Analysis of physiological and chemical changes in wheat crop under salt stress using spectrophotometric techniques

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Abstract

The examination of wheat plant (Triticum aestivum L.) response to salt stress under modern spectroscopic analysis methods is the primary goal of this work. The researchers performed their experiments in Baghdad Governorate during November 2024 using sodium chloride concentrations that ranged from 0 to 50 mM up to 100 mM and 150 mM. Researchers performed assessments through measurements of plant height along with leaf quantity and the detection of chlorophyll content and soluble sugar levels and total proteins and chlorophyll fluorescence performance (Fv/Fm). The experiment showed salinity stress reduced chlorophyll and protein amounts but soluble sugars showed an adaptation to increased salt levels. The exact detection of physiological changes worked best through spectroscopic methods including UV-Vis, FTIR, and Fluorescence testing. There is a recommendation to combine these methods for evaluating environmental stress and selecting salt-tolerant genotypes that are suitable for saline-affected areas.

Keywords: Salt stress, wheat, spectroscopy, chlorophyll, proteins, sugars.

Introduction

Salinity stands as a severe environmental obstacle that damages agricultural land in both arid and semi-arid zones of Iraq along with other regions. [ ] Inactive farmlands expand as a result of three primary factors: irrigation using water of excessive salinity combined with deficient drainage systems and the strong evaporation rates compared to rainfall volumes. Plant growth faces direct harm from salinity because the substance disrupts water absorption and nutrients and damages ionic balance mechanisms in plant cells thereby harming essential biological functions such as photosynthesis and respiration along with protein synthesis [ ] .Wheat (Triticum aestivum L.) serves as a strategic agricultural food source which supports food security for all Iraqi people. Wheat ranks as one of the salt-sensitive crops which fall under this classification. Saline conditions in soil cause severe negative impacts on seed germination rates and lead to restricted growth and worsens yield volume and quality. The analysis of wheat metabolic transformation during salt exposure conditions enables scientists to study genetic adaptations for salt tolerance systems. Knowledge of these developments will aid in creating efficient methods to manage agriculture.

Spectroscopic analysis techniques serve as an efficient rapid non-destructive method to detect biological changes in plants. Three analytical methods including UV-Vis and FTIR spectrometers and fluorescence instruments deliver precise detection of chlorophyll and proteins and sugars and

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phenolic compounds change. The combined analytical tools yield extensive understanding about plant biochemical conduct under stressful conditions [ ] .

A group of sophisticated spectroscopic techniques will be implemented to track salt stress-induced modifications in wheat plants at various salinity levels. The research investigates two aspects: physiological growth modifications and chlorophyll content changes together with essential cellular compound biochemical alterations. This research will expand plant response knowledge which can help develop agricultural and production methods to address salinity impacts in comparable environments.

Theoretical Framework:

.1Concept of Salt Stress in Plants

As a physiological response salt stress develops from the excess accumulation of soluble salts that affect both plant tissue content and root zone sodium (Na^+) chloride (Cl^-) ions. Plants become unable to absorb water and essential nutrients since salt deposits accumulate inside them. Soil accumulation of salts remains the major environmental stressor which decreases agricultural productivity most strongly in dry regions where excessive evaporation rates together with poor irrigation systems create salt buildups in the ground.

Plant systems experience two significant limitations when exposed to salt stress conditions [ ] .

a. Osmotic Stress:

During the first contact with salts the plant stops absorbing water because the soil water pressure becomes lower. Tissue deformation occurs together with growth reduction in plants due to this process.

b. Ionic Stress:

The process occurs after repetitive saline exposure generates toxic salt accumulation in plants which primarily consists of sodium and chloride. The excessive accumulation of toxic ions causes severe disruption of essential physiological activities and enzymatic functions which simultaneously damages photosynthesis and respiration processes.

The productivity decrease of crops cultivated in saline soils results from these two mechanisms thus requiring accurate analytical instruments like spectroscopy for focused studies.

