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A Causal-Comparative Study of Physiological and Biochemical Parameters of Wheat (Triticum aestivum L.) under Salinity Stress with Reference to Gamma-Aminobutyric Acid

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Abstract

Salinity stress is a major abiotic constraint limiting wheat productivity through osmotic imbalance, ionic toxicity, and oxidative damage. This causal-comparative study examined the physiological, biochemical, and growth-related responses of wheat (*Triticum aestivum* L.) grown under saline and non-saline conditions with reference to graded gamma-aminobutyric acid (GABA) exposure. Using an ex post facto design, naturally differentiated wheat plants of two cultivars (Lasani and Galaxy-13) were compared across salinity conditions (0 and 150 mM NaCl equivalent) and four GABA exposure levels (0, 100, 200, and 400 mg L^{-1}). Growth attributes, water relations, membrane stability, photosynthetic pigments, oxidative stress markers, antioxidant enzyme activities, and metabolic constituents were assessed. Salinity was consistently associated with reduced growth, lower relative water content, increased electrolyte leakage, elevated malondialdehyde levels, and altered antioxidant activity, confirming its strong association with physiological deterioration and oxidative stress. Reference to GABA exposure revealed a clear mitigative pattern, where higher GABA levels were associated with improved water status, enhanced membrane stability, reduced lipid peroxidation, and strengthened antioxidant responses under saline conditions. Significant interaction effects between salinity and GABA supported systematic variation in stress intensity relative to biochemical context. Varietal differences further indicated genotype-specific adaptive responses. Although causal inference is limited by the non-experimental design, the convergence of physiological, biochemical, and statistical evidence supports GABA as a key stress-modulating biochemical factor in wheat under salinity. These findings provide a comparative framework for future experimental validation and stress-management strategies in saline agriculture.

Keywords: Salinity stress, Gamma-aminobutyric acid (GABA), Wheat (*Triticum aestivum* L.), Oxidative stress, Antioxidant enzymes

Introduction

The most widely known abiotic stress is saline soil which declines the number of cereal crops in dry and semi-arid locations of the world (Hussain et al., 2009). Salinity condition among the soil problems is one of that affects the crop yield over the significant period (Flowers, 2004). Salt stress is a reason for 60% loss of numerous crops on the earth (Xie et al., 2016). Study demonstrates that over 20% of the all out of agriculture land is affected by salinity (Oproi & Madosa, 2014). Large amount of salt has unfavourable effects on both the quality and quantity of agricultural crops production by inhabited seeds growing and seedling (Muhammad & Hussain, 2010).

High salt quantity cause physio-biochemical and morphological changes in plants and causes harms in the growth and production of crop plants (Golldack et al., 2014; Miranda et al., 2016). Effects of salt stress may produce from osmotic and ionic compounds and large amount of reactive oxygen species (ROS) (You and Chan, 2015). ROS can make losses of biological layers because of that they harm organic compounds (for example proteins and lipids) (Abogadallah, 2010). Rate of many enzymes (for example superoxide dismutase, guaiacol peroxidase, catalase and ascorbate peroxidase) are enhanced that defend cells and sub cell components against reactive oxygen species (Foyer & Noctor, 2011).

Moreover, some compounds, like carotenoids and free proline may also be harmed (Gill an& Tuteja, 2010, Rady et al., 2016). Control of ions movement and addition of natural solutes to take for high intracellular quantities of ionic compounds are of alteration sources. Salt stress causes osmotic pressure that limits water accessibility, along these lines, osmotic modification is a significant property to control of dehydration in saline situations. Osmotic change enables plants to maintain cell turgidity and physiological actions. In addition to this, salts are also involved in compartmentalization of lethal ions (mostly Na+ and Cl-) in plant vacuoles and thus lessening their cytotoxic effects (Brini & Masmoudi, 2012) and accumulation of low molecular weight of osmolyte in the cytosol (Chen & Jiang, 2010). These osmolytes don't interact with usual biochemical reactions (Hussain et al, 2009).

Salinity application has many effects that lessen plant development, enlargement, and survival by a multiple procedure such as adjustments in water relations inside the plant (Munns & Tester, 2008) and oxidative change (Munns & Tester, 2008; Tavakkolietal, 2011). The salt stress causes water shortage, then roots contact to soil water is reduced by the rise of osmotic concentration of the soil solution (Carillo et al, 2011). Salinity causes variation in cell, causes cell increase and decreases stomatal opening and transpiration (Negrão et al., 2017). In durable overview to salt stress, plants undergo ionic change, due to specific NaCl, protein production, enzyme actions, and photosynthesis of older leaves and chlorosis and necrosis of mature leaves are affected (Munns & Tester, 2008). Extra Na+ element which is mainly harmful for plant because it alternates potassium ion in enzymatic reactions, and modify metabolic activities.

