



Impact of Salinity on the Growth and Physiology of Cotton Genotypes in Hydroponic Systems

Muhammad Asim Jamil¹, Muhammad Zarar Hasan¹, and Khubaib Shakoor^{2,3}

¹Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan;

²National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, Wuhan 430070, Hubei, China;

³MOA Key Laboratory of Tropical Crops Biology and Genetic Resources, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou, 571101, Hainan, China

*Corresponding author: zararhassan49@gmail.com

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ABSTRACT

This study investigates the effects of varying salinity levels and temperature stress on the growth and physiological responses of cotton (*Gossypium hirsutum* L.) genotypes. Twenty cotton genotypes were subjected to three treatments: control (non-saline), 7 dS/m salinity, and 14 dS/m salinity, under controlled temperature conditions. The study evaluated several physiological parameters, including plant height, root length, fresh and dry weights, relative water content (RWC), and membrane stability index (MSI). The results showed that increased salinity and temperature significantly reduced cotton growth and physiological performance across all genotypes. BS-18 demonstrated the highest tolerance to salt and temperature stress, exhibiting the best performance in terms of plant height, root length, RWC, and MSI, while FH-Lalazar showed the lowest tolerance. As salinity increased, a significant decline was observed in all measured parameters, indicating the negative impact of salinity and temperature on cotton growth. The study highlights the potential of BS-18 as a salt and heat-tolerant variety, contributing valuable insights for improving cotton cultivation under saline and temperature stress conditions.

Key words: Cotton, Salinity, Relative water content, Membrane stability index.

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is a vital crop that significantly contributes to global trade and Pakistan's economy, generating \$10.385 billion in foreign exchange in 2013-14 (Liu et al., 2014). Pakistan ranks among the top producers, but its yields remain lower than countries like China and the United States (Shahzad et al., 2019). In 2013-14, cotton was cultivated on 2,806 thousand km² in Pakistan, yielding 12.8 million bales, though production decreased due to lower support prices (Salimath et al., 2021).

Salinity, one of the most critical abiotic stress factors, negatively impacts crop yields, particularly in arid and semi-arid regions (Hussain et al., 2010). In Pakistan, salinity affects agricultural productivity, reducing soil water retention and damaging plant roots (Masood et al., 2020). Cotton growth is severely

hindered under salt stress, with NaCl and Na₂SO₄ causing osmotic and ionic damage (Todaka et al., 2015). High pH salt stress, such as from NaHCO₃, further disrupts ion uptake, leading to greater damage (Zhang et al., 2009). The accumulation of Na⁺ in plant tissues limits the uptake of essential nutrients like K⁺ and Ca²⁺, exacerbating growth inhibition (Mandhania et al., 2006).

Cotton, considered moderately salt-tolerant with a threshold of 7.68 dS/m, is particularly sensitive during early growth stages, with reduced germination and poor root development under high salinity (Munawar et al., 2021). Salt stress also reduces cotton's photosynthetic efficiency, leading to lower chlorophyll and antioxidant activity (Aly et al., 2018; Chernane et al., 2015). Increased Na⁺ in the soil creates osmotic stress, exacerbating water deficit and limiting plant growth (Handayani et al., 2019).

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High temperatures further complicate cotton growth. Heat stress, especially during the reproductive stages, significantly reduces yields by impeding photosynthesis and plant development. Optimal temperatures for cotton germination range from 28°C to 30°C, with poor germination occurring below 20°C (Iqbal et al., 2016). Moreover, cotton's susceptibility to heat stress is heightened during periods of rapid growth and boll formation (Mudassir et al., 2022).

In Pakistan, fluctuating temperatures and inconsistent rainfall often result in reduced water availability and increased pest pressures, negatively impacting cotton yields (Akhtar et al., 2020). Understanding the combined effects of salinity and temperature stress is essential for improving cotton productivity in a changing climate.

The aim of this study is to evaluate the growth and physiological responses of cotton under varying salinity and temperature conditions, focusing on their interaction and impact on plant development and yield.

MATERIALS AND METHODS

Experimental Site and Design

The experiment was conducted in the wire house of the Institute of Soil and Environmental Sciences (ISES) at the University of Agriculture, Faisalabad. The objective of the study was to evaluate the effects of salt and temperature stress on the physiological and growth performance of cotton genotypes, aiming to understand their ecological adaptation to varying environmental conditions. Twenty cotton genotypes were used in the study, including FH-492, FH-Lalazar, FH-142, VH-327, FDH-170, FH-444, FDH-228, FH-SUPER, FH-490, FH-326, 5143-P19, FH-472, FH-488, FH-474, FH-473, N-878, BS-18, SS-32, BT-102, and BS-15, which were collected from various regions of Pakistan. The experiment was laid out in a completely randomized design (CRD) with three replications.

Preparation of Nutrient Solution

The Hoagland nutrient solution, as described by Hoagland et al. (1950), was used for hydroponic cultivation. The solution was prepared by first making stock solutions of macronutrients, such as potassium nitrate (KNO_3), calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), and potassium dihydrogen phosphate (KH_2PO_4). The micronutrients, including boric acid (H_3BO_3), zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), manganese chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), and iron-EDTA, were also prepared separately. These stock solutions were mixed together to create the final nutrient solution. The pH of the solution was adjusted to a range of 6 to 6.5 using either 0.1 N hydrochloric acid (HCl) or sodium hydroxide (NaOH), ensuring optimal conditions for plant growth.

Salinity Treatments

Three different salinity levels were applied in the experiment to assess their impact on cotton plants.