.2Physiological Effects of Salt Stress

The vital operations of plants encounter exceptional challenges due to salt stress when they inhabit dry arid areas that experience both inadequate water supplies and detrimental heat conditions. These factors together lead to increased soil salinity. [ ] The adaptation of plants to rising salinity requires complex physiological mechanisms which govern growth development and support photosynthesis functionality together with controlling water intake and maintaining essential ion motion and vital biological systems for their survival and fruitful development.

Salt stress triggers visible changes in photosynthesis early on since this fundamental energy-generating mechanism of plants becomes affected. The main reason behind photosynthetic disruption results from how salts destabilize chloroplast membranes while reducing chlorophyll levels. Plants reduce their intake of carbon dioxide for photosynthesis due to stomatal closure that becomes a water saving method triggered by saline conditions. Plant growth and productivity decrease because photosynthetic efficiency decreases due to reduced activity of Rubisco enzyme [ ] .

Salinity decreases root water absorption because salts present in the root zone generate negative osmotic potential. Plants cannot efficiently absorb available water because of negative osmotic potential which produces drought-like stress similar to drought stress. The combination of tissue wilting with decreased turgor pressure results in field-level reduction of physiological activity.

Salinity causes destructive effects on the development of both roots and shoots. White gloomy symptoms appear throughout plant development because plants grow inefficiently and their leaves decrease in number while their root system grows weakly with sparse tiller production. The observed signs show that cell division and growth procedures experience blockade events. The salt stress leads to hormonal unbalance in plants which shows reduced levels of auxins and gibberellins together with increased abscisic acid (ABA) that functions as a stress response hormone.

Plants under salt stress conditions display a critical physiological change involving disruption of their cellular salt composition. Destructive sodium chlorine ions replace vital potassium and calcium ions when these specific ions are displaced from their original positions. The cellular enzymatic functions become less effective because salt-related ionic replacement disrupts stomatal operation and impairs electrochemical messaging pathways and generates cell-wide oxidative stress .

Plants adapt to salt stress conditions by launching protective measures which enable successful adaptation and tolerance of the environment. Proline along with mannitol and soluble sugars serve as prominent compatible osmolytes which plants produce under salt stress conditions. The compounds function as vital water content protectors which lower cellular osmotic potential for maintaining water balance within cells. The osmolytes also protect proteins and cell membranes from damage. The osmolytes which cells produce help mitigate ROS damage without being affected by salt stress conditions [ ] .

The efficiency of nutrient uptake gets deeply impacted by the stress of salinity which affects the procedure of metabolism. When salt ions invade root absorption sites they force out essential cations like potassium calcium and magnesium thus creating compound deficiencies. When plants experience both saline conditions and imbalanced nutrient supply their growth prospects become diminished while recovery potential decreases.

Association between plant genotypes and salt tolerance effectiveness arises primarily from their different abilities to protect themselves with physiological defense mechanisms. The evaluation of such responses establishes fundamental knowledge to choose the correct cultivars for salt-affected soil cultivation.

.3Biochemical Effects of Salt Stress

The biochemical response to salt affects organic compound structures while modifying their synthesis patterns within plant tissues and holds beyond simple physiological effects. The plant demonstrates its capability to survive or perish based on its tolerance of saline conditions. Biochemical modifications serve as one of the most accurate indicators of salt stress and scientists can analyze them

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effectively through chemical or spectroscopic examination .

The onset of salt stress becomes evident through diminished protein contents which result in decreased amounts of total protein within plant organs. Ionic and oxidative stress inhibits transcription and translation activities thus causing this effect. The excessive presence of sodium and chloride ions destabilizes both endoplasmic reticulum structure and ribosomes which produces malfunctioning or unstable functional proteins needed for protein synthesis. The disruption impacts enzymes whose sensitive tertiary structures shift because of ionic disruptions which results in reduced or complete loss of enzymatic activity.