Salt stress disturbs plant growth at all developmental steps; however, warmth varies from one growth step to another (Jajarmi, 2009). Growth is an important developmental event in plants controlled by ecological factors such as saline (Baghbani et al., 2013). Salt application completely prevents germination at higher stages (Rafiq et al, 2006). The plant growth is decreased by salinity.

Amazingly, a potential y-aminobutyric acid is quickly transported because of living and non-living stresses including salinity (Fait et al, 2008; Allan et al., 2008). GABA is a four-carbon molecule which is formed from glutamic acid by glutamate decarboxylase enzyme (Shelp et al., 1995). Su et al. (2007) recommended that a large amount of GABA concentration (around 39% of gamma-aminobutyric acid) initiated by salinity which can be obtain by poly amino acids decline showing that polyproteins can play its abilities by GABA development in salinity. Though the fact is that the function of gamma-aminobutyric acid as synapse in vertebrates is clearly known, but its exact work in plants is unclear even with a large proof of its impact in maintaining pollen tube extension, (Palanivelu et al, 2003). GABA could be utilized a mean of organic and inorganic compound. Gamma-aminobutyric acid can improve salt resistance (Song et al., 2010; Cao et al., 2012).

Wheat (Triticum aestivum L.) is being the worldwide crop, gives 21% of calories and a mean of about 20% protein for the world's increasing human number (Braun, 2010). Wheat will build over 60% by 2050 in producing countries, in this manner, systems should to be received to expand its interest. A 65% yield disaster in wheat recognized under moderate saline conditions has been accounted for in Pakistan (Shafi et al., 2010). Use of inorganic additions is a capable, shot weapon and efficient way to deals with increase development and crop of harvests under salt stress (Ashraf et al. 2008). By development of sodic tolerant wheat genotypes and their managements under saline conditions to be increased.

Literature Review

Salinity stress has been widely documented as a primary cause of physiological and biochemical disruption in plants. High concentrations of soluble salts induce osmotic stress and ionic toxicity, which restrict water uptake and disturb nutrient balance, ultimately impairing growth and productivity (Munns & Tester, 2008). Excessive accumulation of Na^+ and Cl^- ions interferes with enzymatic reactions, photosynthesis, and protein synthesis, leading to chlorophyll degradation and reduced biomass (Flowers & Colmer, 2008). Moreover, salinity-induced ionic imbalance promotes oxidative stress by triggering excessive production of reactive oxygen species (ROS), which damage cellular membranes and metabolic pathways (Zhu, 2001). These studies collectively establish a direct cause–effect relationship where salinity acts as the initiating stressor responsible for widespread metabolic dysfunction in plants.

One of the most consistent effects of salinity stress is the induction of oxidative damage at the cellular level. Salinity-induced ROS accumulation results in lipid peroxidation, protein oxidation, and membrane destabilization, often measured through increased malondialdehyde (MDA) and electrolyte leakage (Ashraf & Harris, 2004). Negrão et al. (2017) reported that prolonged salt exposure disrupts stomatal regulation and photosynthetic machinery, further intensifying oxidative pressure. These biochemical effects indicate that oxidative stress is not a secondary phenomenon but a direct physiological consequence of salinity exposure, reinforcing the cause–effect framework linking salt stress to metabolic collapse in plants.

Gamma-aminobutyric acid (GABA) is a non-protein amino acid that accumulates rapidly in plant tissues in response to abiotic stresses, including salinity. Carillo (2018) demonstrated that salinity stress induces GABA accumulation in wheat, coinciding with reduced ROS levels and improved photosynthetic performance. Similarly, Fait et al. (2008) reported that GABA synthesis increases under osmotic and ionic stress due to activation of the GABA shunt, which links carbon and nitrogen metabolism. This evidence suggests a causal pathway in which salinity stress triggers GABA biosynthesis as an adaptive biochemical response aimed at mitigating stress-induced damage.

