machado and Serralheiro, 2017, Yan *et al.*, 2019). It alters the enzymatic activates during germination process (Tavares *et al.*, 2021). It not only delays the germination but also affects the

vegetative and propagative growth of the Plant by creating osmotic and oxidative stresses as well as deficiency and toxicity in them (Benedetti *et al.*, 2020).

Salt-affected soils have high concentration of cations ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Na}^+$ ) and anions ( $\text{Cl}^-$ ,  $\text{CO}_3^{2-}$ ,  $\text{SO}_4^{2-}$ , and  $\text{HCO}_3^-$ ) (Geilfus, 2018). Chlorine and sulphur toxicity is widespread in salt-affected soil because it is the most common anion in salt-affected soil (Reich *et al.*, 2015; Tavares *et al.*, 2021). High concentration of  $\text{Cl}^-$  ion in chloroplast or cytosol tissue limits the growth of many glycophytic plants (Geilfus, 2018). It also retards the growth of root and tuber in pea, potato and avocado (Duan *et al.*, 2013). Chlorine ( $\text{Cl}^-$ ) has an antagonistic impact on numerous nutrients, such as nitrate absorption by *Brassica rapa*, which is reduced due to increased soil  $\text{Cl}^-$  concentrations (Reich *et al.*, 2017). When combined with phosphate and sulphate, it has an antagonistic impact on plant cells, decreasing their absorption and transport (Massa *et al.*, 2009). But excessive concentration of sulphur causes toxicity in plants. In many plant species, it is more harmful than chloride salinity (Ranault *et al.*, 2001).

Plants' responses to salt-affected soils have been studied by incorporating a combination of all salts that may be present (Hu *et al.*, 2018; Jixiang, 2012; Yan *et al.*, 2019; Zhang and Zhao, 2011, Zhao *et al.*, 2014). According to published research,  $\text{Cl}^-$  reduces germination in three Mediterranean salt marsh species more severely than  $\text{SO}_4^{2-}$  (Zhao *et al.*, 2014). In salt-stressed areas of Argentina, the germination of an inbred species of salt-affected plants is more severely inhibited by  $\text{SO}_4^{2-}$  than by  $\text{Cl}^-$  (Sosa *et al.*, 2005). When it comes to whether ion is more hazardous to plants, there is a lot of confusion. This research will examine the wheat's ability to withstand  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  salinity.

The objective of this study was:

- To assess the influence of salinity with different ratios of  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  on wheat growth.
- To determine which anion ( $\text{Cl}^-$  or  $\text{SO}_4^{2-}$ ) is more toxic for wheat growth

## **2. Methodology**

### ***2.1. Growth condition and Experimental Layout***

A pot trial was conducted in the wire house of Institute of Soil and Environmental Sciences (ISES), University of Agriculture, Faisalabad (UAF) was conducted during the year 2018-19. The test crop was wheat (Galaxy 2013).

Soil for the experiment taken from farm area of ISES. For the soil preparation, it was first air-dried in a shade to remove the water contents. Then it was grounded and to remove the material other than soil (stones, plant debris) it was sieved through 2 mm sieve. This prepared soil was brought in the lab for different analyses like saturation percentage, texture, carbonate, bicarbonate, chloride, sulphate, calcium, magnesium, pH, EC and SAR before the start of the experiment (Richards, 1954). According to the treatments, salinity ( $EC = 10 \text{ dS m}^{-1}$ ) was created artificially using  $\text{Na}_2\text{SO}_4$  and  $\text{NaCl}$  salts.

## ***2.2. Development of Salinity and Crop Growth***

Soil was weighed, and a quadratic equation was used to calculate the amount of salts per the above treatments. The amount of water for dissolving the salts was equal to SP of the soil. Then soil was saturated by applying different salt solutions according to their treatments. After one month, incubated soil was dried and filled in pots (4.5 kg capacity).

At field capacity, 5 seeds of wheat were sown in each pot (24 pots). Recommended amounts of N, P and K for wheat were applied using urea, DAP and SOP as their sources. After germination, 3 plants were kept in each pot. Canal water was used to irrigate the plants as per crop requirement. At maturity, crop growth parameters were recorded. From each pot, soil samples were obtained with stainless steel tube and were analysed for chemical properties. The plant material was analyzed for N, P, K,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ .

## ***2.3. Plant and Grain Samples Preparation***

The plant shoots and grains samples were also taken to analyse different parameters (N, P, K,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ ).

### ***2.3.1. Grinding***

For the preparation of plant and grain samples, grinding of these samples were done with the help of electric grinder (small in case of grains grinding) to get the material in powder form. After grinding, the digestion of plant material was done.