Plants which demonstrate salt-tolerance tend to accumulate the soluble sugars glucose together with fructose and sucrose. Salts within these sugars enable cells to sustain their osmotic pressure under stress conditions while providing immediate energy when stress appears. Plant cells benefit from soluble sugars which stabilize protein structure and safeguard membrane integrity thus making them crucial elements in the metabolic defense mechanisms under saline exposure.

Salt stress stimulates plants to produce antioxidants and phenolic compounds which represent the most important biochemical stress responses. High salt contents in plant cell milieu increase formation of reactive oxygen species that include hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH). The compounds attack and cause detrimental effects on the structural components of proteins as well as nucleic acids and lipid membranes. Plants activate their secondary metabolic pathways to create antioxidants such as phenolics, flavonoids, carotenoids while simultaneously boosting antioxidant enzyme activity of superoxide dismutase (SOD) together with catalase (CAT) and peroxidase (POD.(

Salt-tolerant plants quickly activate antioxidant enzymes while maintaining efficient operation to restrict cellular destruction during increasing stress levels. The cells of sensitive genotypes experience more substantial damages when subjected to similar conditions [ ] .

Salinity leads to serious disruptions in the ionic composition found within plant tissue structures. Both toxic sodium chloride concentrations and limited supply of vital minerals potassium calcium and magnesium occur simultaneously in plant tissues. Plant survival depends on these ions because they help conduct metabolic functions and maintain membrane health and trigger intracellular signals. Biological processes such as protein synthesis along with photosynthetic electron transport become impaired alongside changes in enzyme stability due to this imbalance.

The adjustments in plant biology make biochemical investigation an essential method for measuring salt stress effects on plants. These detection methods include fluorescence-based procedures in combination with ultraviolet-visible (UV-Vis) spectroscopy and Fourier-transform infrared (FTIR) spectroscopy which achieve both low-invasive detection of changes. Experts in both research and agriculture can initiate prompt appropriate solutions utilizing either better farming practices or using salt-resistant plant varieties through early detection employing such detection methods.

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.4Role of Spectroscopic Techniques in Studying Plant Responses to Salt Stress

Evaluation of physiological along with biochemical plant changes subjected to environmental stresses including salinity depends on spectroscopic techniques. The methods deliver non-invasive fast precise findings which supply qualitative combined with quantitative data regarding modifications to essential biomolecules inside plant tissues.

The analysis of chlorophyll concentration together with the evaluation of sugars and proteins and enzymes and organic molecule structure best utilizes spectroscopic techniques to detect plant reactions to salinity stress.

Most experts utilize UV-Vis Spectroscopy along with other spectroscopic techniques to monitor the composition of various compounds [ ] .

.1Ultraviolet–Visible(UV-Vis) Spectroscopy:

Scientists employ this test to determine compound concentration within the 200–800 nm wavelength light absorption range which includes chlorophylls carotenoids and phenolics. It is useful for:

Total chlorophyll amounts provide an estimation of photosynthetic operational efficiency.

Researchers use the spectroscopic technique to evaluate plant health status and deterioration because of saline exposure.

Testing phenolic compounds functions as a technique for identifying oxidative stress reactions [ ] .

.2Fourier Transform Infrared (FTIR) Spectroscopy:

Through the analysis of chemical bond vibrations researchers can study organic compound molecular structures using this method. It is widely used to:

The method detects protein modifications together with sugar composition and enzymes while measuring lipid alterations.

An investigation of salinity stress should focus on determining which functional groups show changes resulting from the stress.

Study modifications in cell membrane and cell wall composition [ ] .

.3Fluorescence Spectroscopy:

The method detects changes in chlorophyll with high sensitivity and specializes in remarkable detection of Photosystem II (PSII). It is used to:

Examine how efficiently the plant uses light energy through an assessment of illumination-related processes.

The assessment of early stress responses should take place before any visible signs emerge.

The measurement of salinity-related changes that occur within the photosynthetic system requires quantification of functional damage and operational efficiency losses [ ] .