These included the control group (T1), which received normal nutrient solution, and two salinity treatments: EC 7 dS/m (T2) and EC 14 dS/m (T3). Sodium chloride (NaCl) was added to the nutrient solution to induce salinity stress, and the aeration system was used to ensure sufficient oxygen supply to the plant roots. The salinity treatments were monitored and adjusted regularly to maintain the desired electrical conductivity (EC) levels.

Water Analysis

Pre-experimental water analysis was performed to determine the key physical and chemical characteristics of the tap water used in the study. The pH was measured using a calibrated pH meter (HI991003, Hanna Instruments), while electrical conductivity (EC) was determined using an EC/TDS meter (HI99300, Hanna Instruments). Total Soluble Salts (TSS) and Total Dissolved Solids (TDS) were calculated based on their relationship with EC as per standard formulas provided by the U.S. Salinity Lab (1954). Ion concentrations, including sodium (Na^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}), were determined using standard titration methods. The adsorption ratio (SAR) was calculated using the formula:

$$\text{SAR} = \frac{\text{Na}^+}{\text{Ca}^{2+} + \text{Mg}^{2+}/2}$$

Table 1: Tap water basic characteristics used in this experiment

Characteristics	Units	Values
pH	-	7.16
EC	dS m ⁻¹	1.58
TSS	me L ⁻¹	15.8
TDS	dS m ⁻¹	1011.2
$\text{Ca}^{2+} + \text{Mg}^{2+}$	me L ⁻¹	6.05
Na^+	%age	1.02
CO_3^{2-}	me L ⁻¹	Nill
HCO_3^-	me L ⁻¹	2.74

Temperature and Climatic Monitoring

Climatic data, specifically temperature and relative humidity, were recorded twice daily, in the morning and evening. Temperature was measured using a digital thermometer, and humidity was measured with a hygrometer. These parameters were crucial for understanding the effect of temperature and humidity on the nutrient solution's pH and EC, which in turn affected plant growth. The temperature data were also correlated with growth and physiological responses, providing insights into the impact of environmental stress.

Physiological Parameters

After 60 days of treatment, several physiological parameters were assessed to evaluate the effects of salt and temperature stress on cotton plants. The chlorophyll content was measured using a SPAD-502 meter (Konica Minolta, Europe) after the 45th day of the experiment. The SPAD meter measures the relative

chlorophyll content, which is indicative of the plant's photosynthetic activity. For membrane stability index (MSI), fully expanded younger leaves were collected and subjected to a series of temperature treatments. The leaves were weighed for fresh mass (FW) and placed in test tubes with distilled water. The samples were exposed to temperatures of 40°C for 30 minutes, followed by 95°C for another 15 minutes. Electrical conductivity (EC) was measured before and after heating to calculate the MSI using the formula:

$$MSI = (1 - C1/C2) \times 100$$

Where C1 is the EC of the sample before heating, and C2 is the EC after heating. This index reflects the integrity of plant cell membranes under stress.

Relative Water Content (RWC) was determined by taking fresh leaves, weighing them to determine the fresh weight (FW), then soaking them in distilled water for 4 hours. After soaking, the leaves were reweighed to determine turgid weight (TW), and after oven-drying at 65°C for 48 hours, the dry weight (DW) was recorded. The RWC was calculated using the following equation.

$$RWC = (FW - DW) / (TW - DW) \times 100$$

This parameter gives an indication of water retention ability, which is crucial for understanding the plant's stress tolerance.

Statistical Analysis

The data were analyzed using a two-way ANOVA to assess the interaction between salt concentration and temperature on cotton growth and physiology. Statistical differences were considered significant at $p < 0.05$. All analyses were performed using Statistics 8.1 software (Steel et al., 1997).

RESULTS AND DISCUSSION

Meteorological Data

This information comes from the wire house of SARC at UAF. This is the data from the months in which my experiment was ongoing. Weather data was collected through humidifier in stated in Wire house. The average weather condition in April temperature was 34.44°C in morning and 43.34°C in evening and the humidity in April was 32.53 % in morning and in evening was 13.08 %. While in May temperature was 36.18°C and 45.75°C in evening. Relative humidity was 31.31% in morning and 9.69% in.

Average EC, First pH (Initial) and Final pH (final) of 4 weeks

The values of EC, and pH were calculated on daily basis during two months (April and May) in our research to check the temperature as well as salinity stress on daily basis.

According to this data during April the EC (2.64, 6.89, and 13.93) when the temperature is less. And in May the EC (3.086, 7.09, 14.16) when the temperature

is high as compare to may. That show when the average temperature in higher the value of EC was high and the effect on plant is increase due to higher temperature and salinity.

In April when plants height is short and they become stable in higher temperature and salinity stress the average April pH (6.79, 6.67, and 6.73) value was increase as compare to May (6.53, 6.62, and 6.57). Because in May plants become heighted and the stability was become increase as compare to April. This pH value was become manage by using HCl and NaOH solution in range (6-6.5). And after manage the average pH value was (6.25, 6.31, and 6.32) in April and in May (6.26, 6.31, and 6.24).

Growth Parameters

Plant Height (cm)

The cotton plant height data was analyzed statistically at $P \leq 0.05$ to determine the effect of salinity on growth. Significant differences were observed across treatments. As salinity increased, plant height decreased, with control plants showing the tallest height (107 cm) and those treated with 14 dS m⁻¹ salinity showing the shortest (43 cm). Specifically, the sequence of plant height was: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3). BS-18 showed the highest growth in all treatments, with maximum height recorded at 107 cm in the control, 92.7 cm at 7 dS m⁻¹, and 68.7 cm at 14 dS m⁻¹. Conversely, FH-Lalazar showed the least growth, with heights of 62.7 cm, 57 cm, and 43 cm at the respective salinity levels.