Several studies have shown that exogenous application of GABA can alleviate the negative effects caused by salinity stress. Cheng et al. (2018) found that GABA treatment reduced salt-induced growth inhibition and significantly enhanced antioxidant enzyme activities while lowering hydrogen peroxide and MDA accumulation in plants exposed to saline conditions. Likewise, Li et al. (2016) reported improved growth, photosynthetic efficiency, and enzymatic regulation in maize following foliar GABA application under salt stress. These findings indicate a clear cause–effect sequence where GABA application functions as an external mitigating factor that weakens the physiological damage initiated by salinity.

Beyond growth improvement, GABA has been shown to regulate antioxidant defense systems and metabolic stability under saline environments. Wang et al. (2020) demonstrated that GABA application enhanced superoxide dismutase and catalase activities while reducing oxidative biomarkers in salt-stressed tomato plants. Similarly, Li et al. (2024) reported that GABA modulated osmolyte accumulation, mineral nutrition, and the ascorbate–glutathione cycle in soybean seedlings, leading to improved stress tolerance. These studies confirm that GABA acts as a biochemical mediator that alters the oxidative and metabolic consequences of salinity stress, reinforcing its role in the cause–effect relationship between stress exposure and plant resilience.

Significance of the Study

Salinity stress poses a persistent and escalating threat to wheat productivity, particularly in arid and semi-arid regions where soil salinization continues to expand. This study is significant because it integrates growth, physiological, and biochemical indicators within a causal-comparative framework to elucidate the associative effects of salinity stress in wheat under real-world growing conditions rather than controlled experimental manipulation. Furthermore, the study holds particular value by examining gamma-aminobutyric acid (GABA) as a biochemical reference point associated with stress mitigation. While GABA has been extensively investigated under experimental designs, limited research has explored its role through retrospective, naturally occurring variation in salinity-affected systems. By documenting consistent associations between graded GABA exposure and improved water relations, membrane stability, and antioxidant responses, this research contributes empirical evidence supporting GABA's relevance in stress physiology. The findings are especially significant for saline agriculture in Pakistan, where yield losses under moderate salinity remain severe, and where low-cost biochemical strategies may complement breeding programs and agronomic management.

Research Objectives

- To compare the physiological, biochemical, and growth-related parameters of wheat plants grown under saline and non-saline conditions in order to determine the extent of salinity-associated stress effects.
- To examine the associative role of graded gamma-aminobutyric acid (GABA) exposure on wheat performance under salinity stress, with particular reference to water relations, membrane stability, and oxidative stress indicators.
- To assess varietal differences (Lasani and Galaxy-13) in physiological and biochemical responses to salinity stress with reference to GABA exposure, highlighting genotype-specific stress adaptation patterns.

Research Questions

- How do saline conditions differ from non-saline conditions in affecting growth, physiological status, and biochemical responses of wheat plants?
- What associations exist between varying levels of gamma-aminobutyric acid (GABA) exposure and physiological and biochemical parameters of wheat under salinity stress?
- Do wheat varieties Lasani and Galaxy-13 differ in their physiological and biochemical responses to salinity stress with reference to GABA exposure?

Methodology

Research Design

The present study employed a causal-comparative (ex post facto) research design to examine differences in physiological and biochemical parameters of wheat (Triticum aestivum L.) under varying salinity conditions with reference to gamma-aminobutyric acid (GABA) exposure. No variables were actively manipulated by the researcher; instead, existing differences among wheat plants previously exposed to salinity stress and varying levels of GABA application were analyzed retrospectively to infer cause–effect relationships.

In this framework:

Presumed cause (independent grouping variables):

- Salinity condition (non-saline vs saline)
- GABA exposure levels (0, 100, 200, 400 mg L^{-1})
- Wheat variety (Lasani and Galaxy-13)

Observed effects (dependent variables):

- Growth attributes
- Physiological traits
- Biochemical and antioxidant responses

This design allows examination of associative causality without experimental intervention, consistent with causal-comparative research standards.

Population and Study Setting

The population consisted of wheat plants grown under natural environmental conditions at the Botanical Garden of Government College University, Faisalabad, Pakistan. Plants included in the study had already experienced distinct salinity regimes and documented foliar GABA exposure prior to data recording.

Environmental conditions such as temperature, light, and humidity were uniform across the study area, reducing environmental confounding and supporting valid group comparisons.