### ***2.3.2. Di-acid Digestion***

Di-acid digestion was used to prepare the Plant or grain sample for all other analysis except N. For di-acid digestion process, the mixture of two acids (hydrochloric acid ( $\text{HNO}_3$ ) and perchloric acid ( $\text{HClO}_4$ ) at the ratio of 2:1 respectively) were used. 0.5 g plant sample or grain sample was taken in flask. 5 ml of di-acid was used for each sample and keep overnight.

After 24 hours, these flasks were put on the hot plates at 300°C until the color of inside material became white or white fumes started to come out. After that, put out these flasks from the hot plate and cooled them. After cooling, the material inside these flasks was filtrated with Whatman filter paper number 40. 50 ml volume was made in Volumatic flask with DI water of each sample. Now the sample was prepared for further analysis.

#### **2.4. Kjeldahl Method for N Determination in Plant**

Kjeldahl method for N determination has following two steps;

##### **2.4.1. Digestion**

To prepare the sample for N analysis, digestion of that sample was done. 0.5 g grinded plant or grain samples were kept in digestion flasks or tubes. 5 g digestion mixture ( $K_2SO_4 + CuSO_4 \cdot 5H_2O + Se$  at the ratio of 100: 10: 1 respectively) and 15 to 20 ml concentrated  $H_2SO_4$  (0.1 N) were added in these flasks. Put these flasks in digestion block and heat them at 400 °C for 3 to 4 hours until the transparent or light yellowish colored material was observed in these flasks. After that, put out these flasks from the digestion block and cooled them. After cooling, the material inside these flasks was filtrated with Whatman filter paper number 40. 50 ml volume was made in Volumatic flask with DI water of each sample. Now the sample was prepared for further analysis.

##### **2.4.2. Distillation**

After the digestion of the sample, the next step for the N determination was the distillation. For distillation process, 10 ml of digested filtrate was kept in beaker. Added 10 ml of 4 % boric acid ( $H_3BO_3$ ) in this beaker. 2 to 3 drops of indicator (methyl red) was added. The color of the sample became purple. After that, placed the beaker on the distillation unit for 3 min during which the color was changed from purple to light yellow. After 3 min, take the breaker out from the distillation unit and titrate it against the 0.1 N  $H_2SO_4$  until the sample's color again becomes purple. The volume of 0.1 N  $H_2SO_4$  used for titration was noticed, which was used to calculate N concentration in this sample.

The N concentration (%) was determined with following formula;

$$N (\%) = (V_0 - B) * 0.1 * V_1 * 14.01 * 100 / Wt. * V_2 \times 1000$$

Where;

$V_0$  = vol. of  $H_2SO_4$  used for titration of sample

$B$  = vol. of  $H_2SO_4$  used for titration of blank

$V_1$  = total vol. of digest

$W_t$  = weight of sample

14.01 = N atomic weight

### **2.5. Determination of P in Plants**

For P determination, 10 ml of the filtrate sample was put into a flask (100 ml) after di-acid digestion. 10 ml of reagent ammonium-vanadomolybdate (ammonium heptamolybdate  $[(NH_4)_6 Mo_7O_{24}.4H_2O]$  + ammonium vanadate  $(NH_4VO_3)$  + nitric acid  $(HNO_3)$  was added in this flask and made the volume with DI water. The standards were formed by potassium dihydrogen phosphate salt, having the concentration of P (0.5, 1, 1.5, 2 and 2.5 ppm). 10 ml of each standard was also taken in flask (100 ml) and used reagent (10 ml). Samples, standards, and blank were kept for an hour to develop their color. After an hour, the absorbance of all the samples, standards and blank was read on spectrophotometer set at the wavelength of 410 nm (Chapman and Pratt, 1961). A standardized curve was designed against the spectrophotometer reading and respective P concentration in standards. This curve was used to find out the unknown concentration of P in the samples. The following formula was used to determine the P concentration in the sample.

$$P (\%) = (\text{Conc. of P (ppm) from curve}) * (V_0 / V_1 * W_t * 100)$$

Where;

$V_0$  = Total ml of digested sample

$V_1$  = Volume of the sample (ml) used for P determination

$W_t$  = Plant material weight used for digestion (g)

### **2.6. Determination of K in Plants**

For K determination in plants, Filtrate sample after di-acid digestion was used. Standards of K (10, 20, 40, 60, 80 and 100 ppm) were prepared by using KCl salt. The samples and the standards were run on flame photometer and emissions of these samples were noted. A standardized curve was designed against the flame photometer reading and respective K concentration in standards. This curve was used to find out the unknown concentration of K

in the samples. The following formula was used to determine the K concentration in the sample.

$$K \text{ (ppm)} = (\text{Conc. of K (ppm) from curve}) * (V/W_t)$$

Where;

V = Total ml of digested sample.

$W_t$  = Plant material weight used for digestion (g).