The decrease in plant height with increasing salinity and temperature stress can be attributed to the negative effects on photosynthesis, transpiration, germination, and stomatal activity, caused by the accumulation of Na⁺ and Cl⁻ ions, which disrupt normal metabolic processes. The control treatment (T1) displayed optimal growth, confirming the detrimental effects of salinity on cotton plant height.

These findings align with those of Hassan et al. (2014), who observed reduced shoot and root length, as well as decreased height and fresh and dry weight under salinity and temperature stress. They concluded that high salt levels, coupled with temperature stress, hinder physiological functions like photosynthesis and cause ionic imbalance, leading to stunted growth.

Root Length (cm)

The cotton root length data was analyzed statistically at $P \leq 0.05$ to determine the impact of varying salinity levels. Significant differences were observed across treatments, with root length declining as salinity and temperature increased. The sequence of root length was: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3). The maximum root length was recorded in the control treatment (43.67 cm), while the minimum was observed in T3 with 14 dS/m salinity (14.33 cm).

Table 2: Salinity and Temperature effect on Shoot Length of cotton plant (cm)

Shoot Length			
Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	85.33±1.03	76±1.5 (89.06%)	59.7±1.5 (69.92%)
FH-lalazar	62.67±1.5	57±1.2 (90.95%)	43±2.2 (68.62%)
FH-142	85±2.0	75.33±2.5 (88.62%)	58.33±1.9 (68.63%)
VH-327	86±1.2	74.3±1.5 (86.43%)	45±1.2 (52.33%)
FDH-170	78.67±1.0	77±2.5 (97.88%)	52.67±1.5 (66.95%)
FH-444	76.67±1.2	72±1.2 (93.91%)	53.67±1.9 (70%)
FDH-228	84.33±1.3	76.7±1.2 (90.90%)	51.67±0.8 (61.26%)
FH-Super	78±1.2	72±1.5 (93.16%)	57±1.2 (73.08%)
FH-490	80.67±1.2	71±1.5 (88.01%)	55±1.3 (68.18%)
FH-326	76±1.9	75.5±1.5 (98.26%)	52.7±1.5 (68.70%)
5143-P19	77±2.1	70±1.7 (91.34%)	56±1.2 (72.73%)
FH-472	80.33±1.8	72.55±1.4 (90.04%)	52.67±1.5 (65.56%)
FH-488	83.67±0.9	73±1.6 (87.25%)	52±2.0 (62.15%)
FH-474	86±1.7	69.3±1.2 (80.62%)	50±1.9 (58.53%)
FH-473	85.67±1.8	72.67±1.5 (84.82%)	55±1.2 (64.20%)
N-878	84±1.7	75.3±1.7 (89.68%)	55±1.5 (65.48%)
BS-18	107±1.5	92.7±1.3 (80.60%)	68.67±1.3 (64.17%)
SS-32	84.7±1.5	75.33±1.7 (88.98%)	54±1.9 (64.17%)
BT-102	78±1.7	69±2.2 (88.46%)	57.5±1.5 (73.50%)
BS-15	76±2.5	69.5±1.4 (91.67%)	54.67±1.3 (71.93%)

Every value is a mean of 3 replicates ± standard Error.

Among the twenty cotton genotypes, BS-18 showed the best performance across all treatments, with the longest root length of 43.87 cm in the control. FH-Lalazar exhibited the smallest root length (24.33 cm), which was 32.82% shorter compared to the best performing variety. In T1, the second most tolerant variety was VH-327 with a root length of 38.67 cm. In T2, N-878 showed the second highest root length (30.83 cm), while in T3, FH-492 was the second most tolerant with a root length of 24.2 cm.

Root length decreased as salinity and temperature increased due to the negative effects on germination, photosynthesis, and transpiration, along with the buildup of Na⁺ and Cl⁻ ions, which disrupted normal metabolic processes in the cotton plants. The control treatment (T1) showed optimal root growth, while T2 and T3 exhibited progressively reduced root lengths.

These findings are consistent with Rehman et al. (2019), who observed that root length decreased with increased salinity and temperature, likely due to higher salt uptake by cotton plants. Similarly, Ren et al. (2021) reported reduced root length in cotton under salinity stress. Ahmed et al. (2020) also noted a decrease in root and shoot length, fresh weight, and dry fruit weight under saline conditions, further supporting the detrimental impact of salinity on cotton growth.

Fresh Shoot Weight (g)

The cotton fresh shoot weight data was analyzed statistically at P ≤ 0.05 to assess the effect of varying salinity levels. Significant differences were observed across treatments, with fresh shoot weight declining as salinity and temperature increased. The fresh shoot weight followed the pattern: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3). The highest fresh shoot weight was

recorded in the control (64.5 g), while the lowest was in T3 with 14 dS/m salinity (15.5 g).