.4Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS:(

The advanced methods offer detailed analyses of small biomolecules including vitamins and amino acids and secondary metabolites while being used minimally in modern research.

Spectroscopic techniques acquire their importance when they reveal:

The precise trace of biochemical transformations becomes possible with this detection method.

Software should identify stress signals before symptoms start appearing in the plant.

Assessments that encompass plant health conditions across different salinity conditions allow producers to make better cultivation decisions regarding cultivar choices [ ] .

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.5Ionic Balance and Its Role in Plant Response to Salt Stress

The ability of plants to maintain balanced ionic content in their tissues provides the determining power for salt tolerance. Membranes and enzymes function poorly when sodium (Na^+) and chloride (Cl^-) ions elevate and push away necessary potassium (K^+) and calcium (Ca^{2+}) ions which provide membrane stability. When the balance between essential ions shatters it decreases the plant's ability to neutralize environmental pressure as well as harms cellular structures.

Excess sodium uptake becomes reduced in certain salt-tolerant genotypes because these plants improve their sodium extrusion capabilities while storing extra sodium in vacuoles through compartmentalization. Many plant species use a high ratio of potassium to sodium ions as an elementary marker to identify salt tolerance [ ] .

.6Oxidative Stress and Antioxidant Defense Mechanisms

The exposure to salty environments stimulates plant cells to produce reactive oxygen species (ROS) as an indirect response to salinity. The reactive compounds H_2O_2 and •OH generate damage throughout cells by oxidizing DNA along with proteins and lipids.

Plants fight salt damage through their activated defense mechanisms which contain the enzymes:

Superoxide dismutase (SOD): Converts superoxide radicals into hydrogen peroxide.

CAT enzyme performs hydrogen peroxide breakdown through a chemical reaction that forms water and oxygen molecules.

Peroxidase (POD): Decomposes harmful peroxides in the cell.

Research results demonstrate salt-tolerant plants contain elevated antioxidant enzymes while salt-sensitive plants possess lower levels of antioxidant enzymes because this explains their superior ability to fight oxidative stress produced by salinity [ ] .

Practical Study:

.1Location and Duration of Implementation

Researchers conducted the study within the experimental agricultural nursery which belongs to a research center located in Baghdad Governorate Iraq. The trial commenced in November 2024 during the right wheat planting season because wheat operates as a winter crop throughout Iraq and growers typically perform wheat seeding at this time. The experimental site had controlled soiled populations and water supply methods yet experienced natural temperature and humidity patterns of the surrounding region.

Regular measurements of wheat plant growth occurred throughout the six-week period following germination. Research investigators gathered plant examples for testing physiological and biochemical factors at the experimental study termination.

.2Experimental Design

RCBD experimental design used along with three treatment replicates for the study. The research utilized sodium chloride (NaCl) solutions at different concentrations to implement four levels of salinity according to the table below.

Number of Pots: 12 (4 treatments × 3 replicates(

Number of Plants per Pot: 5 plants after thinning

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Table (1): Treatment Distribution According to Salinity Levels

Treatment Code	NaCl Concentration (mM)	Description
T1	0	Control (no salinity)
T2	50	Low salinity
T3	100	Moderate salinity
T4	150	High salinity

.3Soil and Pot Preparation

The experiment utilized pots with dimensions of 30 cm diameter and 40 cm depth made from plastic.

The mix of 60% clay and 40% sand in the soil served to improve oxygen circulation and water evacuation.

Soil received thermal sterilization before each pot received a gravel addition placed under its foundation.

Before planting the medicinal seed requires water irrigation until soil reaches an initial water balance state.

.4Seed Sowing and Salinity Treatments

The researchers used "Ibaa 99" wheat cultivar in their experimental work.

The seed sowing procedure started with ten seeds in each pot but the researchers eventually kept only five uniform seedlings per pot.

The plant received its first salinity treatment exactly 14 days from the beginning of germination.

The NaCl solutions were produced according to the concentrations shown in Table (1) to irrigate the plants with 500 ml solution every three days.