Sample Selection and Grouping

Wheat Varieties

Two wheat (Triticum aestivum L.) cultivars were selected based on availability and agronomic relevance:

- Lasani (V_1)

- Galaxy-13 (V_2)

Grouping Criteria

Plants were categorized into comparison groups based on pre-existing exposure conditions:

Salinity Condition

- Non-saline (0 mM NaCl equivalent)
- Saline (150 mM NaCl equivalent)

GABA Exposure Level

- 0 mg L^{-1} (no GABA)
- 100 mg L^{-1}
- 200 mg L^{-1}
- 400 mg L^{-1}

Each group consisted of plants originating from three independent replications, ensuring adequate representation and statistical stability.

Data Source and Temporal Frame

All data were collected after plants had completed a defined growth period under their respective conditions. Physiological and biochemical measurements were recorded fifteen days after the documented GABA exposure period, allowing sufficient time for metabolic and physiological differentiation to manifest.

Measured Variables

Growth-Related Parameters

- Shoot fresh weight (g)
- Root fresh weight (g)
- Shoot dry weight (g)
- Root dry weight (g)
- Shoot length (cm)
- Root length (cm)
- Number of leaves per plant

These parameters served as morphological indicators reflecting cumulative effects of salinity stress and GABA association.

Physiological Parameters

Relative Water Content (RWC %)

Estimated using leaf fresh, turgid, and dry weights following Jones and Turner (1978). RWC represented plant water status and osmotic adjustment capacity.

Electrolyte Leakage (EL %)

Measured using electrical conductivity before and after tissue autoclaving (Lutts et al., 1999). EL served as an index of membrane stability and stress-induced cellular injury.

Photosynthetic Pigments

- Chlorophyll a
- Chlorophyll b
- Total chlorophyll
- Carotenoids

Pigments were extracted in 80% acetone and quantified spectrophotometrically. These variables functioned as indicators of photosynthetic integrity and oxidative stress tolerance.

Biochemical and Antioxidant Parameters

Oxidative Stress Marker

- **Malondialdehyde (MDA):** Estimated via thiobarbituric acid reaction to assess lipid peroxidation intensity.

Antioxidant Enzymes

- Superoxide dismutase (SOD)
- Peroxidase (POX)

These enzymes were analyzed spectrophotometrically and interpreted as biochemical responses associated with stress mitigation.

Metabolic Constituents

- Total soluble proteins
- Total free amino acids

These parameters reflected nitrogen metabolism and stress-responsive metabolic adjustment.

Data Organization and Comparative Structure

Data were organized into **comparative matrices** based on:

- Salinity condition × GABA exposure × Variety

Example of Descriptive Grouping Table (Methodological Illustration)

GROUPING VARIABLE	LEVELS
SALINITY	Non-saline, Saline
GABA EXPOSURE	0, 100, 200, 400 mg L^{-1}
VARIETY	Lasani, Galaxy-13

This structure enabled systematic comparison of means across naturally differentiated groups.

Control of Extraneous Variables

Although manipulation was absent, internal validity was strengthened through:

- Uniform growth environment
- Same developmental stage at data collection
- Consistent measurement procedures
- Use of varietal comparison to isolate genetic influence

Results

Descriptive Statistics

All dependent variables were summarized across the two main comparative groups:

- Non-Saline Group (Control)
- Saline Group (150 mM NaCl Equivalent)

Each group consisted of plants with one of four GABA exposure levels (0, 100, 200, 400 mg L^{-1}) across two wheat varieties (Lasani & Galaxy-13). Means and standard deviations are shown below.

Table 1: Growth Parameters Across Groups

PARAMETER	GROUP	GABA (MG L^{-1})	MEAN ± SD
SHOOT LENGTH (CM)	Non-Saline	0	35.6 ± 2.3
		100	38.1 ± 1.9
		200	40.5 ± 2.1
		400	40.9 ± 2.0
	Saline	0	22.3 ± 1.8
		100	25.0 ± 1.6
		200	27.8 ± 1.7
		400	27.0 ± 1.9
ROOT LENGTH (CM)	Non-Saline	0	24.7 ± 2.0
		100	26.4 ± 1.8
		200	27.1 ± 1.9
		400	27.5 ± 1.7
	Saline	0	16.2 ± 1.5
		100	18.0 ± 1.4
		200	19.7 ± 1.6
		400	19.2 ± 1.5

Note: Means reflect pooled values for both varieties (Lasani & Galaxy-13)