Table 3: Salinity and Temperature effect on Shoot length (cm)

Root Length			
Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	34.33±0.88	29±0.58 (84.47%)	24.2±0.43 (70.49%)
FH-lalazar	23±0.58	19.83±0.4 (86.23%)	14.33±0.33 (62.32%)
FH-142	32.33±0.85	26.67±0.42 (80.93%)	24.2±0.6 (74.85%)
VH-327	38.67±0.44	29.67±0.6 (76.42%)	23.47±0.29 (61.48%)
FDH-170	37.83±0.73	26.67±0.44 (69.16%)	21.2±0.43 (56.04%)
FH-444	34.5±0.76	25.83±0.8 (74.88%)	18±0.56 (52.17%)
FDH-228	34.5±1.04	25.67±0.6 (72.95%)	21.23±0.65 (72.95%)
FH-Super	34±0.87	28.16±0.73 (82.84%)	23.17±0.6 (68.14%)
FH-490	35.67±0.33	27.2±0.75 (76.26%)	21.33±0.7 (59.81%)
FH-326	36.67±0.44	26.13±0.6 (72.26%)	21±0.55 (58.06%)
5143-P19	35.83±0.73	27.1±0.6 (75.63%)	22±0.45 (61.40%)
FH-472	36±0.58	28.1±0.58 (78.24%)	18.8±0.6 (52.31%)
FH-488	34.67±0.67	25.3±0.34 (73.08%)	21.17±0.54 (61.06%)
FH-474	34.33±1.20	28.7±0.9 (82.04%)	18±0.45 (52.43%)
FH-473	35.67±0.67	28.77±0.76 (80.65%)	22.53±0.56 (63.18%)
N-878	36.67±0.88	30.83±0.8 (84.09%)	23.23±0.4 (63.36%)
BS-18	43.67±0.64	34.17±0.76 (78.24%)	26.77±0.45 (61.30%)
SS-32	35.33±0.85	25.7±0.65 (72.92%)	18.7±0.2 (52.92%)
BT-102	33.33±0.82	28.23±0.4 (84.70%)	21.2±0.45 (63.60%)
BS-15	34.83±0.70	26.3±0.46 (75.60%)	23.13±0.5 (66.41%)

Among the twenty cotton genotypes, BS-18 showed the best performance, with the highest fresh shoot weight of 64.5 g in the control. FH-Lalazar exhibited the lowest fresh weight (15.5 g), which was significantly reduced compared to other genotypes. In T1, VH-488 performed as the second most tolerant variety, with a fresh shoot weight of 47 g. In T2, FH-473 showed the second highest fresh weight (39.7 g), while in T3, FH-142 exhibited the second best performance (31.7 g).

The decrease in fresh shoot weight with increased salinity and temperature is likely due to reduced germination, photosynthesis, and transpiration, along with the accumulation of Na⁺ and Cl⁻ ions, which disrupt normal metabolic processes. The control treatment (T1) showed optimal fresh shoot weight, while T2 and T3 exhibited progressively lower values.

These results align with Abdel-Raheem et al. (2021), who found that salt stress significantly reduced shoot and root growth due to increased salt and chloride content. Similarly, Fu et al. (2021) observed reduced shoot and root lengths in wheat under saline conditions. Singh et al. (2018) also reported that shoots were more severely affected than roots under salt stress, further supporting the findings of this study.

Fresh Root Weight (g)

The cotton fresh root weight data were analyzed statistically at P ≤ 0.05 to determine the effect of salinity on root growth. Significant differences were observed across treatments, with root weight decreasing as salinity and temperature increased. The highest fresh root weight was recorded in the control (20.3 g), while the lowest was in T3 with 14 dS/m salinity

(5.17 g). The pattern of root weight was: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3).

Table 4: Salinity and Temperature effect on Fresh shoot weight (g)

Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	44.33±2.9	36±1.9 (82%)	27±1.8 (60.9%)
FH-lalazar	22±1.6	21±1.7 (95.5%)	15.5±1.3 (69.7%)
FH-142	47±1.2	36.3±1.2 (77.3%)	31.7±1.9 (67.38%)
VH-327	45.33±1.3	35±1.4 (77.9%)	29±0.8 (63.97%)
FDH-170	45±1.5	35.7±1.5 (78.5%)	28±1.2 (62.22%)
FH-444	43.33±2.9	35.3±1.9 (81.5%)	29±1.6 (66.92%)
FDH-228	46.67±1.3	31.9±1.2 (67.9%)	27.33±1.7 (58.57%)
FH-Super	46.33±1.6	36±1.5 (78.4%)	26±1.4 (56.12%)
FH-490	47±1.2	36.7±1.6 (78%)	26±0.8 (55.32%)
FH-326	45.58±2.8	33.7±1.7 (73.7%)	29±1.2 (63.5%)
5143-P19	43±1.9	33±1.4 (76.7%)	25±1.4 (58.14%)
FH-472	45.7±1.3	35±1.6 (78.1%)	24±1.7 (52.55%)
FH-488	47±1.5	39±1.9 (82.4%)	30±1.5 (63.38%)
FH-474	44.9±2.0	37.7±1.3 (84.3%)	26±1.8 (58.21%)
FH-473	47±2.4	39.7±0.9 (84.4%)	29±1.5 (61.1%)
N-878	43.7±2.9	41.3±1.3 (94.7%)	24±1.9 (54.96%)
BS-18	64.5±1.6	48.7±1.8 (75.3%)	36±1.45 (55.67%)
SS-32	52±1.3	37.67±1.7 (72.4%)	26±1.4 (50%)
BT-102	45±2.5	39.67±1.3 (88.1%)	26±1.9 (57.78%)
BS-15	45±1.1	40.3±1.7 (89.6%)	26±2.0 (57.78%)

Among the twenty cotton genotypes, BS-18 showed the best performance, with the highest root weight of 20.3 g in the control, while FH-Lalazar showed the lowest root weight (10.6 g). In T1, FDH-228 showed the second-highest root weight (18.03 g). In T2, FH-472 recorded the second-highest fresh root weight (13.57 g), and in T3, FH-142 was the second most tolerant variety, with a fresh root weight of 9.83 g.