.5Physiological and Biochemical Measurements

At week six researchers gathered information through multiple assessments of which the following features comprised the dataset:

The researcher measured all plant specimens using a regular measuring tape to determine their height in centimeters.

Plant analysis included a record of the number of mature leaves found throughout entire plants.

Total Chlorophyll Content:

This study used a Shimadzu UV-1800 UV-Vis Spectrophotometer (Model: Shimadzu UV-1800) at the Plant Analysis Laboratory, Department of Biology, College of Science, University of Baghdad [ ]

Total Soluble Sugars:

Estimated using the Anthrone method. The plant material underwent extraction using

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water before receiving Anthrone reagent and 10-minute heating at 100°C. Absorbance was recorded at 620 nm. The Biochemistry Laboratory at College of Agriculture [ ] .

Total Protein Content:

The procedure determines total protein content through the combination of protein with Folin-Ciocalteu reagent in the traditional Lowry method. The Biochemical Analysis Laboratory at National Center for Agricultural Research performed the analyses by following Lowry et al. (1951) method [ ] .

Chlorophyll Fluorescence (Fv/Fm:(

A FluorPen FP 110 device from Photon Systems Instruments (Czech Republic) served to measure chlorophyll fluorescence .

The Fv/Fm analyses involved collection of data from upper leaf tissues after PSII reached a stable condition through 30 minutes of dark conditions. The Environmental and Salinity Research Center – Baghdad conducted the analyses at its facilities.

Number of Plants per Pot: 5 plants after thinning [ ]

Results and Discussion

A. Plant Height

The salt treatment in T1 achieved the highest plant heights at 60.99 cm while T4 reached only 44.86 cm in the highest salinity level. Research demonstrates that plant height significantly decreases as salt amounts rise in the soil media.

The osmotic stress created by salinity hinders water intake from the soil because it decreases the plant water potential. The growth restriction impacts cell elongation and reduces general plant growth rate. High salt concentrations build up Na^+ and Cl^- which leads to disrupted hormone signaling particularly decreased levels of auxin and gibberellin needed for stem growing. Effects of salt stress cause cells to age prematurely while simultaneously reducing the operational capacity of growth-regulating meristematic areas.

B. Leaf Count

A progressive decline in the number of leaves was observed with increasing NaCl concentration, although this parameter did not vary as significantly as other metrics. Leaf number dropped from the highest in T1 to the lowest in T4

Leaf production is directly influenced by the plant's energy status and hormonal balance, both of which are negatively impacted by salinity. The reduced leaf number may be

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attributed to a combination of oxidative stress, suppression of photosynthetic efficiency, and decreased cellular turgor, which collectively inhibit new leaf initiation. Furthermore, salinity can lead to early leaf abscission, especially in older leaves, as a defensive strategy to reduce transpiration under water-limiting conditions.

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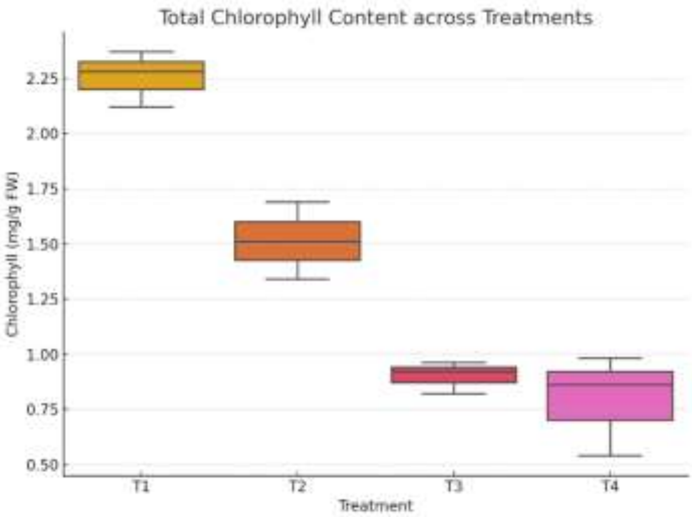
C. Total Chlorophyll Content