### ***2.7. Determination of SO<sub>4</sub> in Plants and Grains***

For the determination of SO<sub>4</sub> in plants and grains material, the extraction process was done first before using the turbidimetric method (discussed on the above portion). For extraction, 1g of sample (plant material and grain) was taken in evaporating basin. 10 ml of magnesium nitrate [Mg(NO<sub>3</sub>)<sub>2</sub>.6H<sub>2</sub>O] was applied on this sample. Then, placed this sample on hot plate at 180 °C. When the sample was completely dried, the hot plate temperature was raised at 280 °C. At this temperature, the color of the sample changed from brown into yellow. Then, sample was kept in a muffle furnace at 450 °C overnight. Sample had put out from the furnace and cooled. Then, 10 ml of HCl was used in this sample and boiled the sample for 3 min. After boiling, 10 ml of DI water was used in it. Then sample was shifted in 100 ml Volumetric flask. It was up to the marked with DI water and then filtered through whatman filter paper number 541. The Extraction process was completed here, further the turbidimetric method was used for SO<sub>4</sub> determination in plant and grains.

### ***2.8. Statistical analysis***

Complete Randomize Design (CRD) with three replications was used. Data from the experiment was analyzed using ANOVA, while the significance of treatment means was separated by applying the LSD test at a 5 % level of significance (Steel and Torrie, 1996).

## **3. Results**

### ***3. Growth Parameters***

#### ***3.1. Plant Height***

Data showed that the treatments significantly ( $P < 0.05$ ). Maximum plant height (98 cm) was noted with T1 and minimum with T2 (95.667 cm). When the ratio between Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> was decreased from T2 to T8, the wheat plant height was increased but remained less than T1 (Control). The percentage decrease in plant height as compared to T1 was 23.8%, 23.8, 23.8

%, 20.4 %, 13.6 %, 13.6 % and 6.8 % in treatments, from T2 to T8 respectively. The comparison between the treatments showed that T2, T3, and T4 treatments were statistically similar. T5, T6, T7 and T8 treatments were in between the response of treatment T1 and treatments T5, T6, T7 and T8. The effect of treatment (T1) was different than the other treatments (Figure (a)).

### **3.2. Yield**

The data disclosed that the impact of treatments on wheat yield was significant at  $P = 0.000$ . Wheat yield was maximum with treatment T1 (27.25 g) while minimum with T2 (17.42 g). When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the yield of wheat was increased but remained less than T1 (Control). The percentage decrease in yield as compared to T1 was 36 %, 34.12 %, 34.75 %, 34.61 %, 29.17 %, 26 % and 25.81 % in treatments, from T2 to T8, respectively. The comparison between the treatments showed that the response of treatments (T3, T4, and T5) and (T7 and T8) on yield was relatively similar (Figure (b)).

### **3.3. 100-Grains Weight**

Data was analyzed statistically, and it showed that the treatment's impact on the 100-grain weight was significant ( $P = 0.000$ ). The T1 treatment has the maximum 100 grains weight (6.2033 g) while the minimum (3.2033g) was with T2. When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the grain's weight of wheat was increased but remained less than T1 (Control). The percentage decrease in gains weight as compared to T1 was 44.7 %, 41.59 %, 34.54 %, 33.9 %, 33.9 %, 26.2 %, and 17.9 % in treatments, from T2 to T8, respectively. The comparison between the treatments showed that the response of T2 and T3 treatments on wheat grain weight was relatively similar, and the T7 and T8 response was also relatively similar (Figure (b)).

### **3.4 Plant Nitrogen (%)**

Data were analyzed statistically, and it showed that the treatment's impact on Plant N was significant ( $P = 0.000$ ). The maximum concentration of N (0.9523%) in Plant was T1 and minimum N concentration (0.4587%) was T2. When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the concentration of N increased but remained less than T1 (Control). The percentage decrease in N concentration as compared to T1 was 51.83%, 50.43%, 49.7%, 48.96%, 46.26%, 42.45% and 40.17% in treatments, from T2 to T8 respectively. Treatments comparison showed that response of treatments (T7 and T8) on N concentration was relatively similar. All the other treatments relative response was different from each other's (Figure (d)).

### **3.5. Plant Phosphorus**

Data was analysed statistical, and it showed that the treatments impact on plant P was significant ( $P = 0.000$ ). Maximum concentration of P (0.0887%) was with T1 and minimum (0.0537%) with T2. The ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the concentration of P increased but remained less than T1 (Control). The percentage decrease in P concentration as compared to T1 was 39.45%, 37.65%, 37.65%, 34.27%, 32.35, 30.1% and 28.97% in treatments, from T2 to T8 respectively. The treatments comparison showed that response of all the treatments remained different from each other (Figure (e)).