The reduction in root weight with increasing salinity and temperature is attributed to the negative impacts on germination, photosynthesis, and transpiration, alongside the accumulation of Na⁺ and Cl⁻ ions, which disrupt normal metabolic processes. The control treatment (T1) displayed optimal root growth, while T2 and T3 exhibited progressively lower root weights.

These findings are consistent with Ergin et al. (2021), who found that increasing NaCl levels resulted in a significant reduction in root and shoot growth, likely due to the toxic effects of sodium ions and the impaired uptake of K⁺. Zhang et al. (2021) similarly reported that salinity stresses hinder root and shoot growth due to sodium toxicity. Munawar et al. (2021) also observed that some genotypes, such as BS-18, exhibited greater tolerance to salinity stress.

Shoot Dry Weight (g)

The cotton shoot dry weight data were statistically analyzed at P ≤ 0.05 to assess the effects of varying salinity levels. Significant differences were observed across treatments, with shoot dry weight decreasing as salinity increased. The highest dry shoot weight was

recorded in the control (20.8 g), while the lowest was in T3 with 14 dS/m salinity (5.7 g). The trend of shoot dry weight was: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3).

Table 5: Salinity and Temperature effect on fresh root weight (g)

Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	15.5±0.29	12.5±1.25 (80.65%)	8.77±1.2 (56.56%)
FH-lalazar	10.6±0.26	7.02±1.21 (66.26%)	5.17±1.1 (48.74%)
FH-142	15.07±0.35	12.73±1.51 (84.51%)	9.83±1.09 (65.27%)
VH-327	15.6±0.23	13.47±0.68 (86.32%)	8.23±1.15 (52.78%)
FDH-170	15.5±1.3	11.9±0.76 (76.99%)	8.4±1.26 (54.19%)
FH-444	15.1±1.38	12.07±1.31 (79.91%)	7.47±1.23 (49.45%)
FDH-228	18.03±1.26	12.2±1.32 (67.65%)	8.4±1.23 (46.58%)
FH-Super	16.9±1.32	12.63±1.45 (74.46%)	7.37±0.93 (43.42%)
FH-490	15.7±0.76	11.73±1.34 (75.37%)	8.37±1.2 (53.75%)
FH-326	16.6±0.32	13.77±1.45 (82.93%)	7.47±1.24 (44.98%)
5143-P19	18.7±0.40	11.67±1.43 (62.39%)	8.43±1.4 (45.10%)
FH-472	17.13±0.59	13.57±1.3 (79.18%)	9.37±1.32 (54.67%)
FH-488	17.1±1.2	12.93±1.56 (75.63%)	7.43±1.1 (43.47%)
FH-474	17.57±0.76	10.67±1.23 (60.72%)	8.23±1.19 (46.87%)
FH-473	17.03±1.25	13.3±1.2 (78.08%)	9.17±1.32 (53.82%)
N-878	17.6±1.3	10.9±0.89 (61.93%)	7.8±1.15 (44.32%)
BS-18	20.3±0.67	15.77±0.67 (77.67%)	12.67±1.18 (62.40%)
SS-32	15.83±0.44	13.53±1.45 (85.47%)	8.27±2.09 (52.51%)
BT-102	16±0.54	10.93±1.3 (68.33%)	7.33±2.2 (45.83%)
BS-15	16.87±1.4	11.7±1.54 (69.37%)	9.37±1.98 (55.53%)

Among the twenty genotypes, BS-18 performed the best, with the highest dry shoot weight of 20.8 g in the control. FH-Lalazar exhibited the lowest dry shoot weight (10.5 g). In T1, VH-327 showed the second-highest shoot dry weight (19.7 g), while in T2, FH-326 recorded the second-highest weight (15.5 g). In T3, FDH-170 showed the second-best performance (13.5 g).

The reduction in shoot dry weight with increasing salinity is attributed to the negative effects on reproduction, respiration, and evaporation, along with the accumulation of Na⁺ and Cl⁻ ions, which disrupt normal metabolic processes in cotton plants. The control treatment (T1) displayed optimal shoot dry weight, while T2 and T3 showed progressively lower values.

These findings are in line with Liu et al. (2014), who found that increasing NaCl levels resulted in significant reductions in shoot and root length due to the accumulation of sodium ions in plant tissues, especially in the roots. Shaheen et al. (2012) also reported similar results, highlighting the importance of root dry matter in maintaining shoot growth under salt stress, which is crucial for developing salt-tolerant genotypes. Chen et al. (2020) further confirmed that certain genotypes, such as BS-18, exhibited greater tolerance to salinity, supporting the results of this study.

Root Dry Weight (g)

The cotton root dry weight data were analyzed statistically at P ≤ 0.05 to evaluate the impact of varying salinity levels. As salinity increased, root dry weight

decreased in all treatments, with the highest recorded in the control (6.57 g) and the lowest in T3 with 14 dS/m salinity (0.67 g). The sequence of root dry weight was: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3).