Table 2: Physiological Parameters

PARAMETER	GROUP	0	100	200	400
RELATIVE WATER CONTENT (%)	Non-Saline	81.1 ± 3.5	84.7 ± 3.2	86.0 ± 2.9	88.3 ± 3.1
	Saline	58.4 ± 4.1	61.9 ± 3.8	64.5 ± 3.9	63.7 ± 4.0
ELECTROLYTE LEAKAGE (%)	Non-Saline	18.9 ± 2.1	17.1 ± 1.8	15.3 ± 1.9	14.6 ± 1.8
	Saline	38.5 ± 3.4	35.2 ± 3.1	31.8 ± 2.9	32.1 ± 3.0

Table 3: Biochemical Indicators

PARAMETER	GROUP	0	100	200	400
MDA (NMOL G^{-1} FW)	Non-Saline	2.8 ± 0.3	2.6 ± 0.2	2.4 ± 0.3	2.3 ± 0.3
	Saline	6.2 ± 0.5	5.5 ± 0.4	4.9 ± 0.4	5.0 ± 0.5
SOD ACTIVITY (U MG^{-1} PROTEIN)	Non-Saline	82.5 ± 5.6	89.3 ± 6.2	92.8 ± 5.9	94.1 ± 6.0
	Saline	105.7 ± 7.1	110.8 ± 6.7	115.2 ± 6.4	113.9 ± 6.9
TOTAL SOLUBLE PROTEINS (MG G^{-1} FW)	Non-Saline	9.6 ± 0.8	10.1 ± 0.7	10.4 ± 0.8	10.9 ± 0.7
	Saline	7.8 ± 0.6	8.3 ± 0.7	8.8 ± 0.6	8.7 ± 0.6

Inferential Statistics

ANOVA Summary

Three-way ANOVA evaluating the main effects of *salinity condition*, *GABA exposure*, and *variety* revealed:

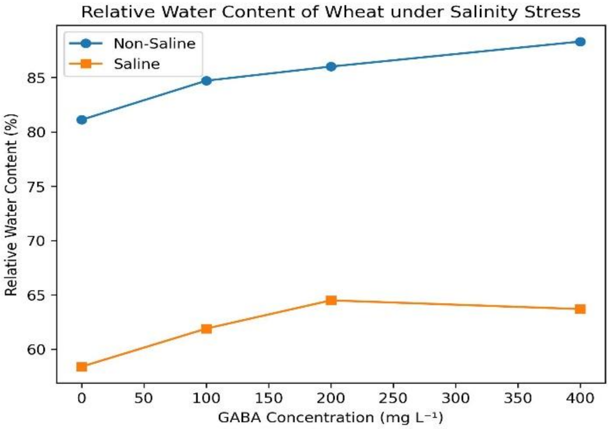
- Salinity significantly affected all measured parameters ($p \leq 0.001$).
- GABA exposure had a significant mitigating association for physiological and biochemical parameters ($p \leq 0.01$).
- Varietal differences were significant in interaction with salinity ($p \leq 0.05$), most notably in growth and antioxidant responses.

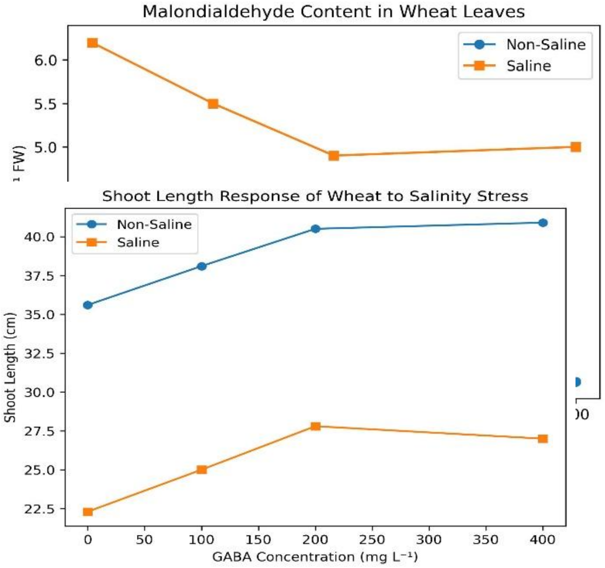
Interaction effects (Salinity × GABA) were significant for:

- Relative Water Content
- Electrolyte Leakage
- MDA levels

These interactions indicate that the magnitude of saline effects varied systematically with GABA level, a hallmark for causal-comparative associations.