The concentration of chlorophyll decreased by 2.37 mg/g FW in T1 and reached 0.67 mg/g FW in T4. Salt stress affects chloroplast structure and pigment biosynthesis in such a manner that results in significant pigment content decline



High salinity causes both chloroplast membrane disintegration and essential mineral ion deficiency which impedes chlorophyll formation. Thylakoid membrane system degradation occurs from both oxidative stress and lipid peroxidation which enhances chlorophyll breakdown. The activity of chlorophyllase enzymes increases under salinity conditions thereby breaking down chlorophyll molecules and resulting in chlorophyll loss together with yellow blade discoloration and decreased photosynthetic performance .

D. Soluble Sugars

An increase in the concentration of soluble sugars was observed with elevated salinity levels. This response is interpreted as an osmotic adjustment mechanism aimed at maintaining water balance within the cells

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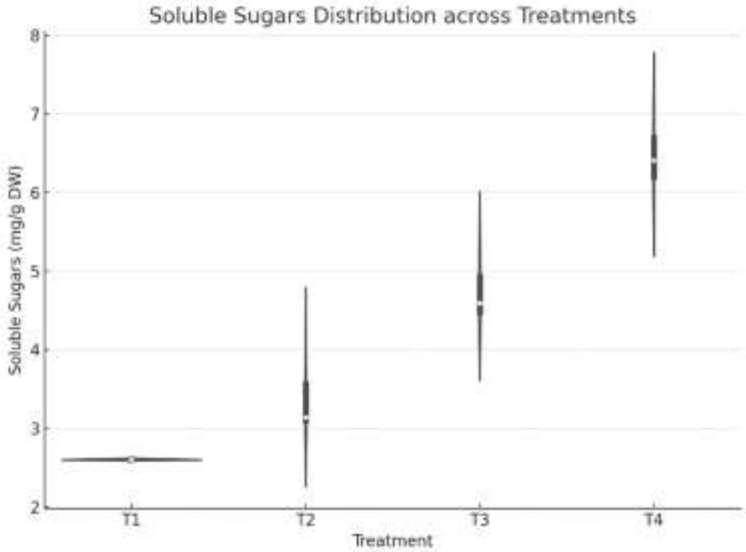

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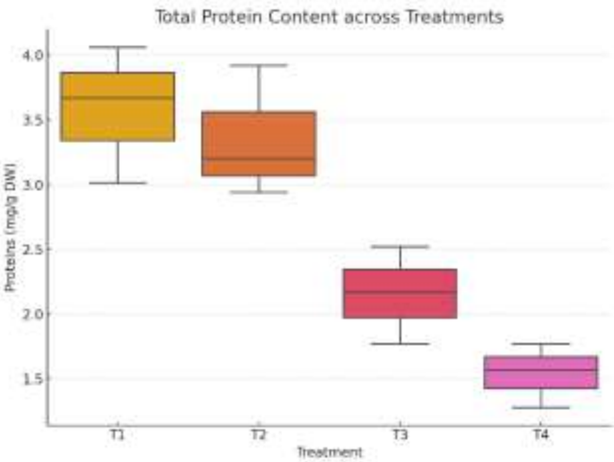
E. Total Proteins

Total protein levels diminished significantly in solutions T3 and T4 when compared to T1. Saline stress causes harm to protein synthesis and turnover processes which leads to diminished protein levels.