### **3.6. Plant Potassium (%)**

The data showed that the treatment's impact on Plant K was significant ( $P = 0.000$ ). The T1 has the maximum concentration of K (0.9057 %), while treatment T2 has a minimum K concentration (0.5643 %). When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the concentration of K was not changed significantly, and it remained less than T1 (Control). The percentage decrease in K concentration as compared to T1 was 37.69 %, 36.7 %, 36.36 %, 36.81%, 35.51 %, 35.07 % and 35 % in treatments, From T2 to T8 respectively. The comparison between the treatments showed that the response of treatments (T2, T3, T4, T5, T6, T7, and T8) on K concentration was relatively similar (Figure (f)).

### **3.7. Soil Chloride (ppm)**

Data were analyzed statistically, and it showed that the impact of the treatment on soil chloride was significant ( $P = 0.000$ ). The maximum concentration of Cl in soil (45.66 ppm) was with T2, and treatment (T1) had a minimum Cl concentration in soil (7.2 ppm). When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the concentration of Cl also decreased but remained more than T1 (Control). The percentage increase in Cl concentration as compared to T1 was 84.23 %, 83.12 %, 77.5 %, 72.65 %, 67.76 %, 62.75 % and 59.24 % in treatments, from T2 to T8 respectively. The comparison between the treatments showed that the response of treatments (T2 and T3) was relatively similar while all treatments' relative response was different form each other's (Figure (g)).

### **3.8. Chloride in Plant (ppm)**

Data was analysed statistical and it showed that the treatments impact on chloride concentration in wheat plant was significant ( $P = 0.000$ ).The treatment (T2) have the maximum concentration of Cl in plant (233.67 ppm) while treatment (T1) have minimum Cl concentration in Plant (94.66 ppm). When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased

from treatment (T2) to treatment (T8), the concentration of Cl also decreased but remained more than T1 (Control). The percentage increase in Cl concentration as compared to T1 was 59.48 %, 52.66 %, 41.8 %, 37.99 %, 33.48 %, 25.06 % and 23.03 % in treatments, from T2 to T8 respectively. The comparison between the treatments presented that response of all the treatments was relatively different from each other's (Figure (h)).

### **3.9. Chloride in Wheat Grain**

Data was analysed statistical and it showed that the treatments impact on soil chloride was significant ( $P = 0.000$ ). The treatment (T2) have the maximum concentration of Cl in wheat grain (195.33 ppm) while treatment (T1) have minimum Cl concentration in wheat grain (73.66 ppm). When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the concentration of Cl also decreased but remained more than T1 (Control). The percentage increase in Cl concentration as compared to T1 was 62.28 %, 60.18 %, 58.84 %, 56.91 %, 53.66 %, 45.29 % 42.14 % in treatments, from T2 to T8 respectively. The comparison between the treatments showed that response of the treatments (T2 and T3) on Cl concentration in wheat grain was relatively similar. T7 and T8 also showed the relatively similar response on Cl concentration in wheat grain (Figure (i)).

### **3.10. Sulphate in Plant (ppm)**

Data was analysed statistical and it showed that the treatments impact on sulphate concentration in wheat plant was significant ( $P = 0.000$ ). The treatment (T8) has the maximum concentration of  $\text{SO}_4$  (1256.9 ppm) while treatment (T2) has minimum  $\text{SO}_4$  concentration (576.28 ppm). When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the concentration of  $\text{SO}_4$  increased in wheat plant. The percentage decrease in  $\text{SO}_4$  concentration as compared to T1 was 14.52%, 9.7% and 3.06% in Treatments, from T2 to T4 respectively. The percentage increase in  $\text{SO}_4$  concentration as compared to T1 was 13.82 %, 30.25 %, 39.16 % and 46.36 % in treatments, from T5 to T8 respectively. The comparison between the treatments showed that response of all the treatments was relatively different from each other (Figure (j)).

### **3.11. Sulphate in Wheat Grains (ppm)**

Data was analysed statistical and it showed that the treatments impact on sulphate concentration in wheat plant was significant ( $P = 0.000$ ). The treatment (T8) has the maximum concentration of  $\text{SO}_4$  (583.09 ppm) while treatment (T2) has minimum  $\text{SO}_4$  concentration (376.43 ppm) in wheat grains. When the ratio between  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  was decreased from treatment (T2) to treatment (T8), the concentration of  $\text{SO}_4$  increased in wheat

grain. The percentage decrease in  $\text{SO}_4$  concentration as compared to T1 was 7.08%, 14.09% and 14.86% in treatments, from T2 to T4 respectively. The percentage increase in  $\text{SO}_4$  concentration as compared to T1 was 7.49 %, 15.16 %, 22.62 % and 24.17 % in treatments, from T5 to T8 respectively. The comparison between the treatments showed that response of the treatments (T2 and T3) on  $\text{SO}_4$  concentration in wheat grain was relatively similar. T7 and T8 also showed the relatively similar response on  $\text{SO}_4$  concentration in wheat grain (Figure (k)).