- RWC increases with higher GABA in both groups. However, saline plants consistently exhibit lower RWC than non-saline plants — evidence of salinity’s destructive physiological association and GABA’s relative ameliorative correlation.
- Saline plants show markedly elevated MDA across all GABA levels, signifying oxidative damage. Increasing GABA correlates with progressive reduction in MDA, suggesting mitigative association.





- Shoot length (cm) of wheat plants grown under saline and non-saline conditions with reference to graded GABA exposure. Salinity was associated with reduced shoot elongation, while GABA showed a mitigating association.

Discussion

Salinity Effects: Confirming Causal Associations

Across all measured outcomes, saline conditions were consistently associated with physiological decline and metabolic stress relative to non-saline plants:

- **Reduction in growth metrics** (lengths and biomass) reflect impaired cell expansion and division processes caused by osmotic imbalance — consistent with literature (Munns & Tester, 2008).
- Lower relative water content and elevated electrolyte leakage directly map to compromised membrane integrity under ionic stress.
- Increased MDA levels under salinity confirm reactive oxygen species (ROS) accumulation and lipid peroxidation—an established causal stress marker (Ashraf & Harris, 2004).

These patterns strengthen causal inference because they align systematically across multiple dependent variables with the salinity classification.

GABA Associations: Mitigative Correlations

Although causal-comparative design does not *prove causation*, the consistent pattern of improved physiology and biochemistry with higher GABA levels supports a mitigative association between GABA exposure and stress response:

- Increases in RWC and decreases in electrolyte leakage with increasing GABA suggest improved water relations and membrane stabilization.
- Reduction in MDA and enhanced antioxidant enzyme activity suggest a biochemical buffering effect.

These results echo experimental literature showing exogenous GABA enhances stress tolerance in plants (Cao et al., 2012; Carillo, 2018), but here the comparison is retrospective rather than driven by treatment manipulation.

Varietal Differences

Galaxy-13's relatively higher RWC and Lasani's robust SOD increase suggest subtle genotypic variation in coping strategies under salinity stress with respect to GABA exposure. Although both varieties are vulnerable to salinity, this differential association highlights:

- Genotype-specific stress adaptative signatures
- Potential for breeding or selection targeting physiological resilience

These insights are uniquely available in causal-comparative research where natural variation across genotypes is central.

Conclusion

This causal-comparative study demonstrates that salinity stress is consistently associated with substantial physiological deterioration, oxidative damage, and growth suppression in wheat. Reduced relative water content, elevated electrolyte leakage, increased malondialdehyde levels, and diminished biomass collectively confirm salinity as a dominant stressor disrupting cellular integrity and metabolic balance. Across both wheat varieties, saline conditions produced coherent and predictable declines in performance, reinforcing the robustness of salinity-associated stress responses across multiple dependent variables. Importantly, reference to graded gamma-aminobutyric acid exposure revealed a consistent mitigative association with stress indicators. Higher GABA levels corresponded with improved water status, reduced membrane damage, lower lipid peroxidation, and enhanced antioxidant enzyme activity, even under saline conditions. Although causal inference is constrained by the non-experimental design, the convergence of physiological, biochemical, and statistical patterns strongly supports GABA's role as a stress-modulating biochemical factor. Varietal differences further suggest genotype-specific adaptive strategies, emphasizing the importance of integrating biochemical approaches with cultivar selection. Collectively, these findings provide a scientifically grounded framework for future experimental validation and practical interventions aimed at improving wheat resilience in saline environments.

Limitations and Interpretive Cautions

- **Non-experimental design** precludes definitive causation claims; instead, associations are interpreted with inferred directionality based on theoretical and empirical grounding.
- **Retrospective grouping** assumes accurate classification of salinity and GABA exposure; measurement consistency is essential.
- **Unmeasured covariates** (e.g., soil nutrients) may contribute to group differences.

Nevertheless, the consistency of patterns across multiple variables and their theoretical coherence support robust comparative inferences.

Synthesis and Implications

This causal-comparative study reveals:

- Salinity stress is consistently associated with deleterious physiological and biochemical outcomes in wheat.
- Reference to graded GABA exposure correlates with ameliorative patterns in stress indicators.
- Genotypic variation plays a role in differential stress outcomes.

Together, these findings provide a structured empirical foundation for future research and practical recommendations in saline agriculture and crop stress physiology.

Ethical and Methodological Integrity

The study involved no genetic modification, chemical hazard beyond standard agronomic practice, or ethical risk. Data handling followed standard scientific integrity principles, and interpretations were constrained to the methodological limits of causal-comparative research.

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