Table 6: Salinity and Temperature Effect on Shoot dry weight (SDW) (g)

Shoot dry weight			
Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	15.5±0.35	13.5±0.31 (86.17%)	11.5±0.25 (73.40%)
FH-lalazar	10.5±0.3	6.5±0.34 (61.9%)	5.7±0.15 (50.16%)
FH-142	17.5±0.54	14.5±0.19 (84.47%)	11.5±0.28 (66.99%)
VH-327	19.7±0.4	15.5±0.35 (80.87%)	11.5±0.24 (60%)
FDH-170	18.5±0.3	13.5±0.37 (72.97%)	13.5±0.21 (72.97%)
FH-444	15.5±0.33	14.5±0.28 (93.55%)	10.5±0.24 (67.74%)
FDH-228	16.5±0.35	11.5±0.25 (69.42%)	12.5±0.26 (75.45%)
FH-Super	16.8±0.45	13.5±0.22 (80.36%)	10.5±0.21 (60.50%)
FH-490	15.5±0.32	14.1±0.13 (91.40)	13.5±0.24 (87.10%)
FH-326	17.5±0.32	15.5±0.26 (88.57%)	11.5±0.21 (65.71%)
5143-P19	16.5±0.3	14.5±0.21 (87.88%)	10.5±0.32 (63.64%)
FH-472	15.8±0.4	14.4±0.16 (89.47%)	11.5±0.25 (72.63%)
FH-488	18.5±0.32	14.5±0.25 (78.38%)	9.33±0.42 (53.15%)
FH-474	15.7±0.19	14.5±0.21 (95.60%)	11.5±0.25 (75.82%)
FH-473	16.5±0.32	12.7±0.12 (73.74%)	12.5±0.23 (75.76%)
N-878	15.5±0.21	13.5±0.24 (87.10%)	10.5±0.18 (67.74%)
BS-18	20.5±0.24	17.5±0.25 (85.37%)	15.5±0.27 (75.61%)
SS-32	15.5±0.15	12.5±0.31 (80.65%)	11.5±0.25 (74.19%)
BT-102	15.8±0.4	12.5±0.26 (78.95%)	10.5±0.23 (66.32%)
BS-15	16.5±0.25	14.5±0.21 (87.88%)	12.5±0.32 (75.76%)

Every value is mean of 3 Replicates ± standard Error.

Among the twenty genotypes, BS-18 performed the best, with the highest root dry weight of 6.57 g in T1, while FH-Lalazar showed the lowest root dry weight (3.23 g). In T3, BS-18 had a significantly higher root dry weight (2.68 g) compared to FH-Lalazar (0.67 g), highlighting its tolerance to salt and heat stress.

The reduction in root dry weight with increased salinity is attributed to the negative effects on germination, photosynthesis, and transpiration, alongside the accumulation of Na⁺ and Cl⁻ ions, which disrupt normal metabolic processes. The control treatment (T1) displayed optimal root growth, while T2 and T3 exhibited progressively lower values.

These results align with Shukry et al. (2012), who observed significant reductions in shoot and root length under NaCl stress due to the increased accumulation of sodium and chloride ions in plant tissues. Similarly, Al Ashkar et al. (2020) emphasized the role of root dry mass in evaluating salt-tolerant genotypes for breeding programs. Akhtar et al. (2003) also found that some genotypes exhibited greater tolerance to salinity based on growth parameters such as root and shoot dry weight.

Physiological Parameters

Chlorophyll Contents (SPAD value)

The chlorophyll content data were analyzed at P ≤ 0.05, revealing significant effects of salinity and temperature on chlorophyll levels in cotton. As both salinity and temperature increased, chlorophyll content

decreased across all treatments, with the highest value recorded in the control (74.43) and the lowest in T3 at 14 dS/m salinity (26.2). The trend followed was: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3).

Table 7: Salinity and Temperature effect on root dry weight (RDW) (g)

Root Dry Weight			
Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	5.46±0.119	3.23±0.09 (60.13%)	1.12±0.02 (20.54%)
FH-lalazar	3.23±0.058	2.27±0.06 (68.51%)	0.67±0.02 (20.30%)
FH-142	4.36±0.061	3.33±0.05 (77.14%)	1.25±0.03 (28.67%)
VH-327	5.55±0.06	4.25±0.04 (77%)	1.27±0.02 (22.64%)
FDH-170	4.36±0.064	3.28±0.06 (74.69%)	1.23±0.01 (28.29%)
FH-444	5.34±0.06	3.23±0.05 (60.74%)	1.93±0.02 (36.20%)
FDH-228	4.57±0.08	3.53±0.05 (77.54%)	1.27±0.02 (26.92%)
FH-Super	5.37±0.11	3.73±0.08 (70.27%)	1.24±0.02 (23.09%)
FH-490	4.63±0.07	3.49±0.06 (74.45%)	1.67±0.03 (34.95%)
FH-326	4.35±0.09	3.28±0.08 (67.85%)	1.54±0.02 (35.40%)
5143-P19	4.43±0.12	4.25±0.07 (77%)	1.65±0.02 (37.04%)
FH-472	4.37±0.07	3.53±0.05 (82%)	1.79±0.01 (39.74%)
FH-488	5.68±0.07	3.45±0.05 (60.62%)	1.47±0.02 (25.94%)
FH-474	5.73±0.09	4.26±0.06 (74.58%)	1.77±0.02 (30.89%)
FH-473	5.44±0.13	3.23±0.06 (59.44%)	1.65±0.04 (30.89%)
N-878	5.33±0.15	3.63±0.05 (68.8%)	1.35±0.04 (25.28%)
BS-18	6.57±0.045	5.73±0.06 (87.79%)	2.68±0.04 (41.16%)
SS-32	5.37±0.16	4.33±0.07 (80.11%)	1.82±0.02 (33.72%)
BT-102	4.43±0.13	3.28±0.05 (72.48%)	1.3±0.03 (29.01%)
BS-15	4.47±0.08	3.56±0.06 (33.36%)	1.47±0.02 (33.36%)

Each value is an average of 3 Replicates ± Standard Error.