## 4. Discussion

### 4.1. Physiological Parameters

The major problem of an irrigated area is soil salinity. The agricultural potential of arid and semiarid regions like in Pakistan is decreasing due to continues increase in salinity. The annual rainfall of these regains is low and net evaporation rate is high. Different types of salts ( $\text{NaCl}$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{NaHCO}_3$  and  $\text{Na}_2\text{CO}_3$ ), mostly dominated with  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  are accumulated on soil surface and causes the soil salinity (Blaylock *et al.*, 1994). The effects of salts on the growth and production of the Plant are investigated using single salt ( $\text{NaCl}$ ) but in salinized area, different types of cations and anions are present (Song *et al.*, 2005; Hanif *et al.*, 2017, Qadir *et al.*, 2000). There are some studies, those investigate the response of different plants using mixture of all possible salts that may occur in salt-affected soils (Sosa *et al.*, 2005; Yang *et al.*, 2007; Zhang and Mu, 2009; Li *et al.*, 2010; Guo *et al.*, 2011; Lin *et al.*, 2014; Zhao *et al.*, 2014; Zhang *et al.*, 2015). There is lot of contradiction that which ion ( $\text{Cl}^-$  or  $\text{SO}_4^{2-}$ ) is more toxic for plant growth and development under saline conditions. The present study examined the wheat tolerance against  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  salinity.

The parameters like respiration rate, photosynthetic rate, evaporation and evapotranspiration were affected by both types of salinities (chloride and sulphate salinity). The decrease in these parameters was due to specific ion toxicity, osmotic effect and deficiency of some nutrients. Under salinity stress, the mount of some ions ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ ) were increased. This increase was toxic for plant development because more concentration of these ions inhibits the function of many enzymes during photosynthesis. Moreover, the relatively more numbers of these ions in soil solution were reduced the uptake of many important nutrients (like  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{K}^+$ ,  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^{2-}$ ) so, the deficiency of these nutrients were occurred during the salt stress. Salinity also create osmotic and oxidative stress. The osmotic

stress severely reduced the roots' uptake of major nutrients and water (Munns and Tester, 2008). These stresses reduced the stomatal conductance, photosynthesis, respiration and evapotranspiration of the wheat plant (Bie *et al.*, 2004; Akbarimoghaddam *et al.*, 2011; Hussain *et al.*, 2013; Chen *et al.*, 2016). When we compared between the two types of salinities (chloride and sulphate salinity), It was found that the effect of chloride salinity (treatments having the dominant ion was chloride) have a more negative affect on wheat physiological parameters than sulphate salinity (treatments have dominant ion was sulphate). These results favour the results of Geilfus (2018) as  $\text{Cl}^-$  ion was more mobile than  $\text{SO}_4^{2-}$  ion, the uptake of  $\text{Cl}^-$  ion was also more than  $\text{SO}_4^{2-}$  ion. The plasma membrane was more permeable for  $\text{Cl}^-$  ion than  $\text{SO}_4^{2-}$  ion.  $\text{Cl}^-$  also have an antagonistic effect with many plant nutrients (N, P and  $\text{SO}_4$ ). So, it used their pathways as its concentration in soil solution was increased. When the uptake of  $\text{Cl}^-$  was increased in xylem cell, it caused an injury of leaf cells of the wheat plant. This damage of leaf cells ultimately reduced the process of photosynthesis. Slabu and his colleagues (2009) found that the field bean showed the signs of chlorosis (yellowing of the leaves) or necrosis (leaves burring) due to higher storage of  $\text{Cl}^-$  ion in chloroplast. Tavakkoli and his co-workers (2010) found that the higher concentration of  $\text{Cl}^-$  ion in chloroplast was related with the reduction or degradation of chlorophyll contents in Plant. This accumulation of  $\text{Cl}^-$  ion in chloroplast and reduction in chlorophyll contents causes several problems for photosynthesis. Higher concentration of  $\text{Cl}^-$  also caused a photo inhibitory damage of photosystem II reaction centre (Foyer *et al.*, 1994). Bose and his colleagues (2017) found that due to excessive concentration of  $\text{Cl}^-$  in chloroplast, the electron acceptors were destroyed during the photosynthesis process, which ultimately enhanced ROS production. The process of photosynthesis is an energy producing process used during respiration and other growth functions. Moreover, the chloride salinity affected the respiration and electron transport pathway during respiration (Jacoby *et al.*, 2016). Overall, the physiological parameters were more damaged by chloride salinity than sulphate salinity in my research.