Salt stress interferes with both transcriptional and translational processes due to ionic toxicity and oxidative stress. The elevated Na^+ and Cl^- concentrations disrupt ribosomes while damaging the endoplasmic reticulum to create structural and enzymatic protein misfolding that results in denaturation. The oxidizing effect of oxidative stress causes amino acids to become dysfunctional through oxidation. The measurement of reduced total proteins during salt stress conditions marks a specific indicator of metabolism dysfunction



F. Chlorophyll Fluorescence Efficiency (Fv/Fm(

Photosystem II efficiency receives measurement through this parameter. The measurement data showed that Fv/Fm significantly decreased in treatment T4 which

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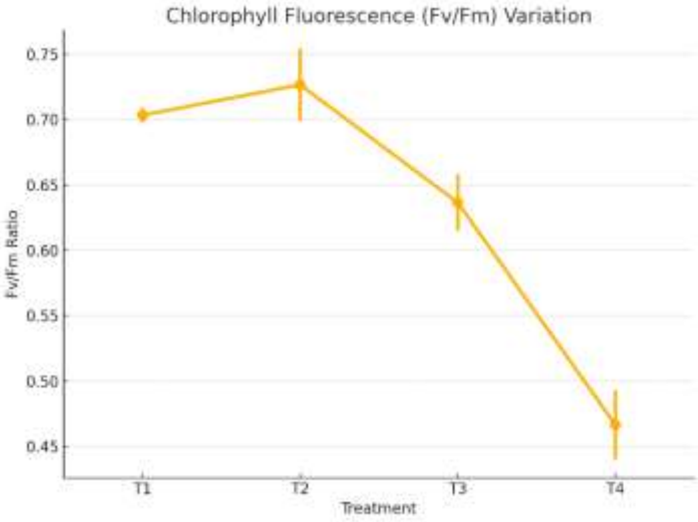
indicated that high salinity stress causes functional damage to the photosynthetic apparatus.



.7Statistical Analysis

SPSS version 25 served for analyzing the data. Research used One-way Analysis of Variance (ANOVA) as its analytical method.

The relevant differences in treatments underwent evaluation with Tukey's Honestly Significant Difference (HSD) test.

Results and Discussion

.1Analysis of Physiological Growth Parameters

The research demonstrated that salt stress created negative effects on wheat plant development. The measurement of plant height together with leaf number decreased systematically when salt concentration levels rose. The control treatment (T1) achieved maximum plant height of 60.99 cm but Plant treatment 4 (T4) reached only 44.86 cm indicating the negative effect of salts on cellular growth and organ growth.

Previous investigations have shown that high salt conditions reduce water absorption thus causing restriction in growth length because of osmotic effects. The disturbed intracellular ionic balance causes hindrance to growth-regulating enzymes and hormones in their activity.

.2Chlorophyll and Photosynthesis

The chlorophyll measurement in the leaf tissue reached its maximum level at 2.37 mg/g FW in treatment T1 and its minimum at 0.67 mg/g FW in treatment T4. The results demonstrate that salt stress negatively impacts chloroplast structure which causes chlorophyll to degrade because sodium and chloride substances accumulate within the plant system. High salt stress in T4 causes severe damage to Photosystem II since the chlorophyll fluorescence efficiency (Fv/Fm) falls below 0.5.

Salt stress generates two negative effects on plants by preventing chlorophyll synthesis and reducing its ability to produce energy in the photosynthetic process thus representing an early sign of stress in plants

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.3Soluble Sugars as an Adaptive Mechanism

The concentrations of soluble sugar in T4 increased substantially compared to T1 due to NaCl treatment resulting in 6.84 mg/g DW as the average value. Results confirm that plants produce soluble sugars as cellular adaptation for maintaining osmotic pressure while protecting cell dehydration processes. Under stressful conditions plants manifest this characteristic adaptive mechanism which demonstrates their ability to adapt.

An increase in sugar content demonstrates that the plant strives to stabilize its proteins and protect its water balance within its cells.

.4Total Proteins

The protein content levels in T3 along with T4 decreased substantially when compared to T1. The observation stems from salt concentrations' negative influence on both protein translation and ribosome structure thus affecting enzyme and structural protein production.

The sensitivity of proteins to salt changes makes their reduction serve as a direct indicator for decreased metabolic functions under stressful situations.

The research used $p < 0.05$ as the significance threshold.

All recorded data points differed significantly from one another with the exception of leaf number.

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Footnotes