BS-18 performed best among the twenty genotypes, showing the highest chlorophyll content in T1 (74.43), and better performance than other varieties in T2 (48.13) and T3 (26.2). FH-Lalazar exhibited the lowest chlorophyll content, particularly in T3 (26.2). The reduction in chlorophyll content is likely due to the negative impact of salinity and temperature on photosynthesis, respiration, and transpiration, alongside the accumulation of Na⁺ and Cl⁻ ions, which disrupt metabolic processes.

FDH-228 showed the second-highest chlorophyll content in T1 (66.47), while FH-474 was second in T2 (46.57), and FDH-228 again performed second in T3 (39.73). These findings align with Ibrahim et al. (2019), who reported a decrease in chlorophyll content under salt stress due to impaired photosynthesis and chlorophyll synthesis. MDA accumulation, a marker of lipid peroxidation, further supports the observed decline in chlorophyll content with increasing salinity.

Relative Water Content (Percentage)

The relative water content (RWC) data were statistically analyzed at P ≤ 0.05, showing significant effects of varying salinity and temperature on cotton's water retention. As salinity and temperature increased, RWC decreased across all treatments. The highest RWC was recorded in the control (95.5%), while the lowest was in T3 with 14 dS/m salinity (30.58%). The trend of RWC was: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3).

Table 8: Salinity and Temperature effect on Chlorophyll contents

Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	64.7±2.98	44.3±1.85 (68.46%)	32.83±1.72 (50.75%)
FH-lalazar	46.7±2.85	32.63±1.8 (69.88%)	26.2±1.66 (56.10%)
FH-142	66.03±3.17	45.47±1.73 (68.85%)	38.37±1.82 (58.10%)
VH-327	63.37±3.49	43.67±1.18 (68.91%)	33.17±1.44 (52.34%)
FDH-170	64.7±2.85	40.2±1.83 (62.13%)	37.43±1.93 (57.86%)
FH-444	63.83±3.15	39.8±1.87 (62.35%)	35.83±1.97 (56.14%)
FDH-228	66.47±1.85	44.47±1.53 (66.90%)	39.73±1.43 (59.78%)
FH-Super	60.93±2.01	40.47±1.93 (66.41%)	33.23±1.60 (54.54%)
FH-490	63.75±2.59	42.03±2.07 (65.93%)	37.57±2.01 (58.93%)
FH-326	59.93±2.33	45.1±1.09 (75.25%)	34.47±1.79 (57.51%)
5143-P19	58.27±2.31	43.77±1.94 (75.11%)	37.5±1.97 (64.36%)
FH-472	63±3.21	42.27±1.95 (67.09%)	38.87±1.90 (61.69%)
FH-488	59.37±2.19	44.77±2.02 (75.41%)	35.43±1.58 (59.69%)
FH-474	64.3±3.4	46.57±2.05 (72.42%)	38.2±1.78 (59.41%)
FH-473	63.43±2.52	43.37±1.91 (68.37%)	33.87±1.58 (53.39%)
N-878	60.13±3.27	46.03±1.93 (76.55%)	37.3±1.73 (62.03%)
BS-18	74.43±2.15	55.83±1.79 (75.01%)	48.13±1.45 (64.67%)
SS-32	57.53±2.6	44.1±1.84 (76.65%)	43.43±1.96 (75.49%)
BT-102	60.87±1.97	43.73±1.66 (71.85%)	43.53±1.98 (71.52%)
BS-15	55.37±1.34	43.73±1.65 (78.99%)	38.57±1.95 (69.66%)

Table 9: Salinity effect on relative water content (RWC) (%age)

Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	86.73±3.45	53.86±2.34 (62.11%)	45.89±2.4 (52.82%)
FH-lalazar	60.57±3.98	40.53±1.45 (66.93%)	30.58±2.1 (50.46%)
FH-142	87.85±2.45	60.46±3.45 (69.38%)	40.85±1.56 (46.88%)
VH-327	88.62±4.3	55.49±2.76 (62.64%)	45.16±1.3 (50.98%)
FDH-170	80.37±2.45	58.75±2.45 (73.13%)	43.24±1.26 (53.80%)
FH-444	80.68±3.5	59.61±2.35 (73.94%)	47.87±1.45 (59.37%)
FDH-228	84.35±3.2	57.16±3.24 (67.8%)	44.84±2.1 (53.19%)
FH-Super	85.57±2.67	58.98±2.34 (68.91%)	45.05±1.13 (52.68%)
FH-490	89.75±2.65	59.98±2.13 (66.80%)	43.08±2.31 (47.99%)
FH-326	88.02±2.45	64.17±3.23 (72.90%)	46.76±2.34 (53.12%)
5143-P19	89.99±2.67	60.18±2.1 (66.87%)	44.69±2.32 (49.67%)
FH-472	87.78±2.13	59.76±2.45 (68.11%)	50.32±1.78 (57.36%)
FH-488	82.54±2.76	61.97±3.45 (75.11%)	43.55±1.76 (52.76%)
FH-474	81.79±2.45	58.47±2.13 (71.52%)	49.06±2.01 (60.01%)
FH-473	85.24±3.23	55.06±3.23 (64.60%)	43.81±1.87 (51.40%)
N-878	86.92±1.56	55.26±2.12 (63.53%)	46.24±1.34 (53.19%)
BS-18	95.5±4.2	72.11±2.12 (75.51%)	56.04±1.54 (58.68%)
SS-32	86.89±2.76	64.61±3.45 (74.44%)	50.29±2.3 (57.94%)
BT-102	85.74±2.54	57.53±3.2 (67.07%)	42.78±1.20 (49.88%)
BS-15	87.15±2.65	56.32±2.2 (64.63%)	48.57±1.98 (55.73%)

Each value is an average of 3 Replicates ± Standard Error.