#### **4.2. Growth Parameters**

Weight of the spike and stalk, plant height, number of spikes and grains weight were affected by both types of salinities (chloride and sulphate salinity). The decrease in these parameters was due to osmotic effect, specific ion toxicity and deficiency of some nutrients (Afzal *et al.*, 2006; Iqbal and Ashraf, 2007; Khan *et al.*, 2009). Under salinity stress, the concentration of certain ions ( $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$ ) was increased. This increase was toxic for plant

growth because higher amount of these ions inhibits the function of many enzymes during photosynthesis. Moreover, the higher concentrations of these ions in soil solution reduced the uptake of many important nutrients ( $\text{NH}_4$ ,  $\text{NO}_3$ ,  $\text{K}^+$ ,  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^{2-}$ ). So, the deficiency of these nutrients were occurred during the salt stress. Salinity also create osmotic and oxidative stress. These stresses reduced the photosynthesis process in the wheat plant (Bie *et al*, 2004; Hussain *et al*, 2013; Alom *et al*, 2016). When we compared between the two types of salinities (chloride and sulphate salinity), it was found that the effect of chloride salinity (treatments having the dominant ion was chloride) have a more negative affect on wheat growth parameters than sulphate salinity (treatments have dominant ion was sulphate) (Minhas *et al*, 2007). Moreover, they found that with salinity level at the same electrolyte concentration,  $\text{SO}_4^{2-}$  had less impact than  $\text{Cl}^-$  on the yield of rice (*Oryza sativa* L.). Munns and Rawson (1999) found that chloride salinity had more damaging effect on wheat growth during reproductive phase. They stressed the wheat plant by applying different concentration of NaCl (100 to 170 mM). These treatments showed significant effect on the number of spikelet on per spike, emergence of the spike was delayed, and the fertility of the seeds reduced. This all resulted in poor grain yield of wheat. The possible reason behind this result was the plasma membrane was more permeable for  $\text{Cl}^-$  ion than  $\text{SO}_4^{2-}$  ion.  $\text{Cl}^-$  also have an antagonistic effect with many plant nutrients (N, P and  $\text{SO}_4$ ) so, it used their pathways as its concentration in soil solution was increased. When the uptake of  $\text{Cl}^-$  was increased in xylem cell, it caused an injury of leaf cells of the wheat plant. This damage of leaf cells ultimately reduced the process of photosynthesis. As photosynthesis is an energy producing process, which was used during respiration and other plant growth functions, all the growth processes were affected by chloride salinity.

### **4.3. Plant Nutrients**

Nitrogen (N) concentration was decreased in wheat plant under salinity stress. As the ratio between  $\text{Cl}^-$ :  $\text{SO}_4^{2-}$  increased (4:1, 3:1), the concentration of nitrogen in Plant decreased more. An antagonistic effect between  $\text{Cl}^-$  and  $\text{NO}_3^-$  was reported in many studied. This effect based on the nitrate transporters inhibition. These transporters unable to differentiate the  $\text{NO}_3^-$  over  $\text{Cl}^-$  especially when the  $\text{Cl}^-$  concentration was enhanced in soil solution (Wang *et al.*, 2012). So,  $\text{Cl}^-$  ion was taken up due to its higher concentration by these transporters instead of  $\text{NO}_3^-$ . While  $\text{SO}_4^{2-}$  did not have such an antagonistic effect with nitrogen, the concentration of N was more affected by chloride salinity than the sulphate salinity. It was concluded that chloride salinity has more negative effect for plant nitrogen than sulphate salinity.

Phosphorus (P) was reduced in wheat plant under salinity. As the ratio between  $\text{Cl}^-$ :  $\text{SO}_4^{2-}$  increased (4:1, 3:1), the concentration of nitrogen in Plant decreased more. An antagonistic effect between  $\text{Cl}^-$  and  $\text{H}_2\text{PO}_4^-$  or  $\text{HPO}_4^{2-}$  was reported in many studied. This effect was based on either the competition between these anions to occupy the binding site at phosphate transporter protein or due to more concentration of  $\text{Cl}^-$  in soil solution was displaced the phosphate from binding site. While  $\text{SO}_4^{2-}$  did not have such kind of antagonistic effect with phosphate so, the concentration of P was more affected by chloride salinity than the sulphate salinity. It was concluded that chloride salinity has more negative effect for plant phosphorus than sulphate salinity.

Potassium (K) concentration decreased in wheat plant under salinity. The concentration of K was decreased in all the treatment except control in almost same trend. It was because Plant take up K in  $\text{K}^+$  ion so, various ratio of  $\text{Cl}^-$ :  $\text{SO}_4^{2-}$  at same concentration of  $\text{Na}^+$ . There was a direct relation between  $\text{K}^+$  and  $\text{Na}^+$  and because all the treatments, the reduction in K concentration showed a similar trend in all the treatments except control. Munns and his colleagues (2006) find out that, when the  $\text{Na}^+$  concentration enhanced, the  $\text{K}^+$  concentration was decreased in wheat. This reduction in  $\text{K}^+$  concentration in wheat caused to reduce its growth.

Overall, it was concluded that the chloride ion has more toxic effect than sulphate ion for wheat development. These results favored the work done by Minhas and his co-workers (2007), who concluded that the impact of  $\text{Cl}^-$  on rice yield was more than  $\text{SO}_4^{2-}$  when the electrolyte concentration was kept content.  $\text{SO}_4^{2-}$  salinity has more toxic effect than  $\text{Cl}^-$  salinity in *Brassica rapa* and also  $\text{Cl}^-$  salt resulted in less decrease of pepper plant growth than  $\text{SO}_4^{2-}$  salt (Navarro *et al.*, 2003; Reich *et al.* 2018). These results also contradict the conclusion of work done by Datta and his co-workers (1995) who found that the  $\text{Cl}^-$  was less damaging than  $\text{SO}_4^{2-}$  to barley yield.

## 5. Conclusion

Agriculture lands are at great threat due to increase in salt affected areas. It not only disturb the soil, but also change chemical and physical processes going on in plant bodies. So, this experiment was done to check the effect of salinity with different  $\text{Cl}^-$ : $\text{SO}_4^{2-}$  ratios on the growth parameters of wheat. It was concluded that the both types of salinities (chloride and sulphate) significantly reduced the growth of the wheat. Maximum growth reduction was observed in T2 and T3 (treatments with 4:1 and 3:1 of  $\text{Cl}^-$  and  $\text{SO}_4^{2-}$  ratios). Chloride salinity

was more injurious than sulphate salinity for wheat growth. Overall, it was concluded that the chloride ion has more toxic effect than sulphate ion for wheat growth.