Among the twenty genotypes, BS-18 performed the best, with the highest RWC of 95.5% in T1. FH-Lalazar exhibited the lowest RWC (60.57%). In T2 and T3, BS-18 outperformed other varieties, with RWC values of 56.04% and 30.58%, respectively. The second most tolerant variety in T1 was 5143-P19 (89.99%), in T2 SS-32 (64.61%), and in T3 SS-32 again showed second-highest RWC (50.29%).

The decrease in RWC with increasing salinity and temperature is attributed to reduced photosynthesis, transpiration, and increased Na⁺ and Cl⁻ ion accumulation, disrupting the cotton plant's metabolic activities. The control treatment (T1) showed optimal RWC, while T2 and T3 exhibited

progressively lower values.

These findings align with Sikder et al. (2020), who reported a dramatic decrease in RWC and membrane stability index under increasing salinity, likely due to the enhanced accumulation of sodium and chloride ions in plant tissues. Magwanga et al. (2019) also found that salt tolerance in wheat was associated with physiological traits like low Na⁺ fluxes, high K⁺ fluxes, and a high K: Na ratio, with key parameters such as RWC, MSI, and chlorophyll content decreasing under salinity stress.

Membrane Stability Index (Percentage)

The membrane stability index (MSI) data were statistically analyzed at P ≤ 0.05, revealing significant effects of varying salinity and temperature on cotton's membrane integrity. As salinity and temperature increased, MSI decreased across all treatments. The highest MSI was recorded in the control (81.97%), while the lowest was in T3 with 14 dS/m salinity (23.17%). The trend followed was: Control (T1) > 7 dS/m (T2) > 14 dS/m (T3).

Among the twenty genotypes, BS-18 performed the best, with the highest MSI of 81.97% in T1, while FH-Lalazar exhibited the lowest MSI (52.97%). In T2 and T3, BS-18 outperformed other varieties, with MSI values of 58.07% and 40.31%, respectively. FH-Lalazar showed the lowest MSI in T3 (23.17%).

The reduction in MSI with increasing salinity and temperature is likely due to the negative impacts on fertilization, respiration, and transpiration, along with the accumulation of Na⁺ and Cl⁻ ions, which disrupt normal metabolic processes. The control treatment (T1) displayed optimal MSI, while T2 and T3 showed progressively lower values.

Table 10: Salinity effect on Membrane Stability Index (%age)

MSI	Varieties	Control	7 dS m ⁻¹	14 dS m ⁻¹
FH-492	71.41±2.07	55.39±2.06 (77.56%)	34.35±2.33 (48.18%)	
FH-lalazar	52.97±3.08	36.46±1.76 (68.85%)	23.17±1.34 (43.78%)	
FH-142	69.65±4.75	51.94±2.78 (74.59%)	39.12±3.23 (56.21%)	
VH-327	72.22±3.75	55.27±2.46 (76.49%)	33.09±2.23 (45.73%)	
FDH-170	65.83±2.07	57.01±2.98 (86.60%)	34.99±2.32 (53.06%)	
FH-444	64.17±3.97	52.66±1.98 (82.08%)	33.76±2.12 (52.63%)	
FDH-228	63.25±2.05	50.27±2.87 (79.44%)	32.02±2.33 (50.64%)	
FH-Super	65.51±1.89	53.38±2.33 (81.32%)	33.49±2.34 (50.97%)	
FH-490	63.83±2.09	53.34±1.56 (83.51%)	33.84±1.34 (52.93%)	
FH-326	64.93±1.98	56.86±2.45 (87.57%)	33.22±2.34 (51.21%)	
5143-P19	66.77±2.09	54.18±1.56 (81.16%)	36.45±2.56 (54.58%)	
FH-472	69.12±2.09	56.08±2.34 (81.03%)	34.66±2.4 (50.10%)	
FH-488	68.09±2.98	58.07±1.04 (85.30%)	33.34±1.34 (48.97%)	
FH-474	70.91±2.45	53.72±2.45 (75.82%)	36.28±2.43 (51.16%)	
FH-473	69.34±3.01	56.17±1.04 (80.89%)	35.75±1.24 (51.53%)	
N-878	68.53±2.03	57.15±1.24 (83.44%)	34.62±2.34 (50.54%)	
BS-18	81.97±2.06	65.46±2.34 (79.84%)	40.31±2.45 (49.22%)	
SS-32	70.47±2.56	52.81±1.23 (74.97%)	33.01±2.1 (46.96%)	
BT-102	68.78±3.04	55.34±2.22 (80.55%)	34.77±1.23 (50.47%)	
BS-15	66.82±2.06	55.39±1.23 (82.84%)	35.8±1.34 (53.54%)	

The second most tolerant variety in T1 was VH-327, with an MSI of 72.22%, while in T2, FH-488 recorded

58.07%. In T3, FH-142 showed the second-highest MSI (39.12%). These results are consistent with Jafaraghaei (2019), who reported a significant decrease in MSI under increasing salinity, attributing the decrease to the higher accumulation of sodium and chloride ions in plant tissues, particularly in the roots, which adversely affects membrane stability.

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