**Abbreviations:**

Cl<sup>-</sup>= Chloride ion

CRD= Coplete Randomized Design

DI= De-Ionized

dS m<sup>-1</sup>= deciSiemens per meter

EC<sub>e</sub> = Electrical Conductivity

ESP= Exchangeable Sodium Percentage

g= gram

K= Potassium

LSD= Least Significant Difference

ml= mililiter

N= Nitrogen

Na<sub>2</sub>SO<sub>4</sub>= Sodium Sulfate

NaCl= Sodium Chloride

nm= nanometer

NWFP= North West Frontier Province

P= Phosphorus

ppm= parts per million

SO<sub>4</sub><sup>2-</sup>= Sulfate ion

SP= Saturation Percentage

**Declaration:**

**Ethics approval and consent to participate: not applicable**

**Consent for publication: Not applicable**

**Availability of data and material: The author will share data on demand.**

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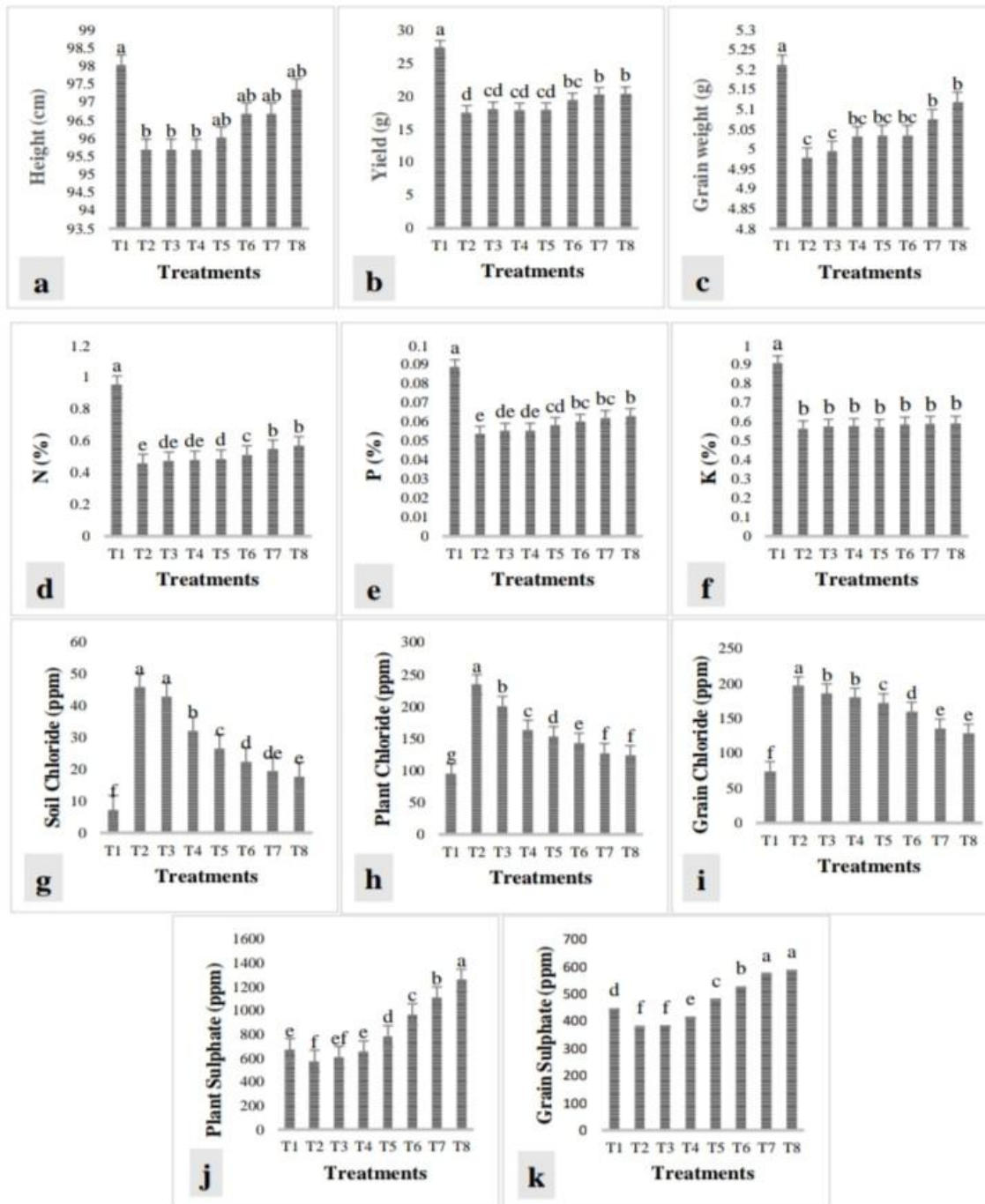
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UNDER PEER REVIEW

**Table 1. Characteristics of Experimental Soil.**

Characteristics	Unit	Results
<b>Texture</b>		Sandy clay loam
<b>EC<sub>e</sub></b>	dS m <sup>-1</sup>	2.2
<b>TSS</b>	mmol <sub>C</sub> L <sup>-1</sup>	22
<b>pH<sub>s</sub></b>		7.48
<b>CO<sub>3</sub><sup>-</sup></b>	mmol <sub>C</sub> L <sup>-1</sup>	0
<b>HCO<sub>3</sub><sup>-</sup></b>	mmol <sub>C</sub> L <sup>-1</sup>	6
<b>Cl<sup>-</sup></b>	mmol <sub>C</sub> L <sup>-1</sup>	11
<b>SO<sub>4</sub><sup>2-</sup></b>	mmol <sub>C</sub> L <sup>-1</sup>	5
<b>Ca+Mg<sup>2+</sup></b>	mmol <sub>C</sub> L <sup>-1</sup>	14
<b>Na<sup>+</sup></b>	mmol <sub>C</sub> L <sup>-1</sup>	8
<b>SAR</b>		2.65
<b>Saturation percentage</b>	%	34



**Figure 1.** T1= Control, T2=4:1 of Cl<sup>-</sup>: SO<sub>4</sub><sup>2-</sup>, T3=3:1 of Cl<sup>-</sup>: SO<sub>4</sub><sup>2-</sup>, T4=2:1 of Cl<sup>-</sup>: SO<sub>4</sub><sup>2-</sup>, T5=1:1 of Cl<sup>-</sup>: SO<sub>4</sub><sup>2-</sup>, T6=1:2 of Cl<sup>-</sup>: SO<sub>4</sub><sup>2-</sup>, T7=1:3 of Cl<sup>-</sup>: SO<sub>4</sub><sup>2-</sup> and T8=1:4 of Cl<sup>-</sup>: SO<sub>4</sub><sup>2-</sup>. Mean sharing the same letter(s) do not differ significantly at P≤ 0.05. (a) Effect of treatments on plant height. (b) Effect of treatments on wheat yield. (c) Effect of treatments on 100 grain weight of wheat crop. (d) Effect of treatments on N concentration in wheat straw. (e) Effect of treatments on P concentration in wheat. (f) Effect of treatments on the concentration of K in wheat. (g) Effect of treatment on the concentration of Cl in soil. (h) Effect of treatments on the concentration of Cl in wheat plant. (i) Effect of treatments on the concentration of Cl in wheat grains. (j) Effect of treatments on the concentration of SO<sub>4</sub> in wheat plant. (k) Effect of treatments on the concentration of SO<sub>4</sub> wheat